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The effect of cambial zone isolation upon the autolytic system in maturing tracheids of pine (*Pinus silvestris* L.)

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Abstract

The autolytic protease system in maturing tracheids of the main stem of *Pinus silvestris* was investigated after separation (using surgical methods) of the cambial zone from the layer of differentiating xylem, in combination with decapitation and IAA application. Separation of the cambium prevented autolysis of the protoplast in maturing tracheids, although the specific activity of proteases was little reduced. It was found that a radial or longitudinal concentration gradient of exogenously applied auxin was not responsible for autolysis, but that it could influence the level of extracted protein, and proteolytic activity. Similarly, decapitation modified, only to small degree, the effects of the cambium separation. Thus, the data from this experiment lead to the conclusion that integration of all cells in the region of xylem formation is a crucial factor for the start of autolytic protoplast breakdown. Possible involvement of auxin waves in the transfer of the positional information for this process is suggested.

Key words: *auxin, proteolytic activity, xylem maturation*

INTRODUCTION

The breakdown of the protoplast terminates the differentiation of pine tracheids. The mechanism which controls the time when this process starts determines the duration of the tracheid maturation phase and, simultaneously, the period of deposition of the secondary cell wall. Studies of cell ultrastructure (Wodzicki and Brown 1973, Wodzicki and Humphreys

1973) led to the conclusion that autolysis of the protoplast in maturing pine tracheids is a rapid process initiated by degradation of vacuolar membranes. The cytoplasm is then exposed to vacuolar sap containing digestive enzymes.

Other data indicate the possibility that proteolytic activity in extracts from differentiating xylem of pine stems may be specific for the xylem morphogenetic pathway. This activity was correlated tentatively with auxin-stimulated xylem formation, but was not found during cambial dormancy, it decreased during mid-summer depression of cambial activity (Rakowski, in preparation). Reduction of the auxin supply to decapitated trees resulted in a delay of the autolysis of protoplast, while continuous availability of auxin causes differentiation to cease earlier (Porandowski et al. 1982). In this case, auxin specifies the positional information essential for autolytic processes, starting probably with breakdown of the vacuolar membranes. This type of information transfer by auxin has been proposed by Zajączkowski and Wodzicki (1978) and Wodzicki and Wodzicki (1980), who suggested that autolytic destruction of the protoplast is determined by the vectorial field resulting from the polar transport of auxin in association with oscillatory phenomena in the cambial zone. Owing to the possible important role in the mechanism determining the period of tracheid maturation, the autolytic protease system in the differentiating xylem of the pine stem was investigated in the experiments presented below.

MATERIAL AND METHODS

Eight-year-old pine trees (*Pinus silvestris* L.) were selected from a forest plantation in the Experimental Forest at Rogów (Central Poland) for experiments performed in June, 1983, and repeated in 1984. In each experiment, 40 trees were selected. Eight were untreated controls (Group A). Thirty-two (Group B–E) were subjected to the following surgical treatments (Fig. 1). Two strips of tissue, each approximately 5 mm wide, 10 cm long, and 2 cm apart from one another were removed from the sixth youngest annual increment of the main stem. Removed tissue included the bark, phloem, and cambial zone. In the area between the two stripped regions (isolated cambial zone) a polyethylene film was tangentially inserted separating the cambial zone from the maturing xylem. Isolation was performed in two opposite places on the stem circumference. The wounded stem portions were then covered with lanolin and plastic bags. The 32 trees were divided into 4 groups (Group B–E) and subjected to one of four treatments. Eight trees of Group B were not decapitated or

subjected to hormones. In 8 trees (Group C) 0.5% IAA in lanolin was applied to the side of the maturing xylem. Sixteen trees (Group D and E) were decapitated 5 cm above the wound; in Group E, 0.5% IAA in lanolin was applied to the cut surface of the stem and in Group D the surface was covered with lanolin paste without hormone.

The stem segments with separated cambial tissues from intact trees were collected for enzymatic and anatomical investigation after 20–40 days.

