Oscillation of stem polarity expression in transport of natural auxin of pine cambium

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Abstract

Simultaneous measurements of acropetal and basipetal efflux of natural auxin from the cambial region of a series of successive sections from *Pinus silvestris* stems revealed an unknown until now variation of polarity expression in the transport of auxin, which occurs in short cycles along the stem. It is suggested that the direction of phase synchronization of this oscillation between the neighboring cambial cells determines the fronts of the auxin waves. Modulations of the auxin-wave caused by apical application of synthetic IAA were shown to depend upon changes in the polarity expression in respect to natural auxin transport. Some evidence of a direct involvement of the exogenous auxin in the mechanism responsible for oscillations of the polarity expression was also obtained in experiments in which ^{14}C-IAA was applied directly into the phloem of living trees of *Pinus taeda L.* Transport of labelled auxin was very slow however, and its direct involvement in polar transport (the oscillation of which was measured) extended only for a short distance from the place of aplication. Simultaneously, the natural auxin wave measured by bioassays extended to further distances. The results corroborate the hypothesis that oscillations of the polarity expression responsible for generation of the auxin wave in cambium depend primarily upon a system autonomous to cells of the cambial region, independent of the direct contribution of the molecules of auxin produced at distant sources. On the other hand, such external to cambium sources of auxin modulate the auxin-wave parameters affecting the system responsible for expression of the polarity in the regions close to their synthesis. These modulations are than propagated in the cambial zone.

Key words: polarity, auxin, Pinus, auxin-wave, morphogenesis

INTRODUCTION

The amounts of natural auxin, collected by basipetal efflux to agar from the cambial region of a vertical series of successive short stem sections, oscillate forming a wave-like pattern. This pattern was interpreted as a manifestation of

the vectorial field which forms when the auxin waves translocate in the cambium, and which may play an important role in a system of positional information for plant morphogenesis (Zajączkowski and Wodzicki 1978a, b). The chemical nature of the natural auxin of pine involved in the wave was proved to be identical with indole-3-acetic acid (IAA) by methods of high performance liquid chromatography (HPLC) and gas chromatography- -mass spectrometry (GC-MS). The amounts of IAA from cambium originally measured by Went's oat coleoptile curvature stimulation test directly in the agar strips were confirmed by a quantifying method of selected ion monitoring (SIM) (Wodzicki et al. 1987). In this study, no natural inhibitors or other than IAA stimulators were found diffused into agar to interfere significantly with bioassays of the natural auxin from the cambial region of *P. silvestris*. Synthetic growth regulators, especially IAA, however, modulate parameters of the auxin-wave when applied at physiological concentrations in agar to the apical cut-surface of the stem segments (Wodzicki et al. 1979, Wodzicki and Wodzicki 1981). In the case of IAA, the effect is recorded first of all as a local increases of the wave amplitude at a distance of 50-60 mm from the place of application in 66-72 mm long stem sections after as little as 60-100 min. Thus, propagation of such signals is several times faster than basipetal transport of the ^{14}C-labelled IAA in the same system (Wodzicki et al. 1984), which excludes any direct molecular action of the synthetic phytohormone.

Knowledge of the mechanism generating auxin-waves and propagation of the auxin-wave modulations in the stem cambium is essential for further study of a possible role of the system in specification of a morphogenetic information in plants. The results presented in this paper contribute to understanding of these processes.

MATERIAL AND METHODS

Stem segments comprising the cambial region were obtained from ca. 80-year old trees of *Pinus silvestris* L. grown in Experimental Forests of the Warsaw Agricultural University in Rogów (Poland), and from about 40- -year old *Pinus taeda* L. in the forest stands near Athens, Georgia, supervised by the School of Forest Resources, University of Georgia (USA).

Strips of 1% agar, about 1.5 mm thick, 2 mm wide and 22 mm long were applied to both apical and basal ends of resectioned stem segments to various number of 6-mm high sections comprising phloem, cambium and about 2-3 mm of the recently formed xylem. After 10-30 min of contact with the stem tissue, during which periods the amounts of natural auxin collected in agar conform to strict logarithmic relation with time (Wodzicki 1978), the agar strips from basal and apical, or only from basal ends were bioassayed by the slightly modified Went's oat coleoptile curvature test (Funke 1939).

