Change in Fatty Acid Content of Fungus-Host Tissue During Pathogenesis of Uromyces phaseoli typica on Phaseolus vulgaris

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Germination of uredospores of *Puccinia graminis* f. sp. *tritici* has been postulated to be at the expense of endogenous lipid reserves synthesized and stored in the spore during their formation in the uredium (1). More recently, carbohydrates were shown to be metabolized rapidly during the initial phases of uredospore germination (3), indicating that germination is dependent upon both carbohydrate and lipid metabolism rather than upon lipids alone. Approximately 20% of the fresh wt of uredospores is made up of lipids, suggesting that fatty acid synthesis is high during uredospore formation. Since the technique of gas-liquid chromatography has refined the analysis of fatty acids, we undertook a study of the major fatty acid constituents of diseased and healthy bean leaf tissue and bean rust uredospores, and followed their changes during disease development.

The model system employed in our laboratory to study pathogenesis consists of *Uromyces phaseoli* (Pers.) Wint. *typica* Arth., race 32 on *Phaseolus vulgaris* L. In the past, we initiated kinetic studies designed to measure fluctuation in CO_2 fixation in the dark (6), dry wt of infected tissue (7, 14), starch hydrolysis and synthesis (7), photosynthesis (14), respiration (14), and translocation (13) from the time of inoculation of the plant until sporulation, usually 7 days later. To complement and conclude these studies, the synthesis of the major fatty acids in diseased tissue was also determined.

Plants were grown and inoculated as previously described (4, 10, 12, 14). Four replicates of inoculated plants and three of control plants were collected immediately after inoculation and at 24-hr intervals thereafter, and analyzed separately. Each replicate was homogenized (8) in the extraction solvent (2) [$CHCl_3$:CH_3OH(3:1)]. After extraction, each sample was concentrated under a stream of nitrogen to a 2-ml volume. One ml was esterified by the diazomethane method (11). After esterification, the amount of each fatty acid in the extract was determined by gas chromatography, using a column of 15% diethyleneglycol adipate coated on 60/80 mesh Chromosorb W previously washed with 3% phosphoric acid (5). The carrier gas used was helium, and the column temperature was 212°C. Detection of fatty acids was by hydrogen flame ionization. Fatty acids were quantitated by measuring peak height of each acid and determining the concentration of acid that is represented, using standard curves previously constructed through measurement of peak height produced by known quantities of the fatty acid. Fatty acid standards were purchased from the Hormel Institute, Austin, Minn.

The principal fatty acids detected in both diseased and healthy bean leaf tissue were palmitic, stearic, oleic, linoleic, and linolenic. These same acids plus an unidentified acid with a retention time greater than linolenic acid (probably 9, 10-epoxy octadecanoic acid) were found to be the principal constituents of lipids extracted from bean rust uredospores under the conditions used in our experiment. Although other acids were present in both diseased and healthy bean leaf tissue as well as in bean rust uredospores, they were minor. For this reason only palmitic, stearic, oleic, linoleic, and linolenic acids were assayed.

With the environmental conditions used (14), chlorotic flecks were observed 4 days after inoculation, and the leaf epidermis over the uredium was ruptured on the 7th day. Hereafter, the word "days" refers to the number of days after inoculation.

Palmitic acid (16:0) was found at a concentration of approximately 0.5 μg/mg of tissue in inoculated and healthy leaf tissue through the first 6 days (Fig. 1-4). Between the 6th and 9th days, its concentration in diseased tissue rose to approximately 2.0 μg/mg of tissue, four times that of the healthy tissue. Stearic acid (18:0) was found at a concentration of approximately 0.07 μg/mg of tissue in healthy and diseased tissue until 7 days, after which its concentration rose to 0.8 μg/mg of tissue, 11 times that of healthy tissue. Oleic acid (18:1) was found at a concentration of 0.04 μg/mg of tissue in healthy and diseased tissue until 5 days, after which its concentration in diseased tissue rose to 2 μg/mg of tissue, 50 times that of healthy tissue. The concentration of linoleic acid (18:2) fluctuated widely, but no significant increase or decrease in concentration was observed when diseased tissue was compared to healthy tissue. Linolenic acid (18:3) was present at a concentration of 7.36 ± 0.35 μg/mg fresh leaf tissue.

