DISTRIBUTION AND SEASONAL VARIATION OF WOOD PEROXIDASE ACTIVITY IN OAK (*QUERCUS ROBUR*)

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ABSTRACT

The distribution of peroxidase activity and the water and nitrogen content within the cross section of an oak trunk (*Quercus robur*) were measured. After a sharp decline in the outer sapwood, an increase of the peroxidase activity in the inner part of sapwood could be detected. This increase was followed by a sharp fall of the activity in the heartwood. In the inner part of the heartwood only small activity could be found. The quantitative data reported in this paper give for the first time strong evidence that peroxidase enzymes are involved in heartwood formation. The results support the findings of other authors that an increased enzymatic activity exists in the zone adjacent to the sapwood/heartwood junction.

In addition the seasonal variation of peroxidase activity in the zone mentioned above was studied. A maximum of activity could be observed during the dormant season. The results indicate that heartwood formation in *Quercus robur* occurs mainly during this period.

Keywords: Peroxidase distribution, seasonal variation, *Quercus robur*, oak.

INTRODUCTION

Peroxidase isoenzymes are widely distributed in the plant kingdom. Peroxidase plays a significant role in almost all oxidative polymerization processes occurring in plants, i.e., lignification (Ebermann and Stich 1982; Harkin and Obst 1973; Nakamura 1967a, b) and heartwood formation (Ziegler 1968). In addition most peroxidase isoenzymes show IAA-oxidase activity (Aspar et al. 1982; Gebhart et al. 1982; Stich 1983). Another function of peroxidase is its participation in cell differentiation (Jaeger-Wunderer 1980).

The antibiotic activity of oak heartwood is one reason for its great importance for practical purposes. Polymerized polyphenols are mainly responsible for this property. The polymerization of the monomeric polyphenols is mediated by polyphenoloxidases and peroxidases that occur in the wood tissues. Recent investigations on *Quercus robur* showed that the wood peroxidase isoenzymes are multifunctional and can as well act as polyphenoloxidases (Stich and Ebermann 1984).

The aim of this investigation is to follow the course of peroxidase activity across the trunk cross section and its seasonal variation in the sapwood near the sapwood/heartwood junction.

MATERIALS AND METHODS

Plant material

For the determination of peroxidase activity and nitrogen content in the trunk cross section, two oak trees (*Quercus robur*) were used. They were taken from a forest owned by the City of Vienna. The trees were felled at the beginning of March. At a height of one meter from the bottom, trunk pieces with twelve annual rings were used.

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For the determination of the seasonal variation of peroxidase activity in the inner part of the sapwood, two oak trees (*Quercus robur*) were used. Their origin was Hochleitenwald, a forest near Vienna. Every month one branch from each tree (appr. 120 years old) with a diameter between 8 to 10 cm was cut off and used for the further determinations.

Sample preparation for the distribution of peroxidase activity and nitrogen content

The trunk pieces used were milled layer by layer with a slow-turning lathe, in order to prevent excess heating. Just before milling the next layer, the diameter of the remaining wood piece was determined.

Since both oak trees showed very similar results, only the data obtained from one single tree will be described. The spans of each fraction (called sample) were collected, mixed, and used for the following investigations.

Determination of water content

The water content was determined immediately after grinding. Approximately 2.5 g spans were dried at 105 C for 4 hours.

Determination of nitrogen content

The course of nitrogen content in the trunk cross section was studied in order to find out whether there is any relation to the course of peroxidase activity.

The determination of nitrogen content was done according to Merrill and Cowling (1966).

Preparation of samples for the peroxidase activity determination in connection with the annual cycle

Every month the outer layers of sapwood were removed from the used oak branches by a slow-turning lathe. Sapwood at a distance of 1 cm from the sapwood/heartwood boundary was milled and used for the determination of peroxidase activity.

Determination of peroxidase activity

The preparation of wood extracts was done according to Ebermann and Stich (1982). The determination of peroxidase activity was made according to the "Worthington enzyme manual" (1972). The change of absorption was measured by a photometer (Perkin Elmer Spectralphotometer 550) and reported by a recorder (Perkin Elmer Recorder 56).

In order to exclude nonenzymatic reactions simulating peroxidase activity, the wood extracts were heated. No peroxidase activity could be detected in the heated samples.

RESULTS AND DISCUSSION

Figure 1 shows the distribution of peroxidase activity, the nitrogen, and the water content within the trunk cross section.