¹⁴C[2]-IAA (NEC), 50 mCi per mMole was used for the experiments with synthetic auxin applied to the exposed surface of secondary phloem in stems of the 3 living *P. taeda* trees. Radioactivity was measured first in the whole agar strips which previously were applied to the cut surface of the cambial region of the resectioned stem segments collected below the place of ¹⁴C-IAA treatment, and afterwards 2nd in crude methanolic extracts of macerated tissue separated to phloem and xylem in the cambial region. LOBA-Chemie, Wien ¹²C-IAA was used for other experiments.

RESULTS

OSCILLATION OF THE POLARITY IN AUXIN TRANSPORT

The hypothesis that the wave-like pattern of auxin basipetal efflux from a cambial region of series of successive short stem sections depends upon oscillation of the polarity expression was investigated in *P. silvestris* stem longitudinal segments $72 \times 22 \times 4$ mm in size. The segments were cut to twelve 6-mm high sections to which strips $1.5 \times 2 \times 22$ mm of pure 1% agar were applied for 30 min both at the apical and basal ends opposite the cambial region as indicated in Fig. 1. The experiments were repeated several times throughout the season.

Results presented in Fig. 1. indicate that oscillation occurred consistently in the basipetal auxin efflux. The acropetal efflux was always less then the amount of corresponding basipetal transport. In one or two cases, only slight oscillation was recorded in acropetal collections and then it was usually opposite to the corresponding basipetal efflux. Consequently, the polarity of natural auxin transport calculated in agar by acropetal to basipetal efflux closely followed the wave-like pattern specific for the basipetal transport.

