Distribution of Phenols in Specialized Cells of Banana Roots

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ABSTRACT

Localized concentrations of phenols, stored in discrete bodies within randomly scattered parenchyma cells in banana roots, were detected both by light and electron microscopy. The distribution of the phenols was comparable (i) in fresh tissues stained with aqueous methylene blue or by the nitroso reaction; (ii) in glutaraldehyde-preserved tissues similarly stained; or (iii) in glutaraldehyde-preserved tissues postfixed with osmium tetroxide and embedded in diepoxy resins. Phytopathology 60:79-82.

Following injury or infection of plant tissues, phenols appear to play an important role in "sounding the alarm", in inhibiting enzymatic hydrolysis, in directly inhibiting microbial development, and in the process of repair. The alarm reaction is apparently elicited by indoleactic acid (IAA) (14) that can be synthesized from tryptophan through a set of reactions in which orthopolyphenols serve as mediators (10, 14). Hydrolytic enzymes of pathogens are inhibited by phenols in resistance to such diseases as chocolate spot of beans (7), apple scab (18), brown rot (4), fusarial wilt of tomato (6), and others (3, 11, 15). Inhibition of growth of parasites by phenols and phenol derivatives from disease resistant plant varieties has also been established (5, 11, 12, 18). The establishment of physical barriers following injury, such as abscission layers in the "shot hole" disease of cherry (20), periderm formation in potato (24), lignituber formation in hops (21), and the impregnation of pectic and hemicellulosic gels to form gums associated with vascular diseases of tomato (27) and banana (2, 28) all require phenols as precursors in the synthesis of lignins or tannins (22, 23).

Phenols are present in high concentrations in randomly occurring parenchyma cells of banana plants (13), species of eucalyptus (29), and in various genera and species of the Rosaceae (16). The phenol in banana has been identified as 3-hydroxytyramine (13). Very little is known, however, of the modes and sites of synthesis and storage. The purpose of this study was (i) to determine the nature of localization as nearly as could be deduced by the light microscope; (ii) to develop techniques which would insure that the randomly occurring phenol cells could be positively identified in electronphotomicrographs; and (iii) to insure that their structure and nature would be altered as little as possible in the process.

MATERIALS AND METHODS.—Banana plants (*Musa acuminata* L. 'Valery') were grown in glasshouse ground beds in a friable soil, sand, and sphagnum mix. Diseased roots were obtained by placing the severed ends of the attached roots into vials containing a suspension of microspores of *Fusarium oxysporum* Schlecht. f. sp. *cubense* (E.F.Sm.) Snyd. & Hans. and vinyl tracer particles (2). Root tissues were sectioned as reported earlier (2), and stained either with 0.5% aqueous methylene blue or with 10% aqueous nitrous acid (19), both of which react strongly with ortho-dihydroxyphenols (13).

Tissues prepared for electron microscopy were fixed in 2% glutaraldehyde, washed in buffer, postfixed in osmium tetroxide, washed in buffer, dehydrated in ethyl alcohol, placed in propylene oxide, and infiltrated over a period of several days in increasing concentrations (25, 50, 75, and 100%) of resin. The embedding resin consisted of 30 parts of diepoxy resin 332, 15 parts of diepoxy resin 732, 50 parts of dodecenylsuccinic anhydride (DDSA), and 2 parts of dimethylaminomethyl phenol (DMP-10). To the above mixture, 5% dibutyl phthalate was added. The tissue was embedded in aluminum weighing dishes; sections were cut with glass knives, mounted on Formvar-coated 100- or 200-mesh grids, and examined with an RCA EMU-3G electron microscope.

RESULTS.—The scattered phenol-containing cells of the vasicentric parenchyma tissue of banana roots (Fig. 1-5) are small, flat, and closely appressed to the vessel walls. They become progressively more colored (from yellow to reddish-brown) within a few days after inoculation with *F. oxysporum* f. sp. *cubense* (Fig. 1). Phenols diffuse out of these cells into the lumen of the infected vessels (Fig. 2) and ultimately into surrounding cells. The phenol cells of the cortex are much larger, but also occur randomly (Fig. 6-9). The phenols stain dark blue with methylene blue (Fig. 4, 5, 6, and 9) and cherry red with the nitroso reaction (Fig. 7, 8). They occur in very fine, discrete, diffuse bodies (Fig. 4, 8) or in larger globular bodies (Fig. 5, 6, 7) which occur singly or in aggregates (Fig. 5, 6, 7) arising from the dense peripheral cytoplasm (Fig. 9).

