The Possible Role of Peroxidase Isoenzymes in the Infection of English Oak by *Loranthus europaeus*

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ABSTRACT

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Electrophoretic investigations of peroxidase isoenzymes in the hemiparasite-host system *Loranthus europaeus-Quercus robur* showed enhanced activities in the host-parasite junction. The peroxidase of *L. europaeus* was increased manyfold in this location. For use in inhibition studies, the enzyme was concentrated by $(NH_4)_2SO_4$-precipitation and partly purified by preparative gel electrophoresis. The enzyme was inhibited most effectively by 1.0×10^{-3} M S^{--}. Classical peroxidase inhibitors were practically without effect. In preliminary field experiments, treatment of oaks with 5% CaS_2 reduced the rate of implantation by *Loranthus* by 36%.

Additional key words: enzyme inhibition, mistletoe.

The mistletoe, *Loranthus europaeus* L., is a hemiparasite growing specifically on oak (*Quercus* spp.) and on chestnut (*Castanea sativa* Mill.). The epidemic appearance of mistletoe is causing severe damage in oak forests. The reasons for the massive invasions of oaks from time to time by *L. europaeus* remain obscure. Presumably climatic and environmental causes are responsible for the large increase of *L. europaeus* observed in recent years in some European countries.

Peroxidase (EC 1.11.1.7.) enzymes are widespread in the plant kingdom and apparently play an important role in plant infection. It is known that peroxidase activity is enhanced during infection in many cases (10). Frequently, increased peroxidase levels are found in the host as well as the invading organism (7,10).

During our investigations of oak peroxidase (8) we began by studying the course of activity of this enzyme during the infection of oak by *L. europaeus*. The purpose of this work was to characterize the peroxidase isoenzymes of *L. europaeus* electrophoretically and to study the possibility of their inhibition by several substrates. Our hypothesis was that if the peroxidase of *L. europaeus* is important for the infection process, a decrease of the infection rate should be observed when enzyme inhibitor is added to the oak-Loranthus system.

MATERIALS AND METHODS

Branches of English oak (*Quercus robur* L.) infected with mistletoe were collected in August. The diameter of the oak branches was about 5 cm. The infecting *L. europaeus* had a diameter of 2–3 cm at its origin on the oak branch. Samples were collected from the mistletoe branch, 0.2 m from its origin, from the oak branch 0.5 m from the locus of infection, and from the Loranthus-oak junction.

The samples of host, parasite, and interface tissues were prepared as described elsewhere (5). Polyacrylamide gel electrophoresis (PAGE) was performed as described earlier (4). Peroxidase isoenzymes were visualized by staining the gel according to Ornstein (6) after eluting the electrophoresis buffer from the gel for 1 hr in running water.

Extracts from tissue of *L. europaeus* were concentrated and partly purified by preparative PAGE. The peroxidase enzyme was located by staining the end of the gel (6). The zone was extracted with 2 ml of physiological saline solution after slicing the gel. Enzyme activity was assayed by the method of Bergmeyer (1) using 0.1 ml of guaiacol, 0.1 ml of H_2O_2, and 1 ml of eluate. Enzyme inhibitors were tested by adding 0.1 ml of inhibitor solution in each assay to give a final concentration of 0.001 M tris, NaF, NaN_3, tris/glycine, KCN, EDTA-Na_2, Na_2SO_3, Na_2S, or 0.01 M $K_4Fe(CN)_6$ (1).

Every experiment was repeated an average of five times and five samples of different oaks infected with mistletoe were tested.

All chemicals were of analytical grade.

RESULTS

Fig. 1 shows a section through an oak branch parasitized by *L. europaeus*. The dark color indicates that oxidative processes have occurred in the intersection zone. As can be seen from the picture, the dark color is present only in the *Loranthus* tissue. The mistletoe haustorium penetrates the oak wood to gain connection to its sap streams.

The electrophoretic separation of the peroxidases of *Quercus robur* L. and *L. europaeus* is shown in Fig. 2. Lane 0 shows the peroxidase isoenzymes of the oak 0.5 m from the locus of infection. The activity of the peroxidase from *L. europaeus* in the intersection zone (lane 2) with the oak was increased manyfold. On the other hand, the activity of the peroxidase from oak was also enhanced in this zone (lane 2); the isoenzyme at the origin of the electropherogram (probably one with a high molecular weight) showed a much higher activity than the same isoenzyme in tissue collected from a more distant part of the same oak tree (lane 0). The peroxidase isoenzymes extractable from the light colored part of the wood (oak) in the parasite-host junction are shown in lane 1. A very strong peroxidase activity is revealed in the wood of *L. europaeus* by the electrophoretic separation of the wood extract from *Loranthus*. The enzymatic activity of this peroxidase is greatly increased at the site of the infection of *Q. robur*. This indicates strongly a participation of this peroxidase in the infection process with the oak.