The data concerning the nitrogen content are in accordance with the theory of Cowling and Merrill (1966) and Merrill and Cowling (1966). The highest nitrogen content and peroxidase activity are found in samples containing the cambial layer

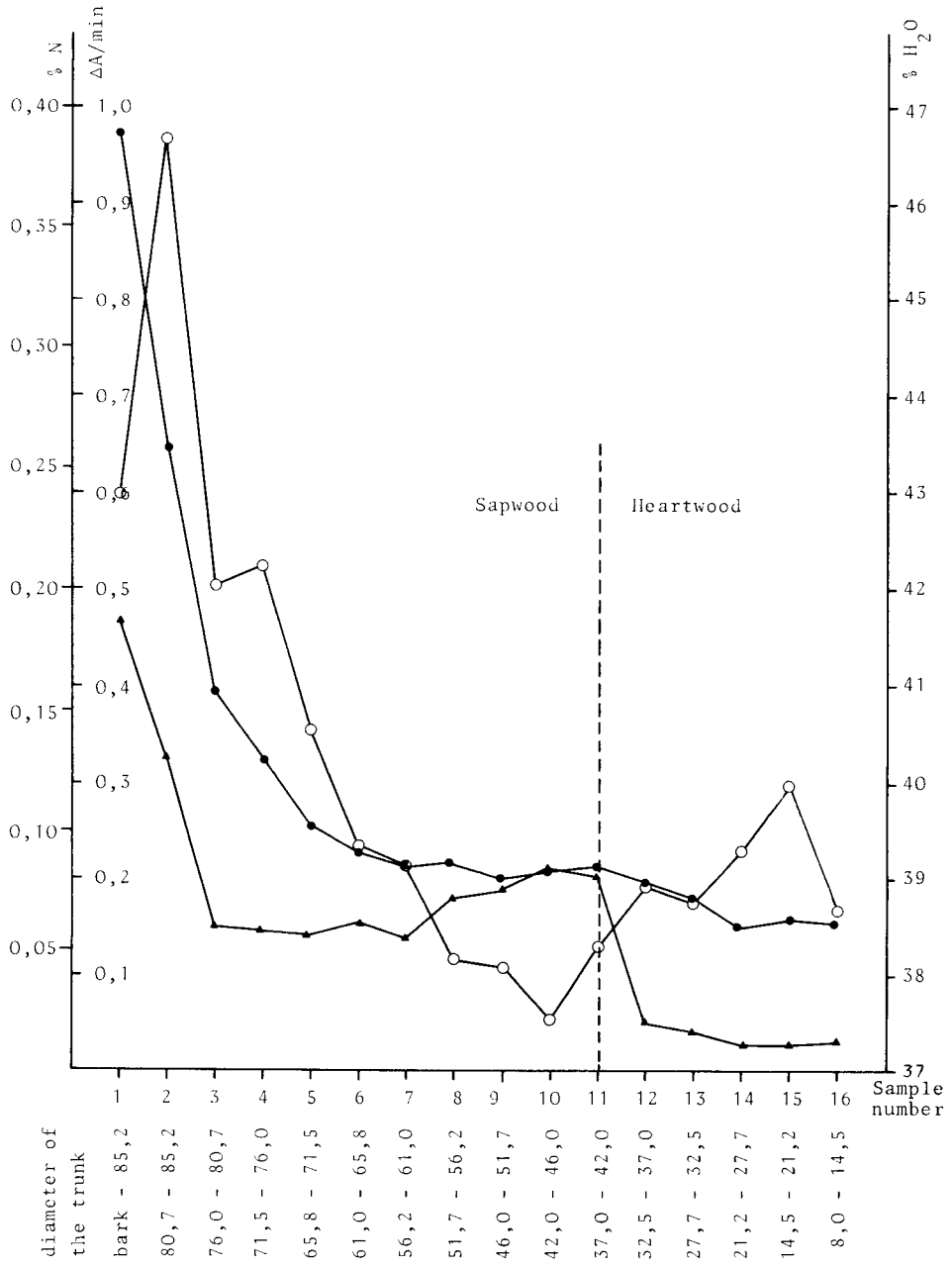

FIG. 1. Distribution of water, nitrogen content and peroxidase activity in the trunk cross section of *Quercus robur*. —○—○— water content (% H_2O); —●—●— nitrogen content (% N); —▲—▲— peroxidase activity (based on dry weight) (ΔA/min).

