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Effects of the Indole-3-Acetic Acid (IAA) Transport Inhibitors *N*-1-Naphthylphthalamic Acid and Morphactin on Endogenous IAA Dynamics in Relation to Compression Wood Formation in 1-Year-Old *Pinus sylvestris* (L.) Shoots[1]

Björn Sundberg*, Hannele Tuominen, and C. H. Anthony Little

Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden (B.S., H.T.); and Natural Resources Canada, Canadian Forest Service, P.O. Box 4000, Fredericton, New Brunswick, E3B 5P7, Canada (C.H.A.L.)

Both *N*-1-naphthylphthalamic acid (NPA) and methyl-2-chloro-9-hydroxyfluorene-9-carboxylic acid (CF) inhibit the polar transport of indole-3-acetic acid (IAA) and, therefore, are attractive tools for investigating IAA's role in the regulation of plant growth. Ringing an intact conifer shoot with lanolin containing NPA or CF induces the formation of compression wood above the ring. This induction has been attributed to a postulated accumulation of IAA above the application site of the IAA transport inhibitor, but the validity of this postulation has never been confirmed. Using gas chromatography-selected ion monitoring-mass spectroscopy with [$^{13}C_6$]IAA as an internal standard, we measured the levels of endogenous free and conjugated IAA in 1-year-old *Pinus sylvestris* (L.) shoots ringed with NPA or CF. The level of free IAA was dramatically decreased below the ring, indicating that the polar transport of endogenous IAA was inhibited by the treatment. However, the free IAA level above the ring, where compression wood was formed, was also slightly lower than in control shoots. The lack of IAA accumulation above the site of the IAA transport inhibitor could not be explained by an increase in IAA conjugation. Furthermore, the turnover of [2-^{14}C]IAA, measured using high-performance liquid chromatography with on-line radioactivity monitoring, was the same in NPA-treated and control shoots. The decrease in IAA level above a NPA or CF ring is attributed to these substances being transported acropetally and interfering with polar IAA transport along the shoot. It is concluded that compression wood formation above a NPA or CF ring is not associated with an overall increase in cambial region IAA level or increased IAA turnover. Instead, we suggest that acropetally transported NPA and CF induce compression wood formation by interacting with the NPA receptor in differentiating tracheids, thereby locally increasing IAA in these cells.

NPA and the morphactin CF interfere with many processes in plant growth and development, with the abolition of tropic responses being the most investigated (Jones et al., 1954; Ching et al., 1956; Schneider, 1970; Katekar and Geissler, 1980). These substances have also been characterized as specific inhibitors of the polar transport of IAA in hypocotyl and coleoptile segments, as measured by the donor-receiver block method (Hertel and Leopold, 1963; Krelle and Libbert, 1968; Parups, 1970; Bridges and Wilkins, 1973; Thomson et al., 1973; Thomson and Leopold, 1974; Gagianas and Berg, 1977; Katekar and Geissler, 1980). In intact plants it has been demonstrated that ringing a stem with NPA or CF inhibits the transport of radiolabeled IAA and that the label accumulates above the ring (Cruz and Audus, 1978; Johnson and Morris, 1989; Little et al., 1990). It is generally believed that NPA inhibits IAA transport by specific binding to the so-called NPA receptor, thereby blocking the carrier-mediated efflux of IAA (Zettl et al., 1992; Muday et al., 1993). Key evidence for this notion is the observation that IAA accumulates in tissue segments, cell cultures, or microsomal vesicles incubated with NPA (Rubery, 1990). It has also been shown that CF binds to the NPA receptor and can cause an accumulation of IAA in corn coleoptiles, suggesting that CF inhibits polar IAA transport by the same mechanism as NPA (Thomson and Leopold, 1974; Sussman and Goldsmith, 1981). Although it is generally agreed that NPA, CF, and other phytotropins (see Katekar and Geissler, 1980, for definition) specifically disrupt polar IAA transport, the exact mechanism by which plant growth and development are affected by these substances is still debated (Firn and Tamimi, 1985; Katekar, 1985; Rubery, 1987; Morris, 1988; Katekar and Geissler, 1992).

