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ROLE OF TRANSITION METALS (IRON, COPPER, MANGANESE) IN THE FORMATION OF PHYTOMELANINES IN SENESCENT LEAVES

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Enzymatic and chemical redox systems (polyphenoloxidase and polyphenols) are present in autumn leaves of many tree species and remain active after defoliation (Tremolieres and Carbiener 1981, 82). In most cases, these sytems are more efficient in the presence of transition metals such as iron, copper and manganese. Both enzymatic and chemical polycondensation of polyphenols lead to the production of phytomelanins. The role of transition metals (Fe III, Cu II, Mn II, Fe II) is studied in both oxidation processes.

It has been suggested by Souchier (1971), Toutain (1974) and Adrian (1985) that the metal ions catalyse the polymerization of phenols into bulky insoluble polymers. Our experiments show that the metal ions react directly with the polyphenols with which they form complexes (Sillen 1964, 1971). Part of these ions are included in the phytomelanins. We also find that at low concentrations, copper acts as a cofactor of polyphenoloxidase (cupric enzyme).

— A first series of experiments demonstrate the role of metal ions in the enzymatic oxidation. On the one hand we have tested the activity of metal ions added to crude powder of senescent leaves : we have studied the enzymatic deoxygenation of leaf powder suspensions (2.5 % W:V), by measuring the oxygen uptake in an oxygraph with an oxygen specific electrode, at pH 6 (phosphate buffer). On the other hand, we have studied the oxidizing activity of an acetone powder (without leaf polyphenolic substrates but with active enzyme) on an exogen substrate (catechol 3 mM) at the same pH (this pH value is close to that of the aqueous litter extracts). Ten deciduous tree species, particularly the black poplar (Populus nigra) harvested in alluvial forest were tested in this study.

— A second series of experiments demonstrate the effect of these metal ions on the chemical oxidation (autooxidation) of leaf polyphenols at acid and alkaline pH : enzyme-free boiled extracts of leaves were used. No more deoxygenation was observed at pH6, showing the importance of the enzymatic process that we have already proved (TREMOLIERES and CARBIENER, 1981 ; TREMOLIERES and BIETH, 1984), but at pH9 the leaf polyphenols were easily oxidized.

RESULTS : Specificity of the action of each the tested metals.

1. — Copper II participates in redox reactions at the polyphenoloxidase active site : at low concentrations (up to 3 meq/l = 6 mM) Copper II is an activator of polyphenoloxidase. But high concentrations (table 2) inhibit the enzymatic oxidation, probably by toxic effects of copper denaturing the enzyme : copper has a high affinity for sulfhydryl groups and peptidic nitrogen, therefore it is a potentially toxic metal (CLARKSON and HANSON, 1980). Cu II ions inhibit the autooxydation of enzyme-free extracts of black poplar at pH 9 (Fig. 6). We may suggest that polyphenol-Cu II complexes occurring in the extracts at alkaline pH are no more reactive.

2. — Fe III appears to have no effect on polycondensation (table 2). Even though the initial deoxygenation rate is not modified by Fe III (table 2) the deoxygenation rate in presence of Fe III decreases with time more rapidly than in the standard extracts at concn >1.5 mM (Fig. 2). Oxidized iron (Fe III) forms very stable complexes with polyphenols, and we hypothesize that these complexes are no more reactive. Thus high concentrations decrease the oxidation rate by decreasing the number of reactive phenolic groups which are complexed by Fe III.

3. — Mn II highly increases the rate of leaf polyphenols oxidation at alkaline pH (autooxidation at pH9) (Fig. 6). It complexes easily the polyphenols (SILLEN, 1964, 1971) and these complexes are more reactive than the ligands alone at alkaline pH, as was also shown by Adrian (1985). But Mn II has no influence on the enzymatic oxidation (table 2).

