Fusarium Wilt of Susceptible and Resistant Tomato Isolines: Histochemistry of Vascular Browning

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ABSTRACT

The substrate for initial vascular browning in wilt-resistant or susceptible tomato isolines infected with *Fusarium oxysporum* f. sp. *lycopersici*, race 1 or 2, is localized in scattered xylem parenchyma cells. Brown products diffuse from these localized sites into surrounding xylem tissues. Phenols were detected histochemically in the xylem parenchyma cells during initial or early stages of browning. The histochemical data indicate that the major type of phenol localized at the sites of vascular browning is an o-dihydric phenol with an unsubstituted position para to one of the hydroxyl groups. Phenols were not detected histochemically in the healthy, nonbrowned xylem parenchyma. These observations suggest that the phenols may occur in the healthy stem xylem in conjugated forms from which free, oxidizable phenols are released after infection. Where localized vascular browning occurred, no differences in the histochemical reactions were noted between the two tomato cultivars inoculated with race 1 or 2 of *F. oxysporum* f. sp. *lycopersici*.

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Additional key words: phenolic glycosides, glycosidases, phenolase, cytochemistry.

Vascular browning is one of the most readily noted symptoms of Fusarium wilt of tomato. Many papers have appeared on various facets of the browning syndrome (12). Davis & Dimond (3), Davis et al. (4), and Waggoner & Dimond (11) presented data in support of the hypothesis that free phenols responsible for vascular browning in tomato stems are derived from conjugated phenols which undergo hydrolysis and enzymatic oxidation in the diseased plant. They detected polyphenol oxidase, phenolic substrate, and β-glycosidase in the diseased stem tissue.

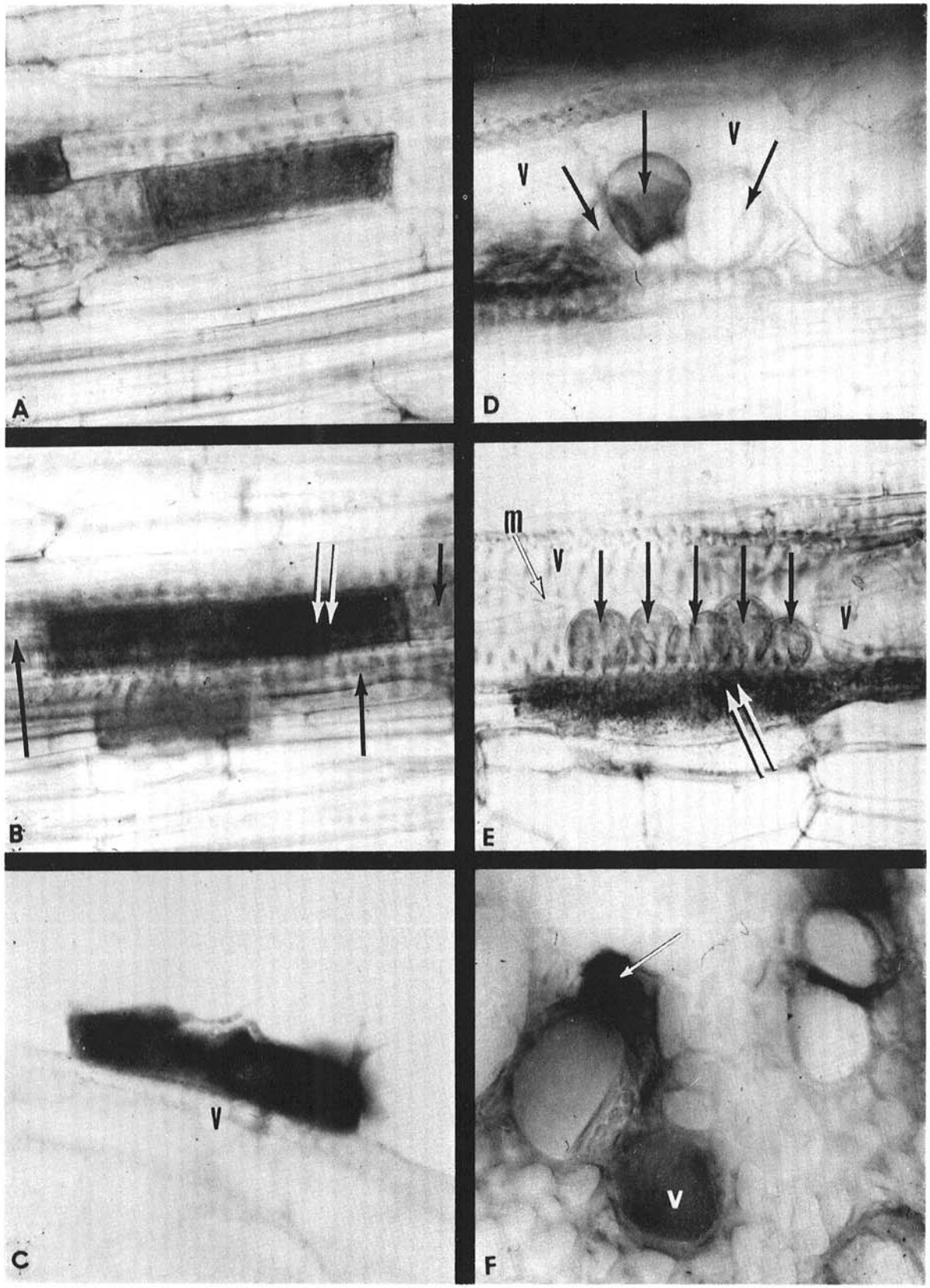
In a study on the colonization of susceptible and resistant tomato stems by *Fusarium oxysporum* f. *lycopersici*, we noted the apparent localization of early vascular browning in scattered xylem parenchyma cells. This paper reports the results of histochemical studies on the localization of the browning process in *Fusarium*-infected tomato stems.