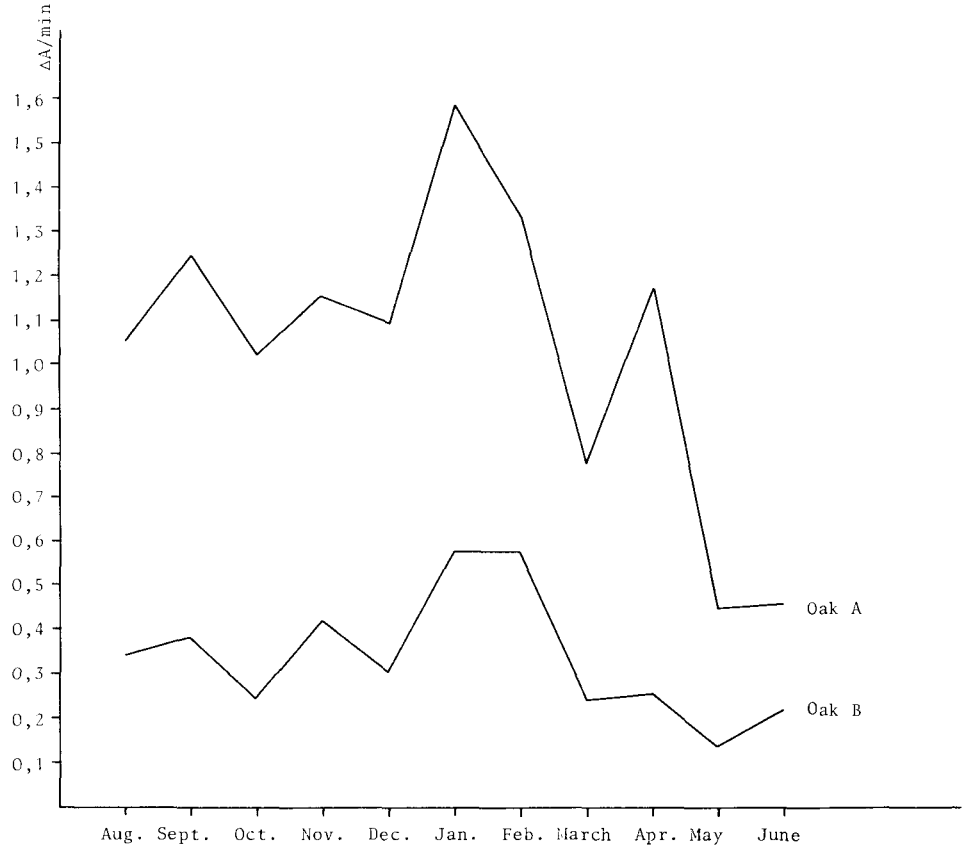

FIG. 2. Seasonal variation of peroxidase activity (based on dry weight) in two oak trees (*Quercus robur*).

and the adjacent xylem. Most of the living cells exist in these tissues. Cell differentiation takes place in the xylem adjacent to the cambial layer and the lignification of the cell wall starts. As mentioned before, peroxidase isoenzymes are involved in both of these processes. This fact is indicated by the high peroxidase activities found in these zones.

Towards the inner part of the trunk, the peroxidase activity and the nitrogen content decrease rapidly and pass a minimum situated in the outer part of the sapwood. Both of the two parameters remain rather constant in the middle part of the sapwood. This zone of constant peroxidase activity is followed by a new increase of the enzymatic activity in the inner part of the sapwood, whereas the nitrogen content shows no remarkable change.

The fact of higher peroxidase activity in the sapwood near the sapwood/heartwood boundary supports the view of several authors (Hillis 1977) that this zone is in general characterized by enhanced enzymatic activities. Dietrichs (1964), Ziegler (1968), Wardrop and Cronshaw (1962) report an increased peroxidase activity in the sapwood/heartwood transition zone of several trees, though no quantitative results were given. Enhanced activities of some other enzymes have been detected in the same zone: malic and glucose-6-phosphate dehydrogenase (Shain and MacKay 1973; Ziegler 1968), amylase and aldolase (Höll 1972).

In the outer part of the heartwood a very rapid decrease of peroxidase activity was detected, whereas the nitrogen content was levelling off much slower. The course of both of these parameters indicates that metabolic activities have almost vanished in the heartwood. A rest of peroxidase activity can be detected in heartwood as Fig. 1 shows. However, a nonenzymatic cause for this activity can be excluded. Recently we were able to detect electrophoretically a peroxidase isoenzyme in heartwood of *Quercus robur* (Stich and Ebermann 1984). This isoenzyme shows the same electrophoretical mobility as the main peroxidase isoenzyme occurring in sapwood.

The significant increase of peroxidase activity outside the sapwood/heartwood boundary gives strong evidence for the participation of peroxidase in heartwood formation. Another question arising in this connection is the seasonal variation of peroxidase activity in the inner sapwood in respect to the sapwood/heartwood transformation.

Preliminary experiments about the distribution of peroxidase activity dependent on the cross section were carried out in the oak branches, which had been used to investigate the seasonal variation of peroxidase. The branches and the trunk pieces showed the same peroxidase distribution.

Figure 2 shows the seasonal variation of peroxidase activity in the innermost part of the sapwood of two different oak trees.

The course of the peroxidase activity throughout the year is analogous in both of the trees. Only quantitative differences exist between them. The reason might be a genetical one. Differences due to the age and environment of the trees as a possible explanation can be excluded, because both were about the same age and from the same stand.

The trees showed an increased peroxidase activity during the winter. This fact indicates that heartwood formation in the oak takes place mainly in the dormant season. This result is in accordance with the results of other authors (Nelson 1978; Shain and Mackay 1973) who reported an increased metabolic activity in the same zone during the dormant period.

The experimental results furnish further evidence that in the inner part of the sapwood there is a zone of enhanced enzymatic activity, which is subjected to seasonal variations. The data obtained underline the important role of the peroxidase and polyphenoloxidase activity showing oxidoreductases of *Quercus robur* in heartwood formation.

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