STUDIES ON WHEAT PEROXIDASE*

By R. W. Parish†

Introduction

Recently, workers in this laboratory have studied the increased synthesis of peroxidase in senescing tobacco leaf disks (Parish 1968*a*, 1968*b*) and the relationship between lignification and peroxidase activity in wheat internodes (Parish and Miller 1968). The present paper reports four simple experiments designed to further clarify the interrelationships between lignification, maturation, and peroxidase activity.

Firstly, the peroxidase activity of wheat coleoptile segments was measured to determine whether this activity increased with maturity. The indoleacetic acid (IAA) oxidase is thought to be a peroxidase (Ray 1958) and its activity is apparently controlled by the level of inhibitors present (Tomaszewski and Thimann 1966). Thus the peroxidase activity of extracts is not necessarily an indication of IAA oxidase activity unless the levels of inhibitors are taken into account. In the second experiment, the effects of extracts from various coleoptile segments on IAA oxidation *in vitro* were determined.

Since peroxidase activity has been found to increase with tissue maturity (Galston and Dalberg 1954; Pilet 1959; Parish 1968*a*, 1968*b*), the distribution of the enzyme along wheat internodes was measured. This provided information about internode development. Peroxidase is thought to be involved in lignin formation (Brown 1961) and the distribution of lignin was measured to discover whether there was a correlation with lignin content.

Finally, to ascertain whether different peroxidases were present in different wheat tissues and whether these enzymes had different substrate specificities, peroxidase isoenzymes were separated by starch gel electrophoresis from a variety of tissues and the gels stained with two substrates.

Methods, Results, and Discussion

(i) *Peroxidase Activity along the Wheat Coleoptile*

Wheat seeds (cv. Gabo) were germinated on moist paper tissues in darkness at 25°C. When approximately 2·5 cm in length, the coleoptiles were removed and divided into fifths. Each fifth was homogenized in 6·7 mM Sorenson's phosphate buffer (pH 7·0) with a Servall Omnimixer at 180 V for 3 min. The homogenate was centrifuged at 1500 *g* for 10 min and the supernatant removed. The precipitate was then re-extracted and the supernatants combined, the final volume being 2·0 ml. The extractions were carried out at 0°C. Peroxidase activity was measured by the method of Lück (1963) using *p*-phenylenediamine as substrate. Soluble protein was estimated according to Lowry *et al.* (1951).

* Manuscript received April 16, 1968.

† Botany Department, University of Melbourne; present address: Eidg. Technische Hochschule, Institut für allgemeine-Botanik, Zurich, 8006. Switzerland.

The activities of segments (numbered from tip to base) from a single coleoptile are shown in the following tabulation, and all coleoptiles tested gave similar trends:

Segment No.	1	2	3	4	5
Specific activity of peroxidase	4·18	4·07	4·75	5·15	6·15

The results support the contention that peroxidase activity increases with maturity.

(ii) *Distribution of IAA Oxidase-inhibiting Activity along the Wheat Coleoptile*

Samples from each coleoptile segment and containing identical protein concentrations were added to the IAA oxidase assay solution, and their effects on IAA oxidation were determined. The assay solution was 10^{-4}M IAA plus 0·1 μM horseradish peroxidase (Sigma type VI, RZ not less than 3·0) in 0·05M KH_2PO_4 buffer (pH 4·5). IAA oxidation was estimated by measuring the increase in absorbance at 248 mμ, readings being taken every 5 min.