MATERIALS AND METHODS.—The materials and cultural methods for hosts and pathogen were described in detail previously (8). Briefly, the hosts consisted of two nearly isogenic (isolines) cultivars of tomato, *Lycopersicon esculentum* Mill., wilt-susceptible Improved Pearson (IP), and wilt-resistant Pearson VF_{11} (VF). Pearson VF_{11} is resistant only to race 1 of the pathogen. Stem cuttings were inoculated with 5×10^4 bud cells/ml of *F. oxysporum* Schlecht. f. sp. *lycopersici* (Sacc.) Snyd. & Hans., race 1 (ATCC No. 16417) or race 2 (ATCC No. 16605) for 30 min.

Except for the diazo red RC reaction, the histochemical procedures for processing fresh freehand sections of tomato stems were like those described for banana roots (7). Sections of stem exhibiting early vascular browning were cut from various levels at 7-20 days after inoculation. Three histochemical reagents, nitrous acid; 2,6-dichloroquinone-4-chloroimine (Gibbs reagent), and diazo red RC, were used to detect phenols. The diazo red RC reagent was prepared by mixing 1 mg of the stable salt/ml of 0.1% aqueous $NaHCO_3$, pH 8.2. In some cases, fresh freehand sections of healthy and diseased stems were preincubated in 0.2% aqueous solution of sodium deoxycholate for 30 min. This procedure disrupts possible membrane barriers to the penetration of the reagents.

RESULTS.—Naturally occurring brown products were found to be localized in xylem parenchyma cells of the stem axis 7-20 days after inoculation. Discrete intracellular localization was observed best in longitudinal sections before appreciable diffusion of brown products occurred (Fig. 1-A). Subsequent diffusion of the brown products out of these cells tended to obscure the initial sites of localization (Fig. 1-B).

Nitroso and diazo red RC reagents yielded deep cherry-red and red-orange colors, respectively, in areas of early vascular browning. The Gibbs reagent failed to react at these localized browning sites. The very intense cherry-red nitroso product was more effective than the less intense red-orange azo dye in masking the pre-existing brown products. The histochemical reactions for the localization of the nitroso derivatives of endogenous phenols in IP and VF stems after inoculation with race 1 or 2 of *F. oxysporum* f. *lycopersici* are illustrated in Fig. 1-C, D, E, F. Histochemical reactions revealed structurally unmodified parenchyma cells appressed to the surface of xylem vessels (Fig. 1-C) or cells from which single (Fig. 1-D) or multiple (Fig. 1-E) tyloses had formed. These tyloses may function to place the browning substrates directly in the vascular stream and facilitate the spread of brown products. As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pathogenesis progressed, unoxidized or oxidized phenols diffused from the initial sites of localization in the xylem parenchyma cells into the gel plugs in vessels (Fig. 1-F). No obvious differences in the reactivity of the browning substrates with histochemical reagents were noted between IP and VF after inoculation with either race 1 or 2 of the pathogen.

Reactions of xylem parenchyma cell contents with the nitroso or diazo red RC reagents were observed only in the presence of vascular browning. The nitroso, diazo red RC, and Gibbs reagents gave faint yellow-orange, red-orange, and blue reaction products, respectively, with compounds in the secondary walls of xylem vessels and the thick walls of collenchymatous xylem parenchyma cells of nonbrowned control and inoculated stems. Treatment of nonbrowned sections from control and inoculated plants with sodium deoxycholate before application of the three histochemical reagents failed to induce positive histochemical reactions in the vascular parenchyma cells. The pretreatment, however, allowed the development of a positive diazo red RC reaction in the trichomes of both cultivars, but had no effect on the Gibbs reaction. The contents of these trichomes gave a positive nitroso reaction without previous treatment with deoxycholate.

