

Peroxidase Distribution in Geotropically Stimulated Candles of *Pinus strobus* L.¹

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The peroxidases are a class of enzymes capable of oxidizing a wide variety of substrates in the presence of peroxide. The results of several investigations (1, 2, 3, 4) suggest that the peroxidases are not only involved in regulating the level of auxin activity in a plant but that their own level is in turn influenced by the amount of auxin present.

A geotropic response is a directed, i.e., an oriented growth response in which the required orientation for the plant is provided by the direction of the force of gravity. There is much evidence to support the supposition that a geotropically stimulated organ tends to right itself by developing a transverse asymmetry of auxin distribution. It was therefore thought desirable to investigate the peroxidase distribution following geotropic stimulation.

As an exploratory step, a limited group of terminal candle clusters (averaging five candles per cluster) was collected from eastern white pine (*Pinus strobus* L.) wildings six to eight years old and ninety to one hundred centimeters tall. The terminal bud clusters had begun to expand about a month before and at the time of collection the shoots averaged twelve centimeters in length, or roughly a quarter of their expected total growth. Leaders and laterals were at this time still indistinguishable in gravitational response, both being strongly negatively geotropic.

Candles of trees bent to the horizontal at this early stage in the growing season exhibit remarkably rapid recovery. Thus the apical half of a candle was found to right itself completely within two days or so (producing an "L" shape). Asymmetric growth actually continues for a short period; the resulting over-recovery is in turn corrected. Recovery is primarily brought about by asymmetric growth in the longitudinally central portion of the candle, the region of most active cell elongation at this time of the growing season.

Candles were collected from trees which had been inclined to the horizontal for two days as well as from untreated, vertical control trees. The candles were frozen immediately upon collection and stored in this condition. The bracts or immature fascicles were removed prior to testing.

The candles were homogenized in one-tenth molar monobasic potassium phosphate and the insoluble matter discarded. Owing to their small size two or three candles from one cluster were pooled for the preparation of a single extract. Extracts were tested for their peroxidase activity by an assay modified from that of Sharpsteen et al. (6). It is based on the ability of peroxidases to combine with hydrogen peroxide and then to oxidize the colorless compound pyrogallol to a stable yellow compound,

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presumably purpurogallin. The rate at which the purpurogallin forms is determined colorimetrically and taken as a measure of the peroxidase activity. Dialysis against one-tenth molar monobasic potassium phosphate (1:500) increased the peroxidase activity of an extract about twenty percent, but was not adopted as a standard procedure because the increase was similar for any extract. The data are presented in relative terms since details of assay technique were changed several times.

Longitudinal distribution of peroxidase activity was determined by cutting the candles into four equally long segments and preparing extracts based on fresh weight from each part. The results of this investigation are presented in Table 1. The data reveal a basipetally increasing gradient of peroxidase activity in the expanding candles. Roughly one-tenth of the total activity is found in the apical quarter of the candle and this increases to roughly one-half in the basal quarter. The gradient thus appears independent of the central region of early elongation, seeming rather to be proportional in magnitude to the distance from the apical meristem. The limited data in the lower portion of Table 1 indicate, moreover, that this distribution is essentially the same in candles inclined for two days as in the erect candles. The gradient for peroxidase activity described here for the candles of eastern white pine is thus similar to the one described by Mirov (5) for the overall auxin activity of the diffusates of candles obtained from ponderosa (*P. ponderosa* Laws.) and Torrey (*P. torreyana* Parry) pine seedlings.

Transverse distribution of peroxidase activity was determined by splitting several candles longitudinally into upper and lower halves. Owing to limited plant material, data for this phase of the investigation are available only for the basal half (i.e., the third and basal quarters), of several candles. The basal half of the candle did, however, receive maximum geotropic stimulation during the treatment since it remained relatively horizontal during this time. This region, furthermore, exhibits most of the peroxidase activity found in a candle. The results of this phase of the study are summarized in Table 2. It is seen that horizontal displacement of the candles resulted in a nonsymmetrical distribution of peroxidase activity with as much as three-fifths of the total activity of a segment being found in its lower horizontal half.

The preliminary findings reported here, therefore, suggest that a correlation exists between the enzyme peroxidase and a geotropic stimulation. A detailed kinetic investigation of the relationship between auxin and peroxidase activities in the upper and lower halves of geotropically stimulated organs would perhaps indicate whether a peroxidase redistribution is responsible for the auxin redistribution found in organs so stimulated. The many candles of a single pine are well suited for such a study.

Summary

Elongating candles of *Pinus strobus* L. were discovered to possess a basipetally increasing gradient of peroxidase activity. This longitudinal gradient appeared to be independent of candle orientation. Inclination of candles did, however, result in a transverse asymmetry with the lower horizontal side of the candle developing the greater peroxidase activity.

Table 1. Longitudinal Distribution of Peroxidase Activity in Erect Candles of *Pinus strobus* L. and in Candles Inclined for Two Days.

Peroxidase Activity in Segment as a Percentage of Activity in Entire Candle				
Position of Candle	Apical Quarter	Second Quarter	Third Quarter	Basal Quarter
Erect	9	27	22	42
Erect	19	17	32	32
Erect	20	15	30	35
Erect	4	8	22	66
Erect	6	4	15	75
<i>Erect Average</i>	<i>11.6</i>	<i>14.2</i>	<i>24.2</i>	<i>50.0</i>
Inclined	12	16	23	49
Inclined	10	11	24	55
<i>Inclined Average</i>	<i>11.0</i>	<i>13.5</i>	<i>23.5</i>	<i>52.0</i>

Note: Two candles were pooled for the preparation of each extract.

Table 2. Transverse Distribution of Peroxidase Activity in the Basal Half of Candles of *Pinus strobus* L. Inclined for Two Days.

Peroxidase Activity in Segment (% Upper Horizontal Half / % Lower Horizontal Half)	
Third Quarter	Basal Quarter
42/58	46/54
40/60	49/51
42/58	45/55
38/62	43/57
Average	
<i>40.5/59.5</i>	<i>45.8/54.2</i>

Note: Three candles were pooled for the preparation of the first two extracts and two for the last two.

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