Compression wood formation normally occurs on the underside of inclined stems and branches of conifers, and its function is to force the organ back to its original position in space (for review, see Timell, 1986). When the stem or branch of a conifer species such as *Pinus sylvestris* (L.) is ringed with NPA or CF in lanolin, compression wood formation is induced around the entire circumference along the shoot above the ring (Smolinski et al., 1972, 1973; Phelps et al., 1974, 1977; Yamaguchi et al., 1980, 1983). All of these investigators attributed this induction to an accumulation of endogenous IAA in the affected part of the shoot, because it has been demonstrated with many conifer species, including 1-year-old shoots of *P. sylvestris* (Sundberg and Little, 1990), that

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* Corresponding author; fax 46–90–165901.

Abbreviations: CF, methyl-2-chloro-9-hydroxyfluorene-9-carboxylic acid; NPA, *N*-1-naphthylphthalamic acid.

applying a high concentration of exogenous IAA results in compression wood formation (Timell, 1986). Although it is well established that NPA and CF inhibit polar IAA transport and cause an accumulation of exogenously applied IAA, endogenous IAA has never been measured in such an experimental system. Moreover, the possibility that NPA and CF affect IAA transport and metabolism at some distance from the application site has rarely been considered. In this study, we show that applying a ring of NPA or CF in lanolin to 1-year-old shoots of *P. sylvestris* causes the expected decrease in the cambial region level of endogenous IAA below the application site. However, above the ring, where compression wood was induced, IAA level was also significantly decreased, and there was no increase in IAA turnover. The significance of these results for the dynamics of IAA and the regulation of compression wood formation in conifer shoots is discussed.

MATERIALS AND METHODS

Plant Materials

Field-grown trees and potted saplings of *Pinus sylvestris* (L.) were used. The field-grown trees, about 10 years old and 2.5 m tall, were selected in a natural stand located on the campus of the Swedish University of Agricultural Sciences, Umeå, Sweden (63°50′ N, 20°20′ E). The saplings were 4 years old and had been reared outdoors in 5-L pots in fertilized peat. They were brought indoors in March, when the peat was still frozen, thawed at 4°C, and reactivated in a greenhouse with a day temperature of 21°C, a night temperature of 15°C, a RH of 50 to 70%, and a photoperiod of 18 h (natural daylight supplemented with Osram HQI-TS 400 W/DH lamps, giving a quantum flux density of 50–70 μmol m^{-2} s^{-1} PAR). During the reactivation and experimental periods, the saplings were watered daily and fertilized once a week with a 1:100 dilution of SUPERBA S (Hydro Supra AB, Landskrona, Sweden).

Application of NPA, CF, and [2-^{14}C]IAA

The treatments were performed on 1-year-old shoots, e.g. the uppermost whorl of branches in field-grown trees and the previous year's terminal shoot in potted saplings. The NPA (Uniroyal Chemical, Middlebury, CT), CF (Celamerk Gmbh. and Co., Ingelheim am Rhein, Germany), and [2-^{14}C]IAA (2.26 GBq $mmol^{-1}$) (Amersham International) were applied at concentrations of 10 g, 1 g, and 10 μg (8×10^6 dpm) g^{-1} lanolin, respectively, around the circumference along 1 cm (0.5 g of lanolin, NPA, CF) or 2 cm (1 g of lanolin; [2-^{14}C]IAA) of the shoot's length. Plain lanolin was used as the control. Prior to application, the periderm was carefully removed with a scalpel from the treatment site, which after the application was covered with aluminum foil. The lanolin mixtures were replaced once a week.

Experimental Design

The first experiment (Fig. 1, A1) was performed to determine the occurrence of compression wood formation and the level of free IAA in the cambial region above and below a site of NPA or CF application. Three groups of five field-grown trees with four 1-year-old shoots of equal size in the uppermost whorl were selected. On June 14, early in the grand period of cambial growth, all four branches in one group of trees were treated as described above with either NPA, CF, or plain lanolin. After 3, 6, 13, and 24 d, one shoot from each of the 15 trees was harvested, immediately frozen in liquid N_2, and stored at −80°C. Samples for the free IAA measurement and the anatomical investigation were obtained as indicated in Figure 1.