4. — Fe II plays an activation role at acid and alkaline pH, though it is unstable and quickly oxidized into Fe III. It has a good affinity for polyphenols (SILLEN, 1964, 1971),

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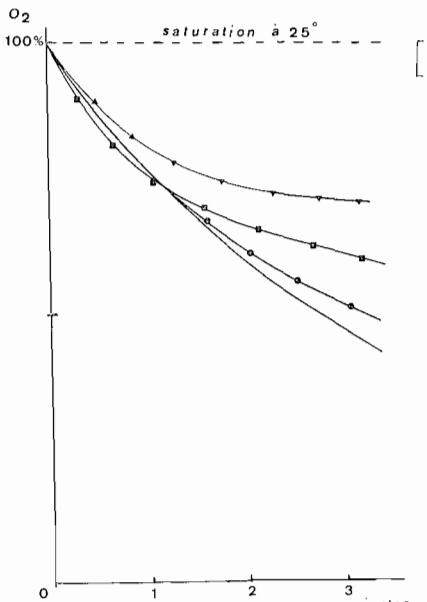
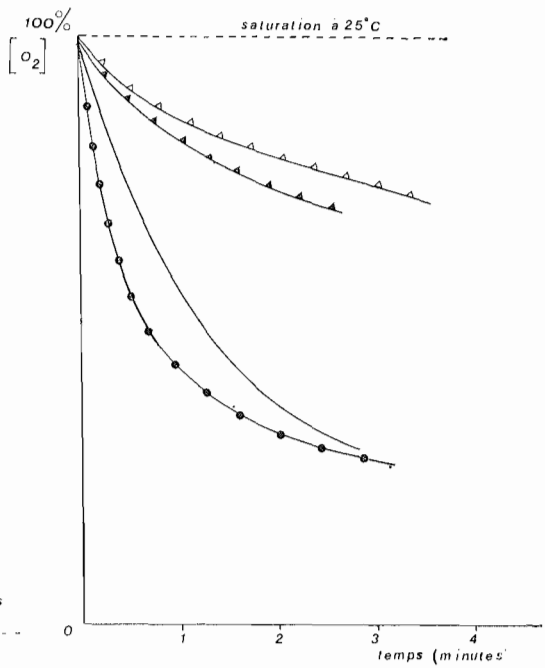
but the affinity is less than that of the ferric ions. However, the polyphenols-Fe II complexes have a more oxido-reducing effect than the free ligands alone but less than the polyphenols-Mn II complexes (Adrian 1985). As Mn II, Fe II is efficient, too, on the enzymatic oxidation (Fig. 4) : if we calculate the oxygen consumptions after one minute, for every test, we observe that the deoxygenation activation of acetone powder by Fe II is in fact due to the catechol-Fe II interaction.

Fe II and Mn II have a similar effect on the phytomelanic polycondensation in that both ions facilitate the oxidation at low concentrations ; however, they differ in that the effect of Fe II takes place both at high and low pH's while the manganese favors the autooxidation at alkaline pH only. Fe II can replace the enzyme at acidic pH, and may therefore be considered indeed as a catalyst of the phytomelanin polycondensation.





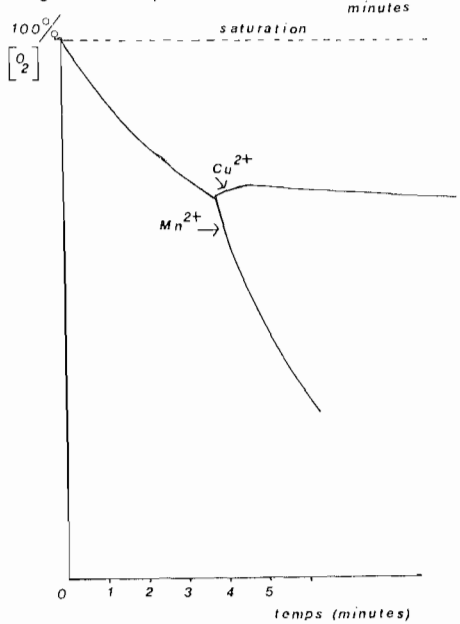


Table 2 :
Initial rates of oxidation (mg/l/min) of black poplar leaf powder suspensions (2.5 %, w/v) as a function of the metal ion concentration (25°C)

Fig. 2 :
Action of Fe III ions ($FeCl_3$, $6H_2O$) on the enzymatic deoxygenation of black poplar leaf powder suspensions (2.5 %, w/v) at 25°C, pH 3.
Standard (———) 12 mM (—▲—▲—) 6 mM (—■—■—) 1.5 mM (—●—●—)

Fig. 4 :
Action of Fe II ions ($FeSO_4$ 0,66 mM) on the oxidizing activity of « acetone powder » of black poplar in the presence of catechol 3.3 mM pH6.
Fe II (—△—△—) Fe II + catechol (—▲—▲—) acetone powder + catechol (———) acetone powder + catechol + Fe II (—●—●—).

Fig. 6 :
Action of Mn II and Cu II ions on the autooxidation of enzyme-free extracts of black poplar at pH 9.