Developing uredospores have been observed in the immature uredium by the 5th day after inoculation; by the 6th day, the concentration of oleic acid in the diseased tissue increased over that of the healthy control tissue. The synthesis of all three fatty acids (palmitic, stearic, oleic) produced during disease development probably started at least 1 day before a rise in concentration was apparent but the relatively large amount of uninfected tissue present at these early stages of uredial development may dilute out the increases in these fatty acids. From the 4th day, when chlorotic flecks appeared, to the end of the study (10 days), samples were selected from obviously infected tissue. Prior to the 4th day, when visible lesions were not apparent, 1-cm^2 samples were selected randomly from over the leaf area.

Increased concentrations of palmitic, stearic, and oleic acids in diseased tissue occurred at the time when uredospores were being rapidly formed in the uredium, and may reflect the lipid content of the uredospores. Because of the extraction method employed, free and

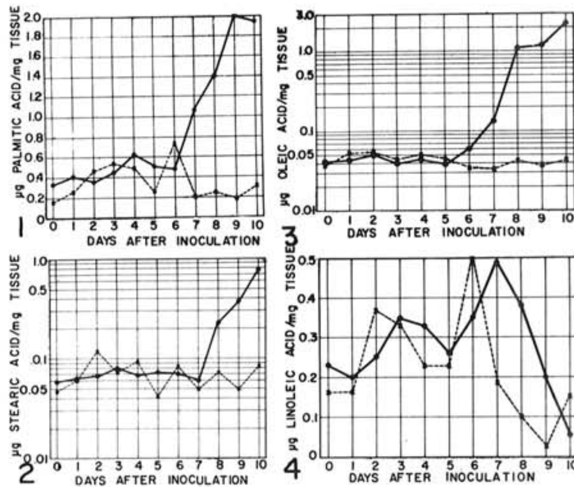

Fig. 1-4. Concentrations of fatty acids in healthy leaves of *Phaseolus vulgaris* var. Topcrop and those infected with *Uromyces phaseoli* var. *typica* race 32. The plants were inoculated on day 0; chlorotic flecks appeared on day 4, and pustules appeared on day 7. Solid line indicates infected tissue; broken line indicates healthy. **1)** Synthesis of palmitic acid, about 4-fold increase in diseased tissue; **2)** synthesis of stearic acid; ordinate is plotted on logarithmic scale; **3)** synthesis of oleic acid, ordinate plotted on logarithmic scale; **4)** synthesis of linolenic acid; decrease in diseased tissue comparable to that in control, but delayed by 1 day.

bound fatty acids could not be differentiated, although much of the fatty acids in uredospores appears to be free.

Although uredospores contained a considerable amount of linoleic (0.6 µg/mg of uredospores) and linolenic acids (1.3 µg/mg of uredospores), comparison of concentrations of these acids in diseased tissue with healthy tissue shows them to be similar. Palmitic, stearic, and oleic acids appear to be synthesized de novo in infected leaves, most likely by the fungus.

Linolenic acid is present in bean leaves in such high concentrations compared to the other acids that it partially masks the relatively large increases in palmitic, stearic, and oleic acids, especially at their initial rise. Methods in which total fatty acids are measured (i.e., by titration rather than as individual components as with gas-liquid chromatography) would not indicate any appreciable quantitative increases in fatty acid content until late in disease development.

The increase in concentration of palmitic, stearic, and oleic acids (6-7 days after inoculation) appeared to coincide with the rise in respiration, rise in starch synthesis, and increase in CO_2 fixation in the dark (6, 9, 14) reported in other studies. It appears that sufficient carbon is available in the area of infection to account for the increase in fatty acid concentration, and can be derived from both starch hydrolysis and carbon formed from CO_2 fixation. The metabolic system favored to account for fatty acid synthesis is the avidin-sensitive biotinyl-acetyl-CoA carboxylase system that forms malonyl-CoA and culminates in the synthesis of palmitic acid.

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