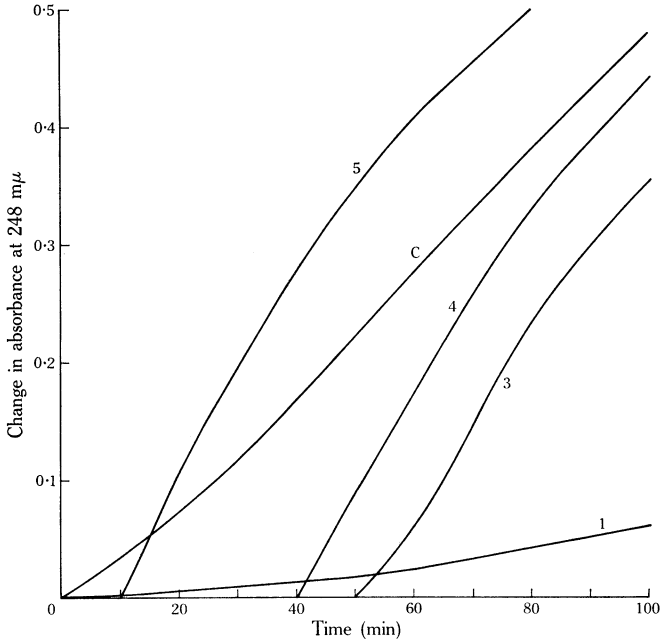

Fig. 1.—Oxidation of IAA in the presence of extracts from segments 1, 3, 4, and 5 of wheat coleoptile (cv. Gabo), measured by the change in optical density at 248 mμ. The coleoptile was segmented into fifths, which were numbered from tip to base. All extracts added to the assay solution contained identical protein concentrations. Readings were taken at 5-min intervals. *C*, control line, representing oxidation rate in the absence of extracts.

The coleoptile extracts caused a lag phase in IAA oxidation, the duration ot which was indirectly proportional to the distance of the segment from the coleoptile tip (Fig. 1). Once the lag phase ended, oxidation was slightly stimulated by extracts from the third, fourth, and fifth segments.

The results resemble those obtained when various concentrations of diphenols, known inhibitors of the IAA oxidase, are added to systems *in vitro* (Schaeffer, Buta, and Sharpe 1967; Parish, unpublished results). The lag phase may, of course, be due to the presence of some alternative substrate which is preferentially oxidized. In actual fact the diphenolic inhibitors may themselves be alternative substrates since, at concentrations which do not inhibit the reaction indefinitely, the diphenols are apparently oxidized before IAA oxidation occurs (Engelsma 1964; Parish, unpublished results). While considerable peroxidase activity is present in the coleoptile tip, the results suggest that IAA is protected from destruction. This is compatible with the theory that the increase of the IAA oxidase activity is partly due to changes in the inhibitor–cofactor balance (Galston and Dalberg 1954; Engelsma 1964).

(iii) *Lignin Content and Peroxidase Activity along the Wheat Internode*

The top three internodes were removed from wheat (cv. Sherpa) in which the top internode had almost completed elongating. The top internode and its leaf sheath were divided into fifths, while the second and third internodes were divided into sevenths. The lignin content and peroxidase activity of internode segments were determined. Lignin soluble in cold alcoholic alkali was extracted by the method of Phillips (1927) modified according to Miller and Anderson (1965). Lignin was expressed as a percentage of dry weight. Peroxidase was extracted and estimated as already described, except that the homogenizer was run for 6 min at 220 V.

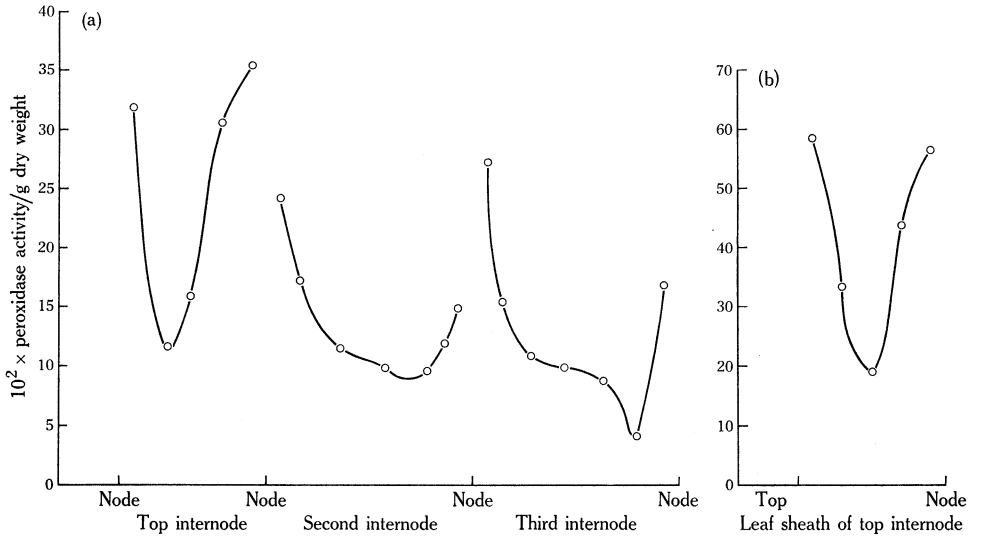

Fig. 2.—Peroxidase activity along the top three measurable internodes (*a*) and along the leaf sheath from the top internode (*b*) of wheat (cv. Sherpa). The top internode and the sheath were divided into fifths and the second and third internodes into sevenths, and the peroxidase activity of each segment estimated. The results are from a single plant, but similar trends were observed in all plants tested.