EFFECT OF APICAL IAA TREATMENT

Two 22 mm wide and 4 mm thick and 80 mm long plate-like stem segments comprising the cambial zone were recut from a larger $44 \times 20 \times 80$ mm piece of stem tissue of *P. silvestris*. Synthetic IAA (0.57 µMole) in a 1% agar strip was applied opposite the cambial region of apical end of one of the two segments for 90 min. Pure agar strips were applied to the apical end of the other segment and to the basal ends of both segments. After 90 min, the stem segments were successively sectioned from the basal ends acropetally to twelve 6-mm long sections to which fresh strips of pure agar were applied at both ends for 10 min. The uppermost 8 mm sections (closest to the surface supplied with synthetic IAA) were discarded. After 10 min, the agar strips were collected and bioassayed, except for those applied at the apical ends of the sections cut from the untreated stem segment.
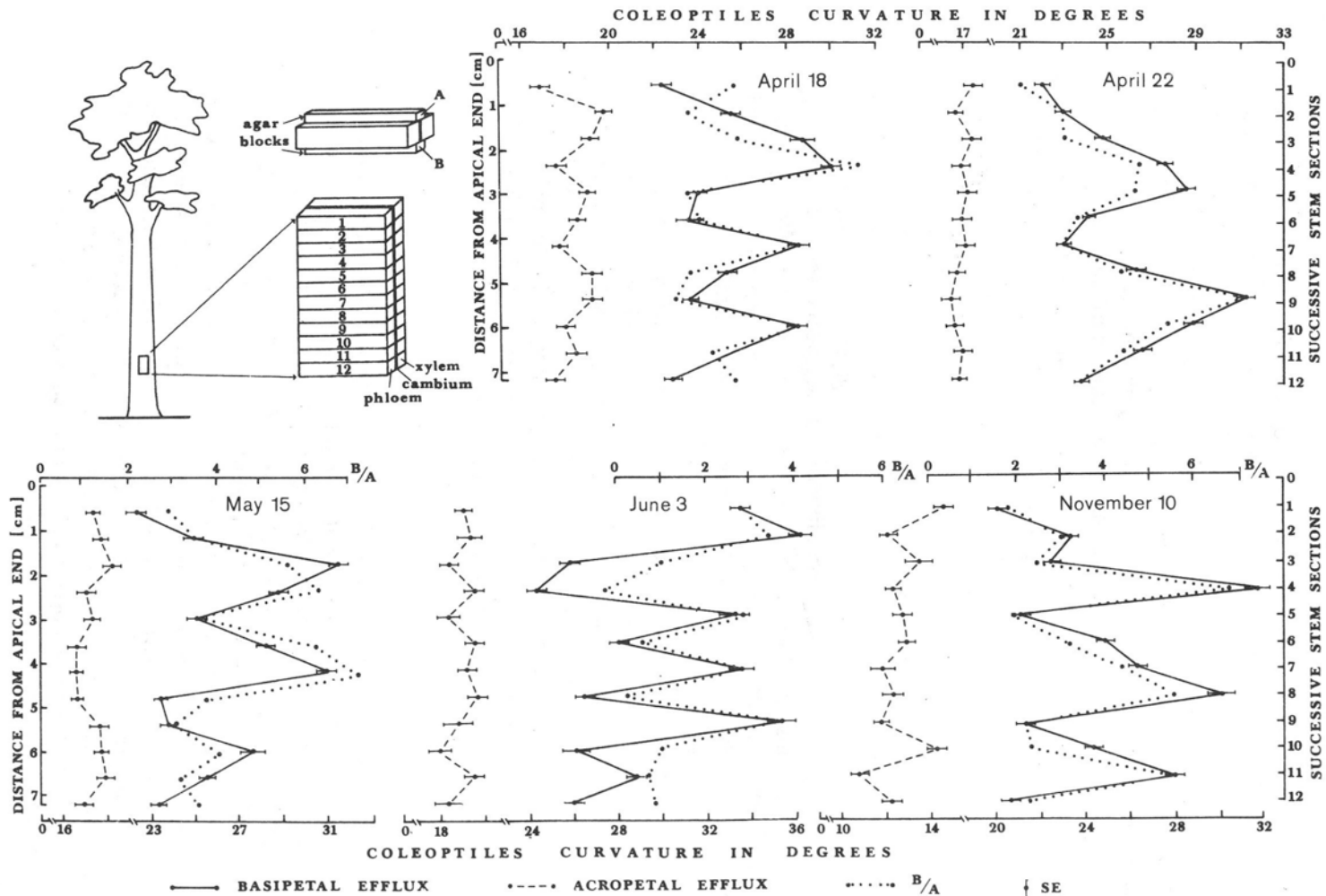
Fig. 1. Bioassay determinations of natural auxin efflux to agar after 30 min from basal (B) and apical (A) ends of twelve 6-mm long sections of stem of *P. silvestris* collected at breast height several times during the 1986 season. Dotted lines denote ratio of apical to basipetal efflux (polarity of auxin transport)

Results of bioassays expressed in concentration equivalents of authentic IAA stimulation are summarized in Fig. 2. It is seen that apical application of IAA modified the pattern of basipetal efflux of the natural auxin (especially its amplitude) while the acropetal efflux from consecutive stem sections remained at the same level. Thus, the calculated polarity of auxin transport (the acropetal to basipetal efflux ratio) in the IAA treated stem segment followed closely the basipetal efflux of natural auxin.
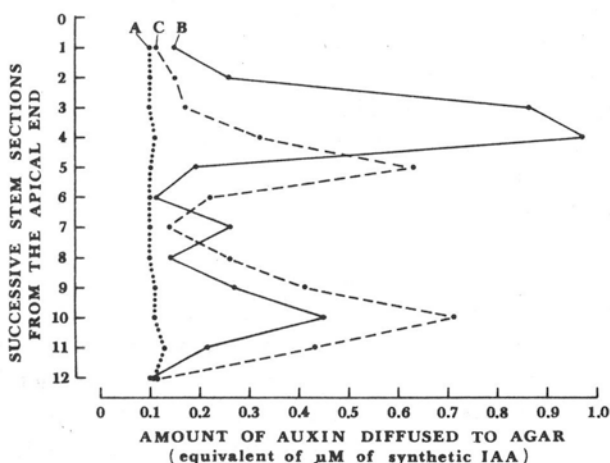