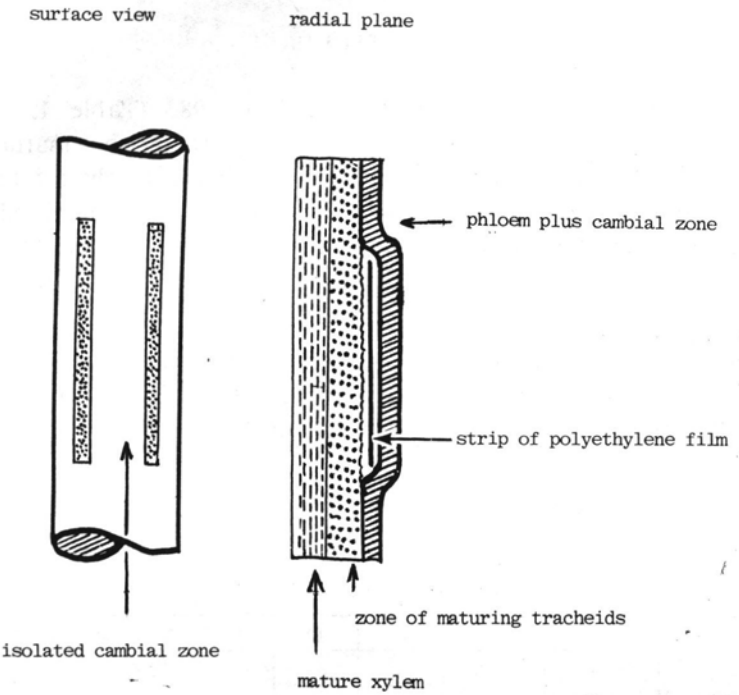

Fig. 1. Schematic drawing of the stem segment showing the area of cambial zone isolation from maturing cells of differentiating xylem, and manner of surgical treatment

Each time, four trees of each series were harvested. For enzymatic investigation, maturing xylem separated from the cambial zone was stripped with a knife and stored in solid CO_2 until November and December. All determinations of protein and proteolytic activity were performed with homogenized tissue extracted with 0.03 M phosphate-citrate buffer, pH 7.2 at the temperature of melting ice. The homogenates were centrifuged at 8000 × g for 10 min at 0°C, the supernatant was acidified to pH 4.1 with phosphate-citrate buffer and centrifuged once more at 9500 × g for 10 min. Protein was percipitated from the supernatant with 10% TCA and determined by Lowry's method at the beginning and end of incubation. Incubation of extracts (with no substrate other than the protein in the extract) was conducted at 37°C and pH 4.1 for 60 min.

For the anatomical investigations, small pieces of stem (1 cm long) from the middle part of the treated area were collected and preserved in 70% ethanol until sectioned. After staining with safranin and light green, the transverse stem sections were mounted in Canada balsam and the radial extent of the zone of maturing xylem (as distinguished according to Wilson et al. 1966) was investigated under the light microscope.

RESULTS

The data of the experiment performed in 1983 (Table 1, upper part) indicated that the proteolytic activity decreased in the maturing xylem if the cambial zone was isolated. It was reduced by half after 20 days and it was 3 times less after the next 20 days as compared with the activity in intact trees. The amount of protein extracted from the outermost

Table 1

Effect of cambial zone isolation, IAA application, and decapitation upon protein content and proteolytic activity in maturing cells of differentiating xylem of 8-year-old pine trees. Experiments performed in two successive years 1983 and 1984. Mean of 4 trees

Date of collection	Analyses I. Yield of protein extracted. $\mu g \times g^{(-1)}$ fr. wt II. Specific activity, $\mu g \times mg^{(-1)}$ prot. $\times hr^{(-1)}$						Extent of the zone of maturing tracheids, cell number per radial file				
1983		A	B	C	D	E	A	B	C	D	E
Start of experiment (June 7)	I	684	–	–	–	–	12	–	–	–	–
	II	920	–	–	–	–					
After 20 days	I	468	860	784	330	140	10	8	9	8	11
	II	987	467	440	664	350					
After 40 days	I	720	648	988	332	422	10	8	9	10	10
	II	750	290	375	559	243					
1984											
Start of experiment (June 12)	I	156	–	–	–	–	8	–	–	–	–
	II	1000	–	–	–	–					
After 20 days	I	140(1)	293	1389	7(2)	226	7	5	6	6	6
	II	744	197(2)	500	0	731					
After 40 days	I	404	70(2)	241	15(1)	78	10	5	6	4	6
	II	983	140(3)	0	0	0					

A, B, C, D, E – experimental treatments; A – intact trees (control), B, C, D, E – separation of the cambial zone from the maturing tracheids. D, E – decapitation 5 cm above the separation. C – application of IAA (0.5%) to the side of the maturing xylem. E – application of IAA (0.5%) to the cut surface of the stem. In a few samples, no proteolytic activity was shown. In these instances, numbers in parenthesis indicate trees not showing activity; the listed figure is still the average for all 4 replicates.