Fig. 3 shows the results of the inhibition experiments conducted with partially purified peroxidase from *L. europaeus*. Inhibition was most complete with sulfide ions. Other classical inhibitors of peroxidase including cyanide, azide, fluoride, or complexing agents like EDTA affected the peroxidase activity very little.

Since our studies were concentrated on the infection of the oak by *L. europaeus* and possible remedies and inhibition possibilities

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for the oak, the peroxidase isoenzymes of the oak were not investigated in this connection. On the other hand, the activity of the oak peroxidase isoenzyme was not greatly enhanced at the site of infection.

DISCUSSION

Strong oxidation reactions seemed to occur in the junction between *Quercus* and *Loranthus*. This hypothesis is supported by the increased peroxidase activity of *L. europaeus* in this zone compared with the activity of *L. europaeus* in a sample 0.2 m from the junction. The peroxidase isoenzymes of oak also have an enhanced activity at the interface but to much lesser extent.

The electropherogram indicates that in a more distant part of the oak branch the peroxidases are not affected by the infection. But the appearance of the peroxidase of *Loranthus* seems not to be strictly confined to the dark part of the junction material (*L. europaeus*). As shown in lane 1 of Fig. 2, it can also be detected in the light-colored material (oak wood). This suggests the possibility that peroxidase of *Loranthus* plays a crucial role in the infection process by weakening the cell wall of the oak by oxidative processes. The reaction of the oak tree seems to consist of an increased proliferation of cells at the locus of infection which becomes evident by callus formation (9).

The mechanism of inhibition of peroxidase of *Loranthus* by sulfide and sulfite remains obscure. Presumably, the sulfide reacts with the metal cofactor of the peroxidase, interfering with the oxygen or H_2O_2 binding site of the enzyme. An inhibition due to a direct reaction of S^{--} with H_2O_2 can be excluded because the molar concentration of H_2O_2 used in the assays was 9.4 times greater than that of S^{--}.

The importance of the peroxidase of *Loranthus* for a successful infection of the oak was further supported by experiments done in vivo (2). A single treatment of the oak with 5% CaS_2 solution reduced the implantation rate of the germs of *Loranthus* by 36%. This result strongly indicates that the peroxidases of the hemiparasite are necessary for the infection, although the chemical mechanism is not known. Treatment with CaS_2 could be an instrument for repelling the hemiparasite by inhibiting its germination. The application of sulfide does not implicate oxidative damages in the oak as revealed by discoloration of the wood, because discoloration can be seen after injection of copper sulfate or of several herbicides in the trunk (3).

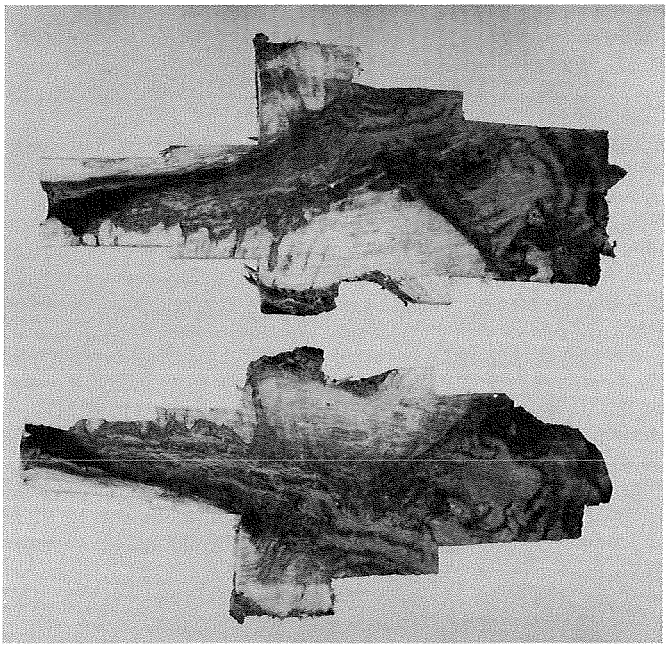

Fig. 1. Section through the junction of *Quercus robur* (light-colored wood) and *Loranthus europaeus* (dark-colored wood).