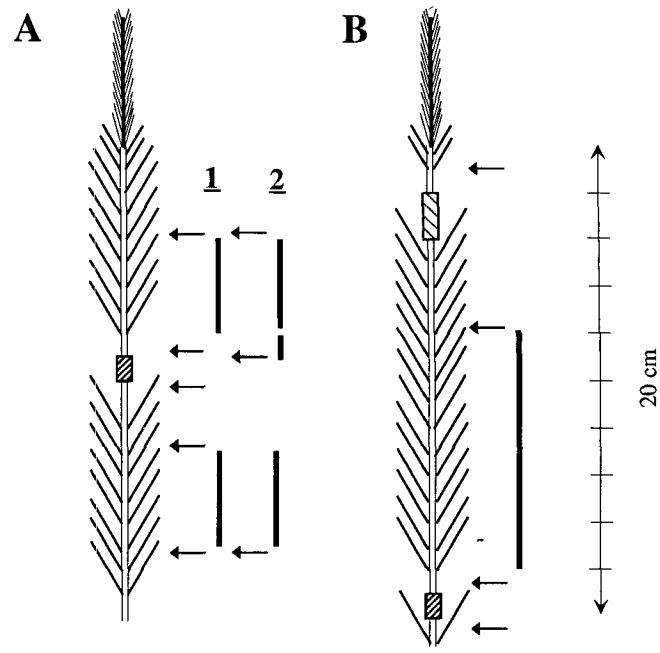

Figure 1. Site of ringing with NPA and CF (▨) and [2-^{14}C]IAA (▧) and of the sample obtained for IAA measurement (|), along the length of the 1-year-old shoot in experiments 1, 2 (A1 and A2), and 3 (B). Arrows indicate where compression wood formation and xylem width were measured.

In the second experiment (Fig. 1, A2), performed with NPA only, both free and conjugated IAA were measured above and below the application site as in experiment 1, and, as well, an additional sampling site was included directly above the application site. Nine field-grown trees with four equal-sized 1-year-old shoots in the uppermost whorl were selected. On June 17, two of the four shoots in each tree were treated with NPA, and plain lanolin was applied to the other two. One shoot per treatment was harvested from each tree after 7 and 14 d, immediately frozen in liquid N_2, and stored at −80°C prior to analysis.

The third experiment (Fig. 1B) investigated whether NPA-induced compression wood was associated with a difference in IAA turnover. Two groups of six similar-sized potted seedlings undergoing rapid cambial growth were selected. One group was treated with NPA, and the other was treated with plain lanolin, both being applied to the previous year's terminal shoot 20 cm below the distal whorl of current shoots. After 8 d [2-^{14}C]IAA was applied as described above to the

same shoot 2 cm below the distal whorl of current shoots. Four days later (i.e. after 12 d of NPA treatment), the 1-year-old shoot was harvested, immediately frozen in liquid N_2, and stored at −80°C. Radiolabeled compounds were monitored in a 10-cm-long segment obtained from above the application site.

Anatomical Investigation

The extent of compression wood formation and xylem production was determined in transverse handcut sections. The sections were stained in a saturated aqueous solution of phloroglucinol in 20% HCl and mounted in Canada balsam. Compression wood was recognized by the presence of tracheids with a round shape and thickened secondary wall and by the occurrence of intercellular spaces. Compression wood formation was expressed as the percentage of the circumference in which the last-formed tracheids had compression wood characteristics. Xylem production was measured as the ratio of current-year xylem width to previous-year xylem width, to obviate initial differences in shoot size. Xylem width was measured at eight equidistant points around the circumference. This measurement included only tracheids reacting with the stain, hence, those tracheids in which at least some secondary wall lignification had occurred.

Measurement of IAA and IAA Conjugates

The procedures for measuring IAA and IAA conjugates were as described by Sundberg (1990). After the shoot was thawed, the cambial region sample was harvested by peeling the bark and combining the scrapings obtained from the exposed xylem side using a scalpel with the surface tissues stripped from the exposed bark side using fine-nosed forceps. Thus, the sample consisted of differentiating xylem cells, cambium zone cells, and differentiating, mature, and 1-year-old phloem cells. After grinding the sample in liquid N_2, it was extracted for 1 h in 5 mL of 0.05 M phosphate buffer, pH 7.0, containing 0.02% sodium diethyldithiocarbamate as an antioxidant and [$^{13}C_6$]IAA (Cambridge Isotope Laboratories, Woburn, MA) as an internal standard. After filtration, free IAA was purified by neutral and acidic ether partitioning, followed by reversed-phase C_{18} HPLC, and quantified by GC-selected ion monitoring-MS. The ions 261/267 were used to check peak homogeneity, and the ions 202/208 were used to quantify endogenous IAA by reference to a standard curve.