Fig. 2. Bioassay determination of natural auxin efflux to agar after 10 min from basal ends of two series of twelve 6-mm long sections of *P. silvestris* stem (solid and broken lines), and acropetal diffusion (dotted line). One of the stem segments (marked B, A) had been treated with 0.57 μM IAA in 1% agar for 90 min before cutting to short sections. The acropetal to basipetal efflux ratio (polarity of auxin transport) follows almost exactly the pattern of basipetal efflux (not shown.)
C — refers to untreated stem segment

MOLECULAR TRANSPORT OF EXOGENOUS IAA AND THE AUXIN WAVE

Three dominant trees of *P. taeda* were selected in the forest stand on September 4th. Synthetic IAA cá 0.2 mMole (36 μg ml^{-1}) was applied in 1 cm^3 of water solution to a cotton plug taped to the surface of exposed secondary phloem in a rectangle 20×30 mm of stem at breast height of two of the trees. One of the two received ^{14}C-IAA (10 μCi) while unlabelled IAA was supplied to the other. A third tree was analogously treated with 1 cm^3 of pure water. After 24 hrs, the stem segments including the treated region were brought to the laboratory and resectioned to smaller blocks of tissue comprising phloem, cambium and recent xylem (66 mm longitudinally, 22 mm tangentially and 4 mm radially) chosen 5 mm below the lower edge of rectangle of debarked tissue.

Agar strips were applied to all three stem segments at the apical and basal ends opposite the cambial zone for 20 min and then each of the segments was cut transversely to eleven 6 mm high sections. Fresh agar strips were immediately applied to both basal and apical cut surfaces of each of the short .sections opposite the cambial region. After 10 min the agar strips detached from the basal ends of the two nonradioactive series of stem sections were taken for bioassays. Agar strips from the series treated with ^{14}C-IAA were collected and replaced with fresh agar strips for another 90 min at both apical and basal ends. Radioactivity of all these agar strips was determined directly in liquid scintillation vials. Simultaneously, the eleven stem sections of the radioactive series were dissected to phloem and xylem in the region of cambium which was still active. The xylem side was scrapped down to the hard mature wood while the phloem was finely chopped and extracted 3 times with 2 cm^3 of MeOH, each section separately. The eluates, combined and evaporated directly in liquid scintillation vials, as well as the residual tissues were separately tested for radioactivity.

Bioassays revealed the wave-like pattern of growth stimulation by the substances diffused to agar from basal ends of the nonradioactive series of stem sections irrespectively of IAA treatment (Fig. 3). Stimulations by substances from the series supplied with IAA were higher than from the nontreated tree, however, these were not confirmed in the experiments repeated later in September and October, while the wave patterns were always evident. Thus, the observed difference in total results of stimulation, in this case, has been ascribed to the individual variability of trees, although this deserves further attention.in seasonal studies because in October the cambium was dormant and its responses to auxin could be different.
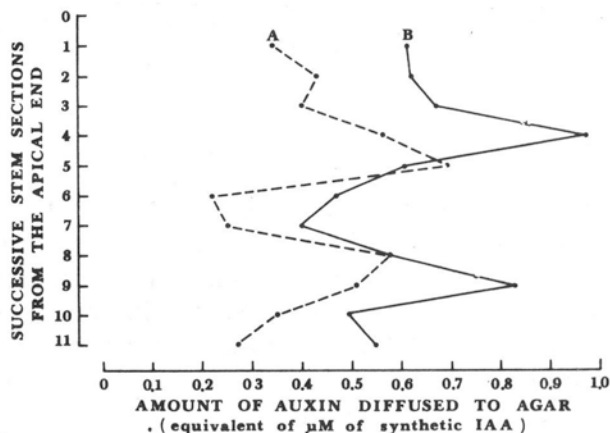

Fig. 3. Bioassay determination of natural auxin efflux after 20 min from basal ends of a series of eleven 6-mm long stem sections, each from a different tree of *P. taeda*. Stem segments sectioned 5 mm below the place of application of pure water (A) or IAA in water solution (B) to outer surface of the nonfunctional phloem.