DISCUSSION.—The constant association between the initial vascular browning in scattered xylem parenchyma cells and the appearance of histochemically reactive phenols establishes that the phenolic substrates for early vascular browning in IP and VF tomatoes are localized in these parenchyma cells. The failure to detect histochemically reactive phenols in the nonbrowned xylem parenchyma of control or inoculated stems indicates, as previously suggested by Davis et al. (4) and Waggoner & Dimond (11), that the phenols may occur naturally in conjugated forms and are released as free, reactive phenols only after enzymatic hydrolysis of the conjugated phenols in the diseased plant. By contrast, 3-hydroxytyramine, the browning substrate in *Fusarium*-infected banana roots, occurs as a free phenol in scattered xylem parenchyma cells (1, 7). Furthermore, the failure to detect reactive phenols in xylem parenchyma of tomato stems after treatment with deoxycholate suggests that a membrane diffusion barrier is not responsible for the negative histochemical reactions.

Inherent limitations of the histochemical methods do not permit an exact identification of the major phenols responsible for early vascular browning in IP or VF tomatoes; however, our data indicates that o-dihydric phenols are involved in the browning process in both cultivars. The persistent cherry-red compound formed in browned xylem parenchyma cells after treatment with the nitroso reagent appears to be specific for the nitroso derivatives of o-dihydric phenols (10). This reaction also establishes the presence of a free position para to one of the hydroxyl groups (5) and the absence of a nitro group. Failure of the phenol(s) to give the Gibbs indophenol reaction suggests the presence of carboxyl, sulfo, or formyl groups on the benzene ring (6). Azo dye formation with the diazo red RC reagent merely demonstrates the presence of a free position para or ortho to one of the hydroxyl groups (2). Matta et al. (9) recently reported the spectrophotometric detection of o-dihydric phenols in nearly equal amounts in healthy stems and those inoculated with race 1 of the pathogen; however, our data suggest that much of this phenol may have been extracted from the glandular trichomes.

Three major steps, diffusion, oxidation, and polymerization are involved in the final distribution of the brown products in the vascular tissue. Polymerization cannot occur before oxidation; otherwise the sequence of events is not positively known. When the brown products are located intercellularly, it is probable that the free unoxidized phenol diffused from the xylem parenchyma. Oxidized phenols rapidly undergo polymerization, and the polymers probably would be too large to pass through the plasma membrane of the xylem parenchyma. A ruptured tylose containing phenolic material could empty its contents into a vessel, and the unoxidized, oxidized, or polymerized material could move to a gel plug.

The localization of early vascular browning in stem xylem parenchyma cells does not preclude the involvement of additional phenolic substrates at later stages of pathogenesis. Davis & Dimond (3) suggest that phenols derived from phenolic glycosides normally destined for lignin biosynthesis may serve as

Fig. 1. The localization of natural brown products (A-B, nonstained) and substrates of early vascular browning (C-F, nitroso stained) in tomato, *Lycopersicon esculentum,* inoculated with race 1 or 2 of the wilt pathogen *Fusarium oxysporum* Schlecht. f. sp. *lycopersici.* All illustrated sections were made 2 cm above the cotyledonary node. **A)** A longitudinal section showing the brown products confined within a xylem parenchyma cell (cultivar VF_{11}, 18 days after inoculation with race 2 of the pathogen). **B)** A longitudinal section showing the browned products within a xylem parenchyma cell (double arrow); adjacent tissue (single arrows) also shows browning as a result of diffusion from the initial site of localization (cultivar VF_{11}, 18 days after inoculation with race 2 of the pathogen). **C)** A xylem vessel (V) with a nitroso-positive parenchyma cell (cultivar VF_{11}, 20 days after inoculation with race 1 of the pathogen). **D)** At least two parenchyma cells below the focal plane were responsible for the formation of the three tyloses (arrows) shown in the vessel lumen (v). One tylose (center arrow) contained nitroso-positive material; the other tyloses were nitroso-negative (cultivar IP, 20 days after inoculation with race 2 of the pathogen). **E)** Multiple tyloses (arrows) from a parenchyma cell (double arrow) are shown in a vessel lumen (v); pathogen mycelium (m) is also present in the vessel. The tyloses and the parent parenchyma cell were nitroso-positive (cultivar VF, 18 days after inoculation with race 1 of the pathogen). Figures D and E illustrate how tyloses may function to deliver browning substrates into the vessel. **F)** A nitroso-positive parenchyma cell (arrow) and a vessel (v) containing a nitroso-positive gel plug into which browning substrates have diffused (cultivar VF_{11}, 7 days after inoculation with race 2 of the pathogen).

browning substrates. Their observation of a decrease in the lignin content of diseased plants lends support to this hypothesis.

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