When IAA conjugates were measured the extract was divided into two portions after the filtration step. Free IAA was determined in one portion. The other portion, used to measure total IAA, was subjected to hydrolysis in 7 N NaOH at 100°C under a stream of water-saturated N_2 for 3 h, neutralized with HCl, and then purified and quantified as described above so as to obtain an estimate of total IAA. The arithmetic difference between free and total IAA is termed IAA conjugates.

Analysis of [2-^{14}C]IAA Metabolism

Cambial region samples (approximately 1 g), obtained as described above, were homogenized in liquid N_2 with a mortar and pestle and extracted under continuous stirring for 1 h at 4°C in 5 mL of 80% methanol containing 0.02% sodium diethyldithiocarbamate. The extract was evaporated at 40°C under reduced pressure in a rotary evaporator to the water phase, which was transferred to a conical test tube and further reduced to approximately 150 μL under a flow of N_2. Methanol and acetic acid were added to give a final concentration of 20% methanol in 1% acetic acid. After the sample was centrifuged at 3000*g* to remove particles, it was analyzed by reversed-phase C_{18} HPLC with on-line radioactivity monitoring.

The liquid chromatograph (Waters Associates AB, Partille, Sweden) consisted of an M680 gradient controller and two M510 pumps. Samples were introduced off-column via a Rheodyne model 7125 injection valve with a 200-μL loop (Rheodyne, Cotati, CA). Ion suppression reversed-phase HPLC utilized a 10-cm × 8-mm i.d. 4-μm Nova-Pak C_{18} cartridge fitted in an RCM 8 × 10 module (Millipore AB, Vàstra Frölunda, Sweden). The mobile phase consisted of a gradient from 20 to 80% methanol in 1% aqueous acetic acid over 30 min at a flow rate of 1 mL min^{-1}. The column eluate was directed to a 1208 Betacord radioactivity monitor (LKB Wallac, Bromma, Sweden) with a heterogeneous flow cell packed with cerium-activated lithium glass scintillator. Fractions (0.5 mL) were collected by a fraction collector, mixed with scintillant, and counted in a Beckman LS 1801 radioactivity counter.

RESULTS

In experiment 1 (Fig. 1, A1) both NPA and CF induced compression wood formation around the entire circumference and along the whole length of the 1-year-old shoot above the application site. In contrast, compression wood was formed only on the lower side in control shoots (Table I). Tracheids with compression wood characteristics were present 6 d after NPA or CF was applied, and typical compression wood was formed during the remainder of the experimental period. The CF also stimulated xylem production well above the application site, whereas NPA increased xylem width only locally above the site (Fig. 2). Below the NPA or CF application site, xylem production was almost completely inhibited. Both substances decreased the elongation of the distal current-year terminal shoot and its needles by an average of 34 and 25%, respectively, and caused the tips of the current-year shoots to curl abnormally, most obviously in the CF treatment. Similar effects of NPA on compression wood formation, xylem production, and current-year shoot growth were observed in experiments 2 and 3 (Fig. 1, A2 and B) (data not shown). Both NPA and CF markedly decreased the level of free IAA below the application site throughout the experimental period (Fig. 3). However, neither substance caused free IAA to accumulate above the application site; rather, they decreased the IAA level relative to the control shoots. This decrease was greater, and occurred sooner, in CF-treated shoots than in NPA-treated shoots.

In the second experiment (Fig. 1, A2), performed with NPA only, both free and conjugated IAA were measured as in experiment 1 and also at an additional sampling site directly

Table I. *Induction of compression wood formation by NPA and CF*

Percentage of compression wood formed around the circumference at different positions above and below the site of application of plain lanolin, 10 mg NPA g^{-1} lanolin, or 1 mg CF g^{-1} lanolin, classified as 1 = 0 to 20%, 2 = 21 to 40%, 3 = 41 to 60%, 4 = 61 to 80%, 5 = 81 to 99%, and 6 = 100%. $n = 5$; the range is within ± 10%.