Table 1

Radioactivity distribution (CPM) along 66-mm stem segment of *Pinus taeda* 5 mm below the place of application, and 24hr after ^{14}C-IAA was applied to nonconducting phloem of living tree. Eleven 6-mm basipetally successive sections

Section No	Distance from IAA application place (mm)	Diffused to agar from				Extracted from		Residual in		Total collected from the section
		apical end		basal end		phloem	xylem	pholem	xylem	
		10 min	90 min	10 min	90 min					
1	5-11	5788	9199	3958	6481	29246	13698	9925	1451	79746
2	11-17	3497	4783	2893	4961	8484	6264	2644	760	34286
3	17-23	1730	2056	1534	2468	3279	3010	985	383	15445
4	23-29	458	692	395	706	1456	1297	495	169	5668
5	29-35	112	275	82	196	448	368	192	49	1722
6	35-41	76	133	61	130	241	168	76	34	919
7	41-47	38	71	49	48	205	135	55	24	625
8	47-53	35	* 53	42	43	165	71	43	16	468
9	53-59	23	* 56	32	* 56	144	65	60	7	443
10	59-65	* 28	50	22	* 59	118	81	54	15	427
11	65-71	* 26	36	50	77	184	79	44	14	510

* Values not significantly different at 95 per cent confidence level. SE of all other means less than 5 per cent.
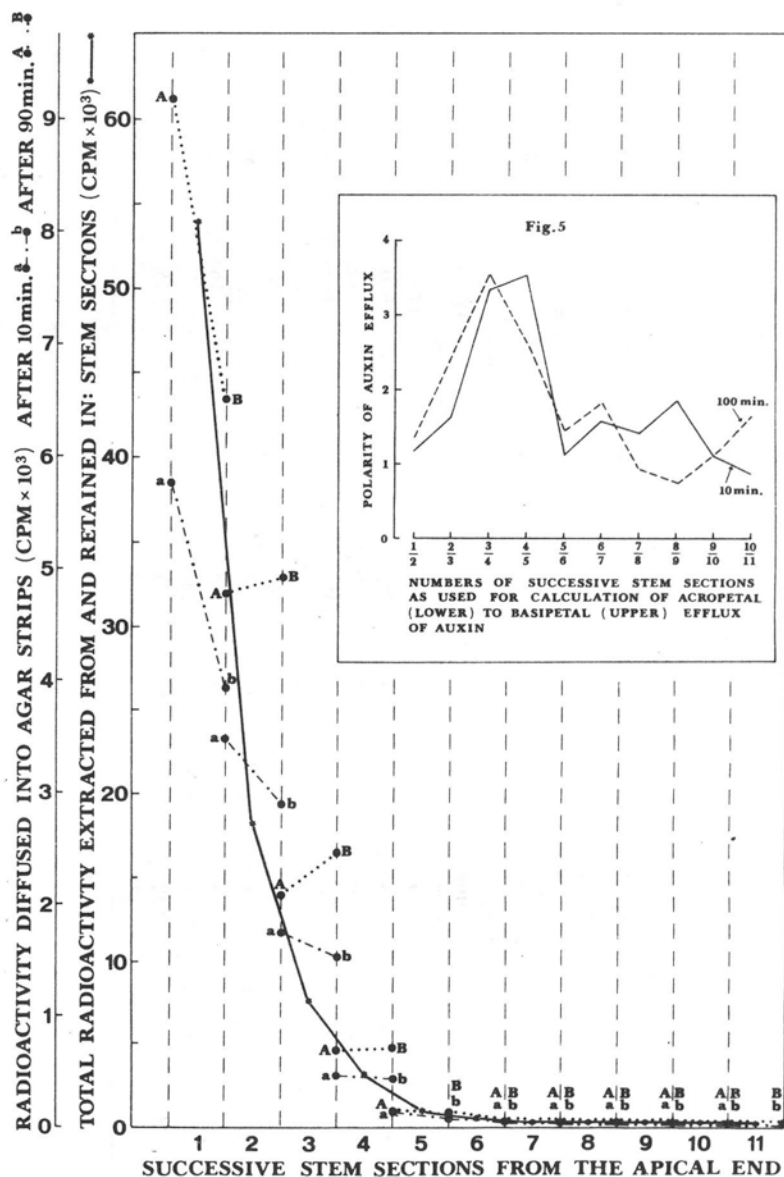
Fig. 4. Distribution of total less diffusible, and the diffused to agar radioactivity of eleven 6-mm successive sections of the 66 mm long stem segment collected 5 mm below the place of application, and 24 hr after ^{14}C-IAA was applied to nonconducting phloem of a living *P. taeda* tree. An acropetal (A, a) and basipetal (B, b) efflux to agar during periods: 0-10 min (a, b) and 10-100 min (A, B)

