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BIOCHEMICAL CHANGES IN TOBACCO INFECTED WITH *COLLETOTRICHUM DESTRUCTIVUM*—II. PEROXIDASES

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Abstract—Tobacco leaf tissue responded to infection by *Colletotrichum destructivum* with the synthesis or activation of at least one and possibly two proteins which exhibited peroxidase activity. One of these proteins was present in very small amounts and could not be detected consistently. The peroxidases common to both healthy and infected tissue were either more concentrated or more active in infected tissue.

INTRODUCTION

A NUMBER of enzymes are known to exist in more than one molecular form, for which the term isozyme has been proposed.[1] The array of isozymes synthesized by an organism varies with the species, with age, and from tissue to tissue in the same organism.[1] That pathogenesis can influence the isozyme content of plant tissues has recently been demonstrated. Uritani and Stahmann[2] detected a protein with peroxidase activity in sweet potato infected with *Ceratocystis fimbriata* which was absent in healthy tissue. Staples and Stahmann[3] found that rust infection of bean leaf resulted in the persistence of a malate dehydrogenase isozyme which was lost during development of the healthy leaf. Neither the peroxidase in diseased sweet potato nor the malate dehydrogenase in bean leaf were contributed by the pathogens.

In a previous paper, a 5- to 10-fold increase in peroxidase activity in tobacco leaf tissue following infection by *Colletotrichum destructivum* O'Gara was reported.[4] The present investigation was undertaken to determine if qualitative as well as quantitative changes in peroxidase activity occur in tobacco leaf tissue following infection with this pathogen.

RESULTS

Several proteins with peroxidase activity were detected in tobacco leaf tissue homogenates by starch gel electrophoresis, but only three, which are referred to as major components, were detected consistently. These are designated a_1, a_3, and c_2 on the basis of their relative mobility toward the anode or cathode. The remaining components—a_2, a_4, c_1, c_3 and c_4—were not detected consistently and gave only a faint reaction when they were detected. Thus, they were designated minor components. The relative positions of these components following electrophoresis in starch gel and their presence in infected and healthy tissue and in mycelial extracts of the pathogen are shown diagrammatically in Fig. 1. Bands a_3 and c_2

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[2] I. URITANI and M. A. STAHMANN, *Plant Physiol.* **36**, 770 (1961).
[3] R. C. STAPLES and M. A. STAHMANN, *Science*, **140**, 1320 (1963).
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were the most prominent in both infected and healthy tissue and were the only components detected in the pathogen, where they were present in only trace amounts. Band a_1 and, in some experiments, band c_4 were detected only in infected tissue. The components common to

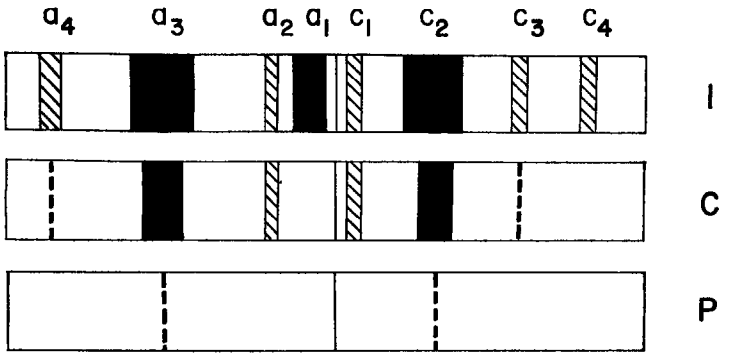

FIG. 1. PEROXIDASES IN HEALTHY AND INFECTED TISSUE AND IN *C. destructivum*.
Solid bands represent major components, shaded bands minor components, and dotted lines trace reactions. Bands a_1 and c_4 were detected only in infected tissue. I, infected tissue; C, healthy; P, pathogen.

both healthy and infected tissue produced much more intense bands in extracts of infected tissue, indicating either a greater concentration or greater activity of each peroxidase in infected tissue.

DISCUSSION

The pronounced increase in peroxidase activity in tobacco leaf tissue following infection by *C. destructivum* results from an increase in the activity in the peroxidases normally present in the tissue and from the synthesis or activation of at least one peroxidase not present in healthy tissue. Whether this peroxidase results from synthesis or from activation of a pro-enzyme already present in the tissue is not known. In either case, the absence of this protein in the pathogen indicates that its production is a response of the host tissue to infection. The only consistent difference between healthy and infected tissue was the greater activity of components a_3 and c_2 and the presence of component a_1 in infected tissue. The minor components, which were not detected in all experiments, might not have been present in the tissue under some conditions or might have been present in concentrations too low to be detected by our procedure. In either case, their activity as compared to the major components was too low to contribute significantly to the overall peroxidase activity of the tissue. Rather, most of the increase in peroxidase activity following infection can be accounted for by the increased activity of a_3 and c_2 and by the appearance of component a_1.

EXPERIMENTAL

Seedlings of *Nicotiana tabacum* L. (var. Ky 26) were inoculated as previously described.[4] Five days after inoculation the leaves were harvested, the mid-veins were removed, and the tissue was frozen at $-15°$. The tissue was then homogenized in cold 0·1 M phosphate buffer at pH 6·0 (3 ml/g fresh wt.) in a Servall omnimixer for 3 min and centrifuged at 3600 rev/min

for 15 min. Tissue homogenates of *C. destructivum* grown in liquid medium were prepared in the same manner. The supernatants were decanted and placed in 2-mm wide slits cut in starch gel strips (19 × 9 × 0·4 cm) perpendicular to the direction of current flow. Ten per cent starch gel prepared by the method of Smithies[5] in the above buffer was employed. A potential of 6·5 V/cm was applied for 18 hr and the temperature was kept at 5° during the electrophoresis run. The gels were then immersed in 0·05 M pyrogallol buffered at pH 6·0 and containing 0·1 % H_2O_2. In some experiments, the H_2O_2 was added 30 min after immersion of the gels in 0·05 M pyrogallol to insure that peroxidase activity and not phenoloxidase activity was responsible for oxidation of the pyrogallol. When this was done, very faint yellow bands appeared which rapidly darkened upon addition of H_2O_2.

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