Treatment	Position (cm)															
	5.25 Above				0.25 Above				2.75 Below				7.25 Below			
	3 d	6 d	13 d	24 d	3 d	6 d	13 d	24 d	3 d	6 d	13 d	24 d	3 d	6 d	13 d	24 d
Control	1	1	1	1	2	1	1	1	2	1	2	1	2	2	2	1
NPA	1	5	6	6	2	5	6	6	2	1	1	1	2	2	2	2
Morphactin	1	6	6	6	1	5	6	6	1	1	1	1	1	2	2	2

above the NPA ring. The NPA affected the free IAA level in the same way as in experiment 1, decreasing it markedly below the application site and to a lesser extent at a distance (1–5 cm) above the site (Fig. 4). However, directly above the NPA ring the level of free IAA was similar to that in control shoots. The amount of IAA conjugates was always less than 15% of the free IAA level. Treatment with NPA elevated the level of IAA conjugates directly above the application site, the extent of the elevation increasing with time. The NPA also significantly increased the IAA conjugate content below the application site after 7 d but not after 14 d.

The possibility that the reduced IAA level above the NPA ring was related to an increase in IAA catabolism was investigated in a third experiment (Fig. 1B). After application of [2-^{14}C]IAA near the apical end of the shoot, basipetally transported radioactivity accumulated above the NPA ring (Fig. 5B). However, the metabolic pattern of [2-^{14}C]IAA was the same in NPA-treated and control shoots and in both cases, approximately 80% of the recovered label was still in the form of free IAA, with the remainder being associated with three major fractions of [^{14}C]IAA catabolites after reversed-phase HPLC (Fig. 5, A and C). The homogeneity and identity of the IAA peak was confirmed by further analysis with ion-pair reversed-phase HPLC (data not shown).

DISCUSSION

The well-established fact that NPA, CF, and other phytotropins inhibit the polar transport of IAA has made them attractive tools for elucidating IAA's roles in regulating plant growth and development. However, the possibility that these substances affect other aspects of endogenous IAA dynamics has received little consideration. The results presented here show that ringing a conifer shoot with NPA or CF decreases the IAA level not only below the ring as expected but also, surprisingly, above the ring. This observation has implica-

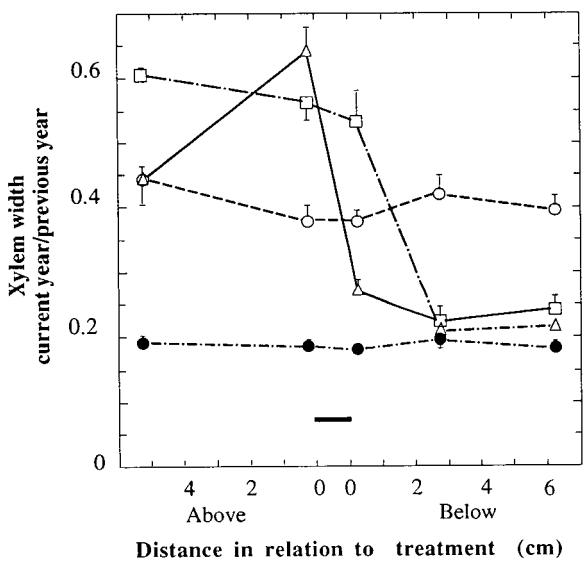

Figure 2. Xylem width at different positions along the 1-year-old shoot at the beginning of the experiment (●) and after 24 d of ringing with plain lanolin (○), 10 mg NPA g^{-1} lanolin (△), or 1 mg CF g^{-1} lanolin (□). Mean ± SE, $n = 5$. Bar indicates the application site.

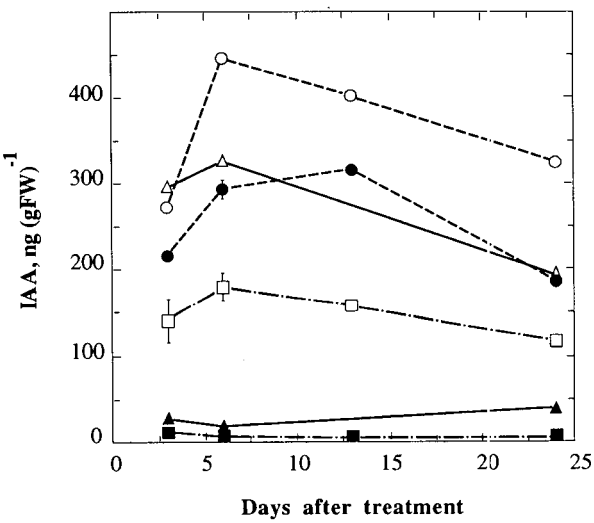

Figure 3. Levels of free IAA in the cambial region measured above (1–5 cm) and below (3–7 cm) the site of ringing with plain lanolin (○, above; ●, below), 10 mg NPA g^{-1} lanolin (△, above; ▲, below), or 1 mg CF g^{-1} lanolin (□, above; ■, below). Each sample consisted of five pooled shoots, and independent duplicates of each sample were measured. The vertical line indicates the range of the duplicate measurements, when larger than the symbol.