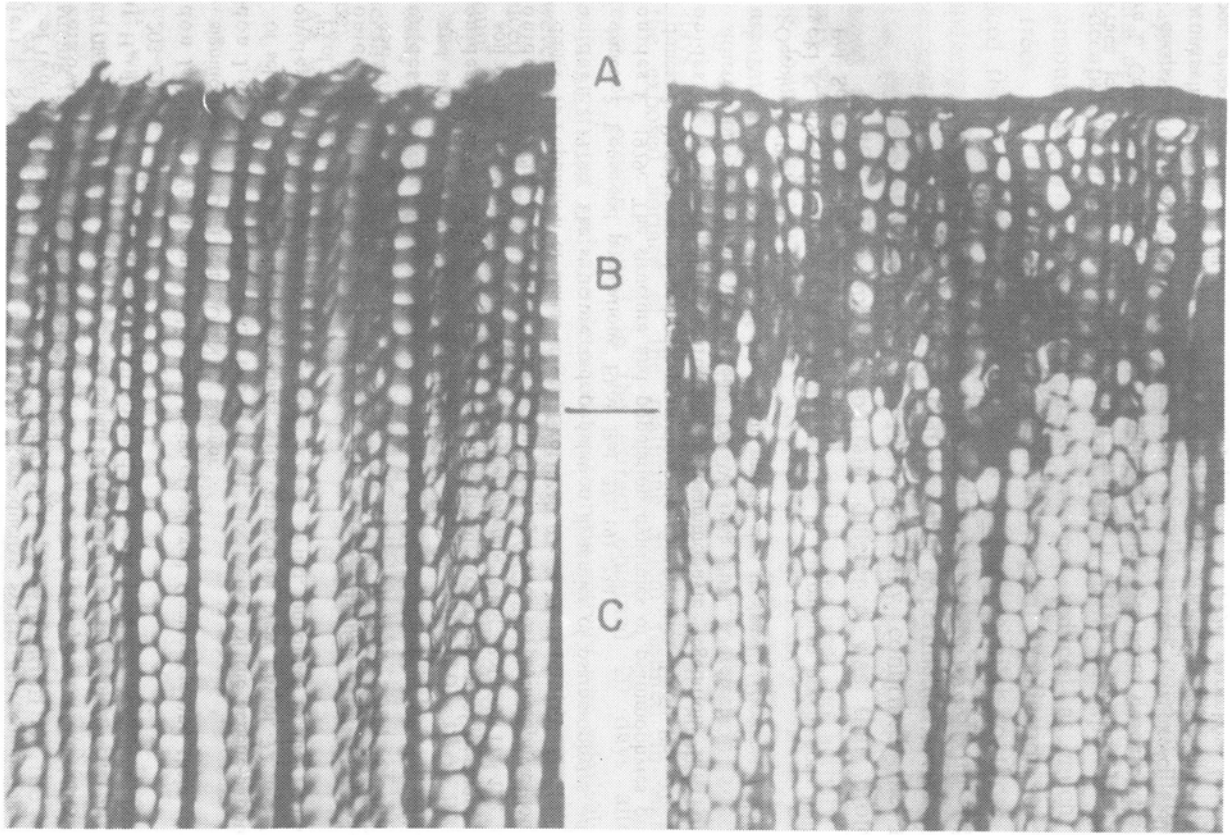

Fig. 2. Transverse sections through the stem of *Pinus silvestris* from the area of maturing tracheids. Left — just after isolation of cambial zone, indicating that the tissue separates to the outside of maturing xylem; Right — the same after 40 days of experiment (treatment B). The extent of the zone of maturation, in both examples is similar. A — surface of tissue breaking, B — zone of maturing xylem, C — mature xylem. ×100

tissue of the xylem side was not reduced or was even somewhat increased. The radial number of maturing tracheids was only slightly reduced, indicating that the protoplast breakdown was completed only in the most differentiated cells and that the process had not started in most of the zone of xylem maturation (Fig. 2). Decapitation of the treated stems reduced the amount of protein extracted, but the specific activity of proteases was not reduced. Decapitation did not change the number of cells in the zone of maturation. Lateral application of IAA scarcely changed the responses described for the non-decapitated trees. On the other hand, when auxin was applied apically, the amount of protein after 40 days was not reduced as much as in the trees not treated with IAA. However, apical application of auxin reduced the specific activity of proteases to levels similar to those found in undecapitated trees. Reduction of the radial number of cells in the zone of maturation was comparable to that in other experimental series.