In stelar tissues fixed with glutaraldehyde, phenol cells were distinguishable from surrounding cells because of their dense appearance, but retained their normal staining characteristics. After postfixing with osmium tetroxide, these cells were clearly distinguishable from surrounding cells because of their black color (Fig. 10). Darkly staining bodies, comparable to the phenol-positive bodies found in methylene blue- and nitroso-stained cells, were clearly visible (Fig. 11). When osmium tetroxide was added to a solution of the

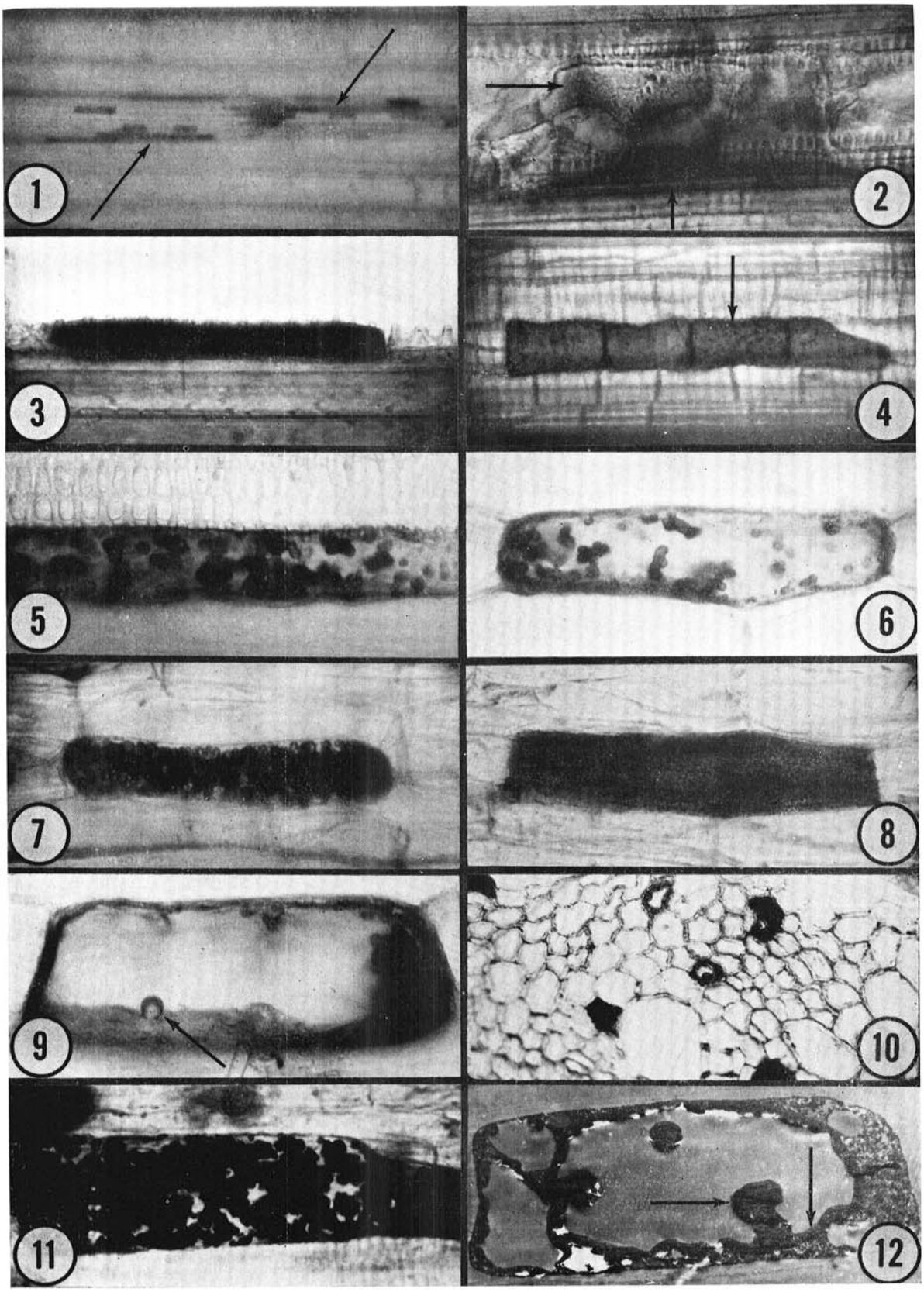
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Fig. 1-12. Photomicrographs of free hand, longitudinal sections of freshly prepared Valery banana roots in aqueous mount showing **1)** coloration of randomly scattered phenol cells (arrows) 5 days after inoculation with *Fusarium oxysporum* f. sp. *cubense* (×57); **2)** diffusion of colored phenol products (horizontal arrow) from a phenol cell (vertical arrow) into the lumen of a vessel occluded with gel and tyloses 5 days after inoculation with f. sp. *cubense* (×300); **3)** a typically thin vasicentric phenol cell of a healthy root closely appressed to the vessel wall and darkly stained with aqueous methylene blue (×600); **4)** a surface view of a vasicentric phenol cell showing fine granular phenol bodies (arrow) stained with aqueous methylene blue (×300); **5)** a side view of a vasicentric phenol cell with large phenol bodies occurring singly and in chains stained with aqueous methylene blue (×680); **6)** a large, cortical, phenol cell with large phenol bodies stained with aqueous methylene blue (×430); **7)** a large cortical phenol cell with large globular phenol bodies stained by the nitroso reaction (×550); **8)** a cortical phenol cell with very fine phenol bodies stained by the nitroso reaction (×550); **9)** a cortical phenol cell stained with methylene blue showing a typically thick layer of peripheral cytoplasm surrounding a large central vacuole and with a large phenol globule (arrow) arising from the peripheral (×500); **10)** photomicrograph of a cross section of banana root tissue prepared for electron microscopy showing the randomly occurring phenol cells with a thick, darkly stained peripheral cytoplasm and occasional phenol globules (×160); **11)** photomicrograph of a longitudinal section of root prepared for electron microscopy showing a large cortical phenol cell with large phenol globules stained densely black with osmium (×300); **12)** electron photomicrograph of a cross section of a long vascicentric phenol cell showing a thick peripheral cytoplasm, several large vacuoles, and phenol in large globular deposits (horizontal arrow) and as a dense layer lining the main vacuole (vertical arrow) (×3,000).