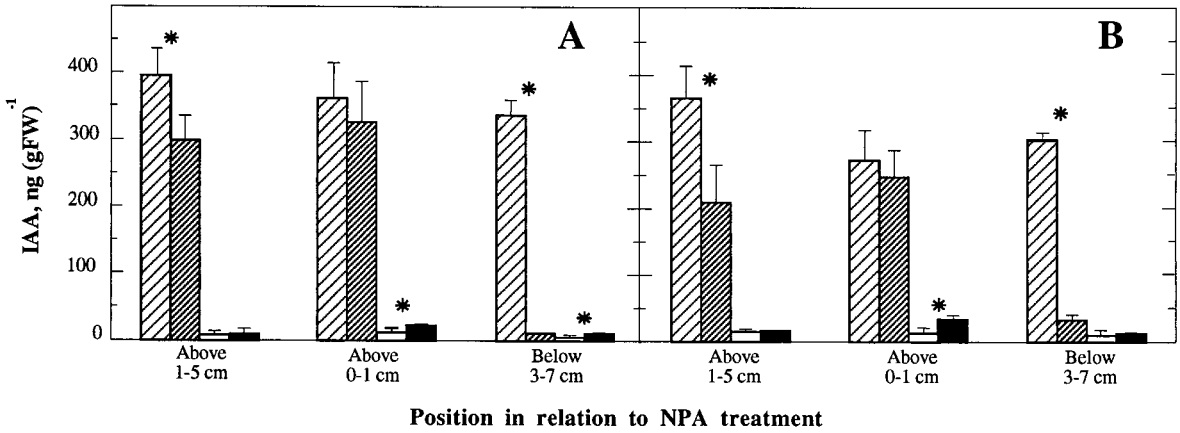

Figure 4. Levels of free and conjugated IAA in the cambial region at different positions along 1-year-old shoots measured 7 d (A) and 14 d (B) after ringing with plain lanolin (▨, free IAA; □, conjugated IAA) or 10 mg NPA g^{-1} lanolin (▨, free IAA; ■, conjugated IAA). Mean ± SD of three independent samples, each consisting of three pooled shoots. An asterisk (*) indicates a significant difference ($P \leq 0.05$, Student's *t* test) between the NPA-treated and control shoots within each sampling position and IAA fraction. FW, Fresh weight.

tions when evaluating the involvement of IAA in compression wood formation in conifers, and it may also influence the interpretation of the results obtained with NPA and CF in other experimental systems.

The finding of a very low endogenous IAA level beneath the NPA or CF application site (Figs. 3 and 4) is consistent with our earlier demonstration that these substances decreased the basipetal transport of exogenously applied [^{14}C]-IAA in *P. sylvestris* shoots (Little et al., 1990). Thus, it can be concluded that the polar transport of endogenous IAA was inhibited at the site of NPA or CF application in the experiments described here. Our finding also suggests that the polar transport system provides the main supply of IAA to the cambial region of the 1-year-old pine shoot. It follows that the transport of IAA by mass flow in the phloem, which has been proposed to be important in trees (Aloni, 1988), was not significant under our experimental conditions. Furthermore, the existence of the markedly decreased IAA level below the NPA or CF ring argues against the hypothesis that differentiating tracheids are a major source of endogenous IAA (Sheldrake, 1973). The nearly complete inhibition of xylem production below the site of NPA and CF application (Fig. 2 and Little et al., 1990) supports earlier observations that a reduced supply of endogenous IAA, induced by debudding or defoliation, is causal to the reduced tracheid production (Little and Wareing, 1981; Sundberg and Little, 1987, 1990).