The experiment performed in 1984 generally confirmed the results obtained in 1983. The most important difference was that proteolytic activity was lower in all treated plants with sometimes no activity at all being found. Proteolytic activity decreased significantly within 20 days of separation of the cambial zone (Table 1, lower part). In two trees (out of 4) protein was not extracted from the maturation zone after 40 days of separation of the cambial zone. Lateral supply of IAA to the exposed surface of the maturing xylem resulted in significant increase of protein found in the extract obtained 20 days after the experiment started and the detected proteolytic activity was relatively high. However, after another 20 days, the amount of extractable proteins was reduced five-fold and no measurable proteolytic activity was found. The amount of protein extracted from the maturing xylem decreased practically to zero if the stem was decapited. Apical supply of IAA prevented or delayed these effects of decapitation. Separation of the cambial zone generally prevented autolysis of 70% of cells in the zone of maturing xylem, even after 40 days, in all cases except for decapitated stems, if IAA was not apically applied.

DISCUSSION

It was found previously that decapitation of the pine tree arrests stem cambial activity near the point of decapitation. However, the successive maturing tracheids still autolyse their protoplast (Porandowski et al. 1982). The experiments presented above showed that separation of the cambial region prevents autolysis, although the specific activity of proteases still exists. In these particular experimental conditions, neither apical nor lateral application of auxin stimulated the maturation of pine tracheids. This finding

excludes the operation of a simple concentration gradient of auxin. The observed effect of lateral IAA application upon the amount of protein extracted is probably due to enhanced proliferation of the surface cells forming a layer of callus. Exceptionally dry weather conditions presumably may have influenced generally low levels of both extracted protein and proteolytic activity during the second year of experimentation.

The presence of the proteolytic enzymes in maturing tracheids is not enough to initiate the process of autolysis. Undisturbed integration (linkage) between the maturing pine tracheids and the cambium plays a particularly important role in switching on the mechanism leading to the final phase of differentiation. Thus, it indicates that factors which control the initiation of the autolytic breakdown of protoplasts have a supracellular nature and are influenced by the cambial zone. According to Zajączkowski et al. (1984) these conditions may be fulfilled by vectors of auxin wave propagation which could be involved in the mechanism of auxin control of the final phase (autolysis) of tracheid maturation in the secondary xylem of the stem.

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REFERENCES

Porandowski J., Rakowski K., Wodzicki T. J., 1982. Apical control of xylem formation in pine stems. II. Responses of differentiating tracheids. Acta Soc. Bot. Pol. 5: 187–207.

Wilson B. F., Wodzicki T. J., Zahner R., 1966. Differentiation of cambial derivatives: proposed terminology. Forest Sci. 12: 438–440.

Wodzicki T. J., Brown C. L., 1973. Organization and break-down of protoplasm in maturing pine tracheids. Amer. J. Bot. 69: 631–640.

Wodzicki T. J., Humphreys W. J., 1973. Maturing pine tracheids: organization of intravacuolar cytoplasm. J. Cell Biol. 56: 263.

Wodzicki T. J., Wodzicki A. B., 1980. Seasonal abscisic acid accumulation in stem cambial region of *Pinus silvestris* and its contribution to the hypothesis of a late-wood control system in conifers. Physiol. Plant 48: 443–447.

Zajączkowski S., Wodzicki T. J., 1978. Auxin and plant morphogenesis — a model of regulation. Acta Soc. Bot. Pol. 47: 233–243.

Zajączkowski S., Wodzicki T. J., Romberger J. A., 1984. Auxin waves and plant morphogenesis. In: Encyclopedia of Plant Physiology, New Series, Vol. 10. Hormonal Regulation of Development. II. Functions of Hormones, T. K. Scott (ed.), Springer Verlag, Berlin-Heidelberg-New York-Tokyo, pp. 244–262.

Wpływ oddzielenia strefy kambialnej na wystąpienie procesu autolizy protoplastu w dojrzewających cewkach sosny (Pinus silvestris L.)

Streszczenie

Badano system proteolityczny w dojrzewających cewkach pędu głównego *Pinus silvestris* L. po oddzieleniu strefy kambialnej od warstwy różnicującego się drewna w połączeniu z dekapitacją i dostarczeniem IAA. Oddzielenie kambium zapobiega autolizie protoplastu w dojrzewających cewkach, chociaż aktywność właściwa enzymów proteolitycznych była tylko niewiele zmniejszona. Dostarczenie IAA na wierzchołek pędu zdekapitowanego, lub na styczną powierzchnię różnicującego się drewna, nie powoduje autolizy, ale może wpływać na poziom białka ekstrahowanego i aktywność proteolityczną. Podobnie, dekapitacja może modyfikować, ale jedynie w małym stopniu, efekt oddzielenia kambium. Tak więc wyniki tych badań prowadzą do konkluzji, że integracja wszystkich komórek w regionie tworzenia się drewna jest czynnikiem niezbędnym do rozpoczęcia autolitycznej destrukcji protoplastu. Wskazana jest możliwość udziału fal auksynowych w przenoszeniu informacji pozycyjnej w tym procesie.