Peroxidase activity was lowest in the middle of the internode segments, increasing as the node was approached [Fig. 2(*a*)]. Activity was lowest in the second and third segments of the top internode, and in the fifth and sixth segments of the

second and third internodes respectively. The pattern of peroxidase activity in the leaf sheath surrounding the top internode resembled that of the internode [Fig. 2(*b*)].

Lignin content was highest in the second segment of the first internode, decreasing down the internode to rise again in the region of the lower node, as the following tabulation shows:

Top internode segment No.	1	2	3	4	5
Lignin content (% dry weight)	6·8	10·5	9·9	2·1	8·0

Lignin was fairly evenly distributed along the second and third internodes, no pattern being apparent.

The activity of peroxidase along the wheat internode suggests that the most mature tissue occurs at the nodal regions. The classical idea of internode development is of a meristem above each node, this intercalary meristem being responsible for the growth of the internode immediately above it. In such a case the youngest tissue will be at the base of the internode and the oldest at the top, with a gradation between the two. In this experiment the top internode had not completed elongating and the area of lowest peroxidase activity was in the middle of the internode. In the mature second and third internodes this area was closer to the lower node. This suggests a development in which the meristem at first adds equally to the tissue above and below it, but later on only to the former tissue. Polukhina (1960) has studied the anatomy of elongating wheat internodes. Her work supports the development suggested by the peroxidase pattern, viz, a zone of intercalary growth is formed in the middle of the internode and the cells above and below the node grow vigorously, eventually disintegrating to form an air chamber. The cell enlargement below the upper node continues after division above the lower node has halted.

Lignin content bears no close relation to peroxidase activity (see also Parish and Miller 1968). While lignin levels increase between the fourth and fifth segments of the internode, they are considerably lower than levels at the top of the internode. Peroxidase activities are similar, however, in the region of both nodes. As the internodes mature, lignin becomes more evenly distributed although the basic peroxidase pattern is unchanged. The major factor controlling lignification would not seem, therefore, to be peroxidase activity. IAA is known to inhibit lignification (Petinov and Urmantsev 1964; Stafford 1965; Parish, unpublished results), and perhaps relatively high levels of IAA accumulate in the lower part of the internode.

(iv) *Peroxidase Isoenzymes in Wheat Tissues*

Starch gels were made from 25 g Connaught hydrolysed potato starch in 190 ml 0·76M Tris–citrate buffer (pH 9·0). The bath buffer was 0·3M borate (pH 9·0). Rectangles of chromatography paper (0·8 by 1·0 cm) were soaked in enzyme extracts and placed in the gel. Extracts were obtained as described above, the ratio of tissue weight to buffer volume being approximately 1 : 3. Gels were run at 0°C for 2 hr and the current maintained at 35 mA. The gels were then cut in two horizontally, and each half stained for peroxidase. Stains used were 1 mM *p*-phenylenediamine and 1 mM guaiacol with 1 mM hydrogen peroxide in 6·7 mM Sorensen's phosphate buffer (pH 7·0).

Ten different isoenzymes were detected in various tissues of wheat (Fig. 3). Different combinations of isoenzymes were present in different tissues and the

isoenzymes demonstrated varying activities towards *p*-phenylenediamine [Fig. 3(*a*)] and guaiacol [Fig. 3(*b*)].