Although the polar transport system of IAA was blocked at the site of NPA or CF application, there was a reduction, instead of an accumulation, of IAA above the site (Figs. 3 and 4). Additional experiments with NPA indicate that this reduction could not be attributed to an increase in IAA conjugation or catabolism (Figs. 4 and 5C). Therefore, in addition to having a local effect on IAA transport at the application site, NPA and CF must also affect IAA transport well above the site, presumably because they are acropetally transported. The likely occurrence of such NPA/CF transport is reflected in aberrant geotropism resulting in curling and disorientation of the current-year shoots in our experiments, as well as in the study of Smolinski et al. (1972). Furthermore, Neumann et al. (1977) demonstrated that bark-applied ^{14}C-labeled morphactin diffused laterally and entered the xylem stream in *Pinus radiata* shoots. However, two lines of evidence indicate that the reduction in IAA transport capacity above the NPA ring was not complete. First, [^{14}C]IAA applied laterally to the top of a shoot ringed basally with NPA was readily transported down to the NPA ring (Fig. 5). Second, the free IAA level was similar in NPA-treated and control shoots directly above the application site, whereas it was significantly lower in NPA-treated shoots well above the site (Fig. 4). A significant increase in IAA conjugates was also observed directly above the application site but not further up. This suggests that basipetally transported IAA accumulated immediately above the NPA ring, where its level was down-regulated by conjugation (Cohen and Bandurski, 1982; Sitbon et al., 1991, 1993). However, the proportion of IAA conjugates to free IAA was small (<15%; Sundberg et al., 1990); thus, the increase in conjugated IAA directly above the application site in NPA-treated shoots did not significantly affect the total IAA level (free plus conjugated) at this position.

Although the reduction in the amount of IAA transported down the NPA-treated 1-year-old shoot can be attributed to an inhibitory effect of acropetally transported NPA on IAA movement in this shoot above the application site, it seems also to be partly due to a decreased supply of IAA from the distal current-year shoot. That is, when an equal amount of radiolabeled IAA was applied to NPA-treated and control 1-year-old shoots, more label was recovered from the NPA-treated shoots (Fig. 5B), whereas the amount of endogenous IAA was smaller in NPA-treated than in control shoots (Figs. 3 and 4). Considered together, our results suggest that the decreased IAA level above the NPA ring in a 1-year-old pine shoot is due to acropetally transported NPA interacting with the NPA receptor, thereby reducing the transport capacity for IAA in both the 1-year-old shoot and the current-year

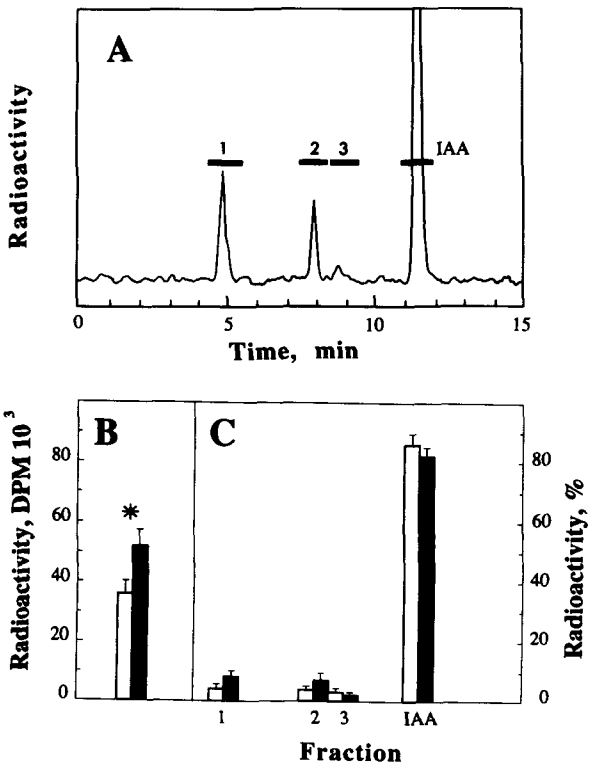

Figure 5. Metabolism of [2-^{14}C]IAA applied laterally for 4 d to the distal end of 1-year-old shoots pretreated near the base for 8 d with a ring of plain lanolin (eight shoots) or 10 mg NPA g^{-1} lanolin (eight shoots) (see Fig. 1B). Compression wood was being formed around the circumference and along the length of the shoot above the NPA application site when the [^{14}C]IAA was supplied. A, Typical reversed-phase C_{18} HPLC trace. Bars indicate peaks corresponding to fractions 1, 2, 3, and IAA. B, Total radioactivity recovered 1 to 10 cm above the site of application of plain lanolin (open bar) or NPA (black bar). C, Percentage of total radioactivity recovered in fractions 1 to 3 and in the IAA peak. Mean ± SE of eight independent samples, one from each shoot. The asterisk (*) indicates significant difference ($P \leq 0.05$, Student's *t* test) between the NPA and control treatments.

shoots. This may in turn decrease the biosynthesis of IAA in the current-year shoots.