Fig. 5. Acropetal to basipetal ratio of radioactivity (CPM) diffused to agar after 0-10 min (10 and 10-100 min (100) from successive eleven 6-mm high sections of 66 mm long *P. taeda* stem segment collected 5 mm below the place of application, and 24 hr. after ^{14}C-IAA was applied to nonconducting phloem. Ratio calculated each time between the basipetal efflux of the preceding and the acropetal efflux of the following stem section

Radioactivity of the successive stem sections, both in soluble and solid tissue fractions decreased rapidly with distance from the place of application of ^{14}C-IAA (Table 1). In the region 5-11 mm below the edge of the exposed phloem to which the labelled auxin was applied, the radioactivity recovered in these fractions was about 0.36% of the total supplied and only 0.002% at the distance of 47-53 mm. This corresponds to 60 ng of IAA per 1 mm^2 of cambial surface at the place of application, and 1 ng mm^{-2} or 5.8 pg mm^{-2} of cambium at the distances of 5-11 or 47-53 mm respectively, it all the recovered radioactivity would be in IAA.

The radioactivity of agar strips which contained the substances diffused from the stem sections decreased rapidly with increasing distance from the source down to 47 mm and then equilibrated at the low level, at which significant differences (if any) could not be distinguished from random variability of measurements (Table 1). General patterns of regression recorded after 0-10 and 10-100 min of diffusion remained similar. Neither basipetal or acropetal diffusion of the labelled substances from the successive stem sections revealed visible oscillations. However, basipetal diffusion from six uppermost sections was more dynamic than the acropetal one which was also closer to the regression pattern of total radioactivity of the soluble and solid fractions (Fig. 4). After 90 min of diffusion from the basal ends of the 2nd, 3rd, 4th, 10th, 11th stem sections, the radioactivity was even higher than that of the corresponding apical agar strips, revealing polarity of the transport. The quotient of the radioactivities obtained by acropetal to basipetal efflux from each of the successive pairs of stem sections disclosed a single cycle of the polarity oscillation within a distance of 5-41 mm from the source of ^{14}C-IAA (Fig. 5). The results of analogous calculation for further distances could not be interpreted with certainty due to the increased error of measurements at the low level of radioactivity recovered. However, a trace of second cycle of the polarity oscillation may be seen in a series of agar strips collected after only 10 min of diffusion.

DISCUSSION

Results of simultaneous measurements of acropetal and basipetal efflux of natural auxin from the cambial region of stem sections of *Pinus silvestris* presented in this study revealed an until now unknown variation of the polarity in transport of auxin which occurs in short cycles along the stem. After the bioassay results were confirmed by analytical and quantifying methods of GC-MS and SIM (Wodzicki et al. 1987), there is little doubt that the demonstrated oscillation referred to polar transport of natural IAA. The close similarity between the cyclic variation in auxin polar transport and the auxin waves (spatial pattern of basipetal efflux of auxin) suggests a casual relationship. Thus, if the phase pattern of the auxin-waves is synchronized in larger

areas of the stem cambial zone, as demonstrated by Wodzicki et al. (1984), this may be the result of the synchronized expression of the polarity in the neighboring clusters of cambial cells. However, the synchronized pattern of auxin waves can be altered by local applications of IAA or abscisic acid (ABA) in physiological concentrations for only 90 min to the cambial region of the larger stem tissue blocks, subsequently resectioned to three tangentially neighboring series of short stem sections (results unpublished prepared for separate publication, Wodzicki 1984). This means that the potential polarity of cells in the cambial region at any time and position may be expressed to various extents and change synchronously in the neighboring cells clusters. When the polarity expression oscillates with the shift of phase between such cell clusters, the supracellular auxin waves form and translocate in the cambial region as described by Zajączkowski and Wodzicki (1978a) and Zają-czkowski et al. (1983, 1984). The direction of phase synchronization of the oscillation of polarity expression determines the fronts of the auxin waves.