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ortho-phenol, i.e., 3-hydroxytyramine, to test the reaction of these two compounds, a dense, black substance formed which rapidly flocculated and settled out of suspension. Furthermore, after osmium tetroxide treatment, no cells in the tissues responded to phenol reagents. It was concluded, therefore, that the phenols in these cells react with osmium tetroxide, and that the black substance seen in such deeply stained cells of osmium-treated tissues is a phenol derivative.

Phenol cells in root tissues treated in the above manner were readily observed with the electron microscope (Fig. 12) without further staining. The cells had a dense peripheral cytoplasm with many large vacuoles frequently bordered by dense, darkly stained bands and globular bodies. Globular bodies were often present as aggregates, and extended well into the vacuoles. In the initial preparations, the phenol cells were torn or shattered, whereas surrounding cells were intact. The phenol cells are apparently difficult to embed, but modifications of the embedding procedure that retarded polymerization and increased plasticity improved the embedding and the sectioning properties. Even in the best sections, however, regions of apparent phenol accumulation showed many "chatter" marks. The most likely cause for the difficulties encountered in sectioning is that embedding is difficult because of the dense cytoplasm of these cells. The "chatter" marks result from differences in the physical properties of the embedding resin polymers themselves and those that have been admixed or combined with the phenols or phenol polymers occurring in loci of high concentrations.

DISCUSSION.—Mace (13) has described specialized cells in banana roots containing high concentrations of phenol which he identified as 3-hydroxytyramine (dopamine). The foregoing results confirm the report of Mace (13) that phenols in banana roots are localized in randomly occurring cells. Within these cells, the phenols are compartmentalized in globular bodies that border the many large vacuoles of these cells. The phenol bodies frequently form aggregates that extend into the vacuoles. These globules may coalesce to form a continuous layer of phenol deposited around the margins of the vacuoles.

Phenol cells present severe problems in tissue preparation and observation by electron microscopy, apparently because the dense peripheral cytoplasm retards embedding. The high concentration of highly reactive, easily polymerized phenols alters the cutting properties of the embedding polymers, resulting in frequent tearing of the protoplasts and an apparent gumming of the polymers to produce a drag across the knife edge during sectioning. This drag results in "chatter" marks in the regions of phenol deposition, making observation of fine structural detail difficult. Frequently, the entire protoplasmic mass of the cell is torn and clumped at one end of the cell. Rapid fixation in glutaraldehyde of small tissue masses and prolonged infiltration before final polymerization of the embedding medium have diminished, but not eliminated, these problems. Even in the best preparations, structure within the phenol cells was often obscured by the dense deposit of phenols, as is the structure of melanosomes in animal cells (9).

The work of Mace (13), Politis (16), Wardrop and Cronshaw (29), and our own suggests that phenols occur in high concentrations in many plants, and that they are somehow compartmentalized within specialized storage cells. Many of the "tannin" cells so widely reported in earlier literature (8, 23) may represent artifacts of tissue preparation. Such cells may, in reality, have been phenol cells in which, through chemical treatment, the phenols have become decompartmentalized and polymerized to form tannins. Other cells in dead tissues (cork layers) are undoubtedly tanned at the time of observation, but presumably were phenol cells during their active life span.

The function of phenols in plants is still poorly understood, but their involvement in many reactions is well documented (17, 26). There have been numerous reports of an increase of phenols in plant tissues following injury or infection (5, 11). Thus, many plants respond to irritation by a rapid activation of phenol synthesis. Many plants, however, have a significant supply of preformed phenols that are carefully packaged and stored, and can be rapidly released to perform numerous regulatory and structural functions when an emergency arises. Further study of the fine structure of the types of phenol bodies and their formation and breakdown will contribute greatly to our understanding of the responses of plants to irritation and of disease resistance.

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