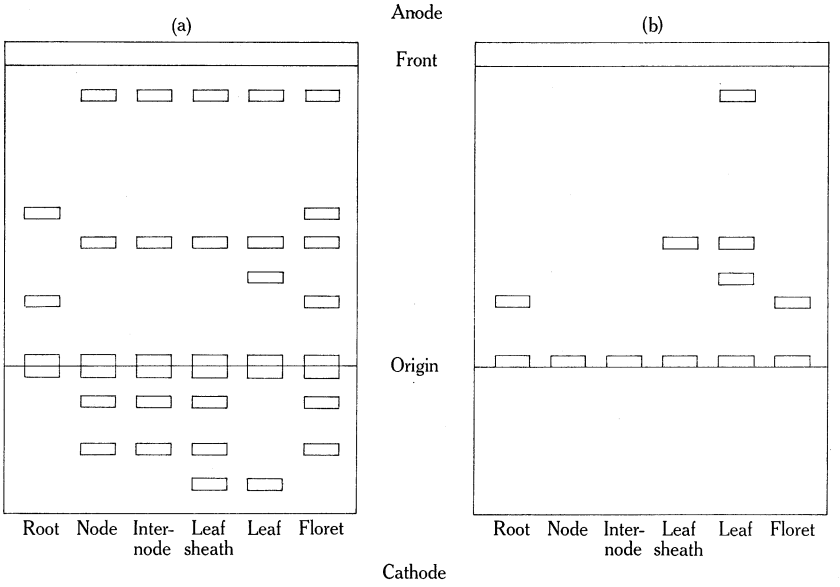

Fig. 3.—Patterns of peroxidase enzymes of wheat tissue extracts obtained on starch gels after electrophoresis. Gels stained with *p*-phenylenediamine (*a*) and guaiacol (*b*).

The results support the theory that, since peroxidase isoenzymes have different activities towards different substrates and isoenzyme patterns change during growth, the physiological role of peroxidase may also change (McCune 1961; Evans and Allridge 1965; Kawashima and Uritani 1965; Goren and Goldschmidt 1966; Macnicol 1966; Racusen and Foote 1966; Kay, Shannon, and Lew 1967; Siegel and Galston 1967).

Acknowledgments

The author thanks Dr. F. L. Miller, Gordon Institute of Technology, Geelong, and Professor J. S. Turner, Botany Department, University of Melbourne, for their encouragement and criticism. The work was supported by a Federal Wheat Research Council Grant.

References

Brown, S. A. (1961).—*Science*, N.Y. **134**, 305–13.
Engelsma, G. (1964).—*Nature, Lond.* **202**, 88–9.
Evans, J. J., and Allridge, N. A. (1965).—*Phytochemistry* **4**, 499–503.
Galston, A. W., and Dalberg, L. Y. (1954).—*Am. J. Bot.* **41**, 373–80.
Goren, R., and Goldschmidt, E. E. (1966).—*Phytochemistry* **5**, 153–9.
Kawashima, N., and Uritani, I. (1965).—*Pl. Cell Physiol., Tokyo* **6**, 247–65.
Kay, E., Shannon, L. M., and Lew, J. Y. (1967).—*J. biol. Chem.* **242**, 2470–8.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951).—*J. biol. Chem.* **193**, 265–75.

LÜCK, H. (1963).—In "Methods of Enzymatic Analysis". p. 895. (Ed. H. U. Bergmeyer.) (Academic Press, Inc.: New York.)
MACNICOL, P. K. (1966).—*Archs Biochem. Biophys.* **117**, 347–56.
MCCUNE, D. C. (1961).—*Ann. N.Y. Acad. Sci.* **94**, 723–30.
MILLER, F. L., and ANDERSON, K. L. (1965).—*Crop Sci.* **5**, 391–4.
PARISH, R. W. (1968*a*).—*Planta* **82**, 1–13.
PARISH, R. W. (1968*b*).—*Planta* **82**, 14–21.
PARISH, R. W., and MILLER, F. L. (1968).—*Aust. J. biol. Sci.* **22**, 77–86.
PETINOV, N. S., and URMANTSEV, YU. A. (1964).—*Soviet Pl. Physiol.* **11**, 559–65.
PHILLIPS, M. (1927).—*J. Am. chem. Soc.* **49**, 2037.
PILET, P. E. (1959).—"Fourth International Conference on Plant Growth Regulation." (Iowa State Univ. Press.)
POLUKHINA, I. N. (1960).—*Dokl.* (*Proc.*) *Acad. Sci. U.S.S.R.* **130**, 2–3.
RACUSEN, D., and FOOTE, M. (1966).—*Can. J. Bot.* **44**, 1633–8.
RAY, P. M. (1958).—*A. Rev. Pl. Physiol.* **9**, 81–118.
SCHAEFFER, G. W., BUTA, J. G., and SHARPE, F. (1967).—*Physiologia Pl.* **20**, 342–7.
SIEGEL, B. Z., and GALSTON, A. W. (1967).—*Pl. Physiol., Lancaster* **42**, 221–6.
STAFFORD, H. A. (1965).—*Pl. Physiol., Lancaster* **40**, 844–51.
TOMASZEWSKI, M., and THIMANN, K. V. (1966).—*Pl. Physiol., Lancaster* **41**, 1443–54.