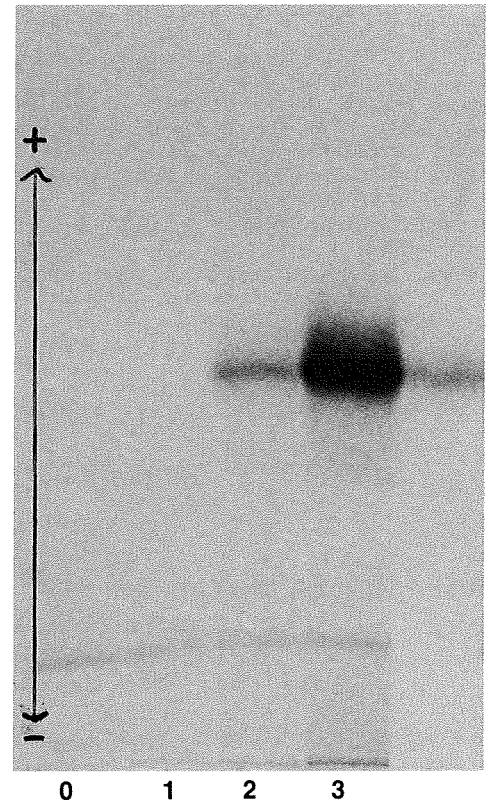

Fig. 2. Electropherogram of extracts of sapwood of *Quercus robur* and *Loranthus europaeus*: lane 0, peroxidase isoenzymes of *Q. robur* 0.5 m from the locus of infection; lane 1, peroxidase isoenzymes of *Q. robur* in the junction between *Q. robur* and *L. europaeus*; lane 2, peroxidase isoenzymes of *Q. robur* and *L. europaeus* in the junction between them; and lane 3, peroxidase isoenzymes of *L. europaeus* 0.2 m from the junction between oak and *Loranthus*.

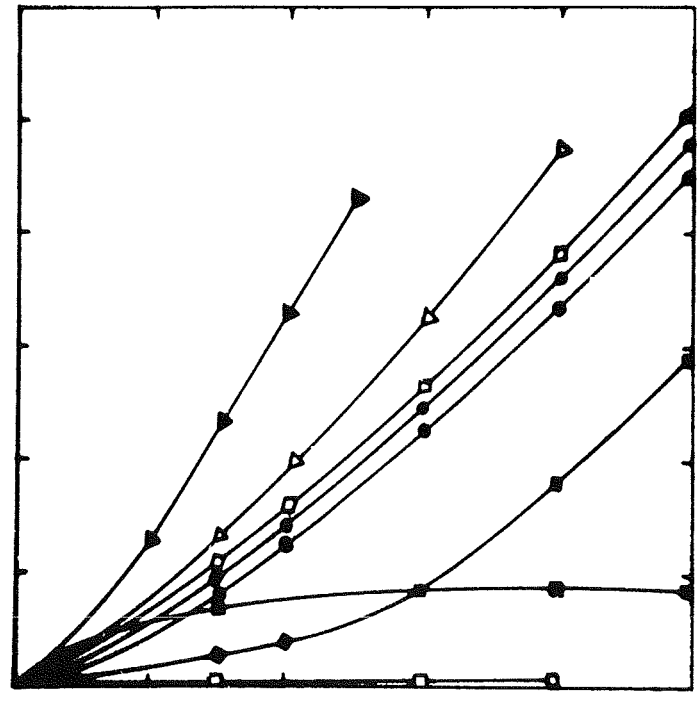

Fig. 3. Inhibition of activity of peroxidase isoenzymes of *Loranthus europaeus*. The reaction mixture contained partly purified enzyme, guaiacol, and H_2O_2. Legend: ▲, without inhibitor; △, + 0.001 M tris, ◇ + 0.001 M NaF; ◇, + 0.001 M NaN_3; ◇ + 0.001 M tris/glycine; ● + 0.001 M KCN; o, + 0.001 M EDTA-Na_2; ■, + 0.001 M Na_2SO_3; □, + 0.001 M Na_2S; and ◆, + 0.01 M $K_4Fe(CN)_6$.

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