It is evident from our results (Table I; Figs. 3 and 4) that compression wood formation above the application site in NPA- or CF-treated shoots was associated with IAA levels in the cambial region that were lower than those in control shoots, where normal tracheids were formed. Thus, it is necessary to re-evaluate the oft-stated supposition that compression wood formation in conifers that is induced by strangling or by IAA transport inhibitors is the result of endogenous IAA accumulation above the treatment point (Blum, 1970; Smolinski et al., 1972, 1973; Phelps et al., 1974, 1977; Yamaguchi et al., 1980, 1983). It also seems unlikely that the decreased IAA level above the NPA or CF ring was the direct cause of the compression wood formation in our experiments, since defoliation treatments, which have been shown to reduce the cambial region IAA level (Little and Wareing, 1981; Sundberg and Little, 1987), have never been reported to induce compression wood formation. On the contrary, the accepted view, arising from results obtained in experiments with exogenous IAA, is that high concentrations of IAA induce compression wood (Timell, 1986). However, saturating the polar transport system with exogenous IAA, which increased the internal IAA concentration 2-fold, did not result in compression wood formation in *P. sylvestris* shoots (Sundberg and Little, 1990). Moreover, although the formation of compression wood in displaced stems was associated with increased cambial region levels of endogenous IAA in *Cryptomeria japonica* (Funada et al., 1990), it was not in *Pseudotsuga menziesii* (Wilson et al., 1989) or *P. sylvestris* (B. Sundberg and C.H.A. Little, unpublished results).

We propose that NPA and CF induce compression wood formation above the application site by being transported acropetally and interacting with the NPA receptor in cambial derivatives that differentiate on the xylem side of the cambium. It is not known whether the signal(s) inducing the differentiation of tracheids with compression wood characteristics must be maintained in the cell for all or only part of the differentiation process. In either event, it is possible that engagement of the NPA receptor in specific cambial region cells could result in an accumulation of IAA in these cells only (Rubery, 1987; Morris, 1988), thereby triggering compression wood formation. Such localized accumulation need not be reflected in an increased IAA level measured in bulk cambial region samples and could even occur when the overall level of IAA in the shoot is reduced. It has been suggested that endogenous IAA transport inhibitors occur in plants (Rubery, 1987), and it can be hypothesized that such substances induce compression wood formation in displaced shoots in the same way as NPA and CF do in our experimental system. This would explain the anomaly that high levels of exogenous IAA induce compression wood formation (Timell, 1986), whereas decreased levels of endogenous IAA have been found in shoots forming compression wood (Figs. 3 and 4; Wilson et al., 1989; B. Sundberg and C.H.A. Little, unpublished results).

In addition to compression wood formation, NPA and CF also stimulated the production of xylem above the application site (Fig. 2). The NPA promoted xylem production for only a few millimeters, whereas CF increased it along the whole shoot above the site, as also noted by Smolinski et al. (1972, 1973). We have previously shown in 1-year-old *P. sylvestris* shoots that increasing the level of IAA in the cambial region stimulated tracheid production (Sundberg and Little, 1990). Although the IAA level was not measured specifically in the part of the shoot where NPA increased xylem production, it was lower along the length of the shoot where the production of xylem was stimulated by CF (Figs. 2 and 3). This relationship may also be attributed to compartmentalization of IAA within the cambial region, as discussed above.

In summary, if the endogenous IAA concentration plays a role in the regulation of compression wood formation, it is not necessarily manifested as a measurable increase in a bulk cambial region sample but, rather, as a more finely tuned change within differentiating tracheids. Our results indicate that the NPA receptor is involved in the formation of compression wood. It is suggested that its function is to modulate the IAA concentration in specific cells.

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