Some evidence of the analogous variation of stem polarity in respect to exogenous synthetic auxin was demonstrated also in spite of the masking effects caused by the steep gradient of radioactivity close to the place of application of ^{14}C-IAA to *Pinus taeda* secondary phloem. There is good reason to believe that most of this radioactivity measured after 24 hrs referred to still unmetabolized IAA, bacause in similar experiments with living trees of *Pinus echinata*, even after 48 hrs, Nix and Wodzicki (1974) recovered about 70 per cent of radioactivity from the cambial region in the IAA fraction. There is, however, a striking difference between the wave pattern revealed by measurements of the basipetal efflux of natural auxin by bioassays and the single cycle of the polarity change in respect to the exogenous ^{14}C-IAA transport which was found only within the limits of a steep gradient of radioactivity. The fraction of radioactive auxin which travelled below the gradient was so small that it practically could not directly contribute to the measurable amounts of natural auxin determined by bioassay, even if it entered the route of polar transport. However, also within the range of the gradient, if there was any significant effect of the exogenous auxin upon the pattern of the auxin wave, it was too small to be distinguished.

The results corroborate the hypothesis that oscillations of the polarity expression responsible for generation of the auxin wave in cambium depend primarily upon the system autonomous to the cells of the cambial region independent of the direct contribution of the molecules of auxin produced at distant sources (eg. apical meristems). Wodzicki et al. (1979) and Wodzic-ki and Wodzicki (1981) demonstrated that IAA and other phytohormones applied to the cambium produce modifications of the auxin wave propagated to distant cambial regions, and Wodzicki et al. (1984) exluded direct contributions of the molecular transport of the synthetic auxin to these regions. Involvement of the exogenous auxin revealed in the present experiments as the

measurable differences of ^{14}C-IAA polar transport only within a close range from the place of application may mean that this primary effect of auxin from the external source concerns alteration of the polarity expression in the closest cells of the cambial region. Any change in the oscillation phase of polarity, its frequency or amplitude due to effects of such a distant source of auxin would than be propagated within a system of cells of the cambial region, acting as a system of coupled oscillators (Zajączkowski and Wodzicki 1978a, Zajączkowski et al. 1983, 1984). The results presented in this paper indicating that the modulations of the auxin-wave caused by apically applied IAA in physiological concentrations depend upon changes of the polarity expression in respect to natural auxin transport, support such a hypothesis.

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Oscylacje przejawu polarności w transporcie naturalnej auksyny w rejonie kambium sosny

Streszczenie

Badano polarność wypływu naturalnej auksyny z serii kolejnych, w kierunku osiowym, krótkich odcinków tkanki z rejonu kambialnego pnia *Pinus silvestris*. Wykazano, że basipetalny wypływ tworzący obraz fali przestrzennej uwarunkowany jest oscylacją ekspresji polarności tkanki rejonu kambialnego. Sugeruje się więc, że kierunek synchronizacji fazy oscylcji przejawu polarności między sąsiadującymi komórkami strefy kambialnej określa fronty fal auksynowych opisanych wcześniej (Zajączkowski i Wodzicki, 1978a, Wodzicki et al. 1987). Również modulacja fali auksynowej powodowana przyłożeniem apikalnym agaru, zawierającego syntetyczne IAA w fizjologicznej koncentracji, uwarunkowana jest modyfikacją przejawu polarności tkanki. Krótki okres przyłożenia (90 min) i wyniki wcześniejszych badań (Wodzicki et al. 1984) wykluczają bezpośredni udział molekuł dostarczanego IAA w obserwowanej modulacji fali auksynowej w dalszej niż 15 mm odległości od miejsca przyłożenia. Badania z zastosowaniem ^{14}C-IAA do łyka wtórnego żywych drzew *Pinus taeda* sugerują jednak, że auksyna ze źródła pozakambialnego włącza się lokalnie do mechanizmu odpowiedzialnego za oscylacje polarności komórek, i tą drogą oddziaływuje na parametry fali auksynowej propagowanej w kambium.