Chemical Characterization of Stress-Induced Vascular Coating in Tomato

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Abstract

Indirect evidence suggests that vascular coatings formed by plants in response to stress consist of suberin-like substances containing lipid and phenolic compounds. To provide more direct chemical evidence that coatings are suberin, we used a natural pathogen, Verticillium albo-atrum, or a stress-responsive hormone, abscisic acid, to induce coating in two isolines of tomato (Lycopersicon esculentum L. cv Craigella) that are resistant or susceptible to the pathogen. Using treated petioles that had been monitored cytologically, chemical depolymerization followed by combined gas-liquid chromatography-mass spectrometry analysis of alkane-ω,ω-diol levels confirmed the presence of suberin after induction of coating and showed quantitative differences between the isolines that correlated with cytological measurements of the coating response. Northern analysis of suberization-associated anionic peroxidase mRNA showed corresponding increases, and tissue blot analysis further indicated that induction of the mRNA was localized in the responding vascular bundles, as determined by suberin histochemistry. Taken together, these results provide chemical evidence that the coatings are mainly suberin.

MATERIALS AND METHODS

Plants

Near-isolines of tomato (Lycopersicon esculentum L.) cv Craigella, susceptible (GCS 26) and resistant (GCR2 218) to Verticillium albo-atrum, were grown as described (25).

Pathogen

V. albo-atrum was maintained on potato dextrose agar at 21°C. Conidial suspensions were prepared as previously described (25) to give a final concentration of 9 × 10⁶ conidia/mL in sterile, distilled water.

Treatment of Plant Material

Adjacent 12-cm-long petioles from the fifth and sixth nodes of 4- to 6-week-old plants were excised in distilled water and infused directly by enhanced transpiration (1, 14). Treatments consisted of: (a) conidia in sterile, distilled water; (b) 10⁻⁴, 10⁻³, or 10⁻² M ABA (Sigma, grade II) in sterile, distilled water; or (c) sterile, distilled water. In preliminary experi-

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2 Abbreviations: GCR, Craigella resistant; h.p.i., hours post-inoculation; LM, light microscopy; TBO, toluidine blue O; TEM, transmission electron microscopy.
mments, red vinyl particles were added to spore suspensions to give a faint pink color at the initial trapping sites (25). After 1 h, petioles infused with conidia were removed from the conidial suspension. The basal portions of the cuttings were washed in sterile water to remove excess inoculum from the surface. Inoculated cuttings were transferred to sterile glass vials containing sterile, distilled water. Petioles infused with water or ABA remained in vials containing the treatment solutions. All vials were then placed randomly in a tray in a humidity chamber (RH near 100%) and returned to a growth cabinet maintained at the standard growth conditions. The petioles were kept under high humidity until sampled. For the tissue blot hybridization experiments, the tomato stems were similarly treated.

Cytological Procedures

For plastic embedding, each sample consisted of a 5-mm segment of petiole severed 2 cm above the cut base. Three samples each of resistant and susceptible plants were taken for each of the three treatments at 72 h.p.i. Three samples each of resistant and susceptible plants infused with the pathogen were also taken 18 h after infusion. Samples were fixed in a solution of 2% (v/v) glutaraldehyde and 1.5% (v/v) acrolein in 0.07 M phosphate buffer, pH 6.8, for 6 h at 4°C. The tissue was washed in the same buffer, retrimmed to a final thickness of 2 to 3 mm, and postfixed in 1% (w/v) OsO4 in the same buffer for 1 h at 4°C. All samples were washed in buffer, dehydrated in a graded acetone series, and embedded in Spurr’s plastic. For LM, thick sections (0.25 mm) were cut with a diamond knife (Diatome), mounted on glass slides, and stained with 1% (w/v) TBO in 1% (w/v) sodium borate (18, 19). Material was photographed with a Nikon Labophot microscope. For TEM, thin sections (80 nm) were cut, stained with 4% (w/v) uranyl acetate in 70% methanol followed by Reynold’s lead citrate, and viewed and photographed with a JEOL 100cx transmission electron microscope operating at 80 kV.

For histochemical studies by LM, fresh freehand sections were cut at a height of 2 to 4 cm above the cut base. Samples were taken from two resistant and two susceptible plants for each of the three treatments at 72 h.p.i. Hand sections were viewed and photographed with a Nikon Labophot microscope. The sections were stained in one of the following ways: (a) sudan III, IV (16): sections were stained in a solution consisting of equal parts saturated sudan III in 70% ethanol and saturated sudan IV in 70% ethanol for 15 min, followed by rinsing in 70% ethanol for 1 min; the sections were floated on distilled water and mounted in 50% glycerol on glass slides; (b) sudan black B (7, 18, 19); (c) 12 N KOH (7); (d) oil red O (16); or (e) ammoniacial gentian violet (7).

Analysis of Suberin

For suberin analysis, samples of each of the three treatments were taken at 72 h. Petioles were monitored for induction by observation of KOH-stained freehand sections. Each sample consisted of a 5-cm segment of petiole severed 0.5 cm above the cut base. The cuticle and epidermis were completely removed physically. For each treatment, six samples were pooled for resistant plants and six for susceptible plants. Three grams of the pooled samples were lyophilized for 48 h and stored at -80°C for suberin analysis. The experiment was repeated twice.

Depolymerization and analysis of the aliphatic components of suberin were as previously described (11). The lyophilized material was finely ground with a Wig-L-Bug amalgamator (Crescent Manufacturing) and extracted in a Soxhlet apparatus for 48 h with CHCl3 and a further 48 h with CH3OH. Weighed portions of the insoluble residue were refluxed overnight in tetrahydrofuran with an excess of LiAlH4. The reaction mixtures were cooled and carefully added into water with stirring. The mixtures were then acidified and extracted three times with CHCl3. The chloroform was evaporated under reduced pressure, and the resulting material was subjected to TLC with diethyl ether:hexane:methanol (8:2:1, v/v) as the developing solvent. The TLC plates were sprayed with 2′,7′- dichlorofluorescin, and the components were visualized under UV light. The silica gel from the alkane-α,ω-diol region was eluted with CHCl3:CH3OH (2:1, v/v) to recover the diol fraction. The diol fraction was then silylated with N,O-bis(trimethylsilyl)acetamide and analyzed by capillary GC-MS on a 15-m SPB-5 (Supelco) column in a Hewlett-Packard 5890 gas chromatograph attached to a Hewlett-Packard 59888A mass spectrometer equipped with a Chemstation computer data system. Mass spectra, recorded at 70 eV, were used for identification of the diols (26).

Northern Blotting

Samples were taken at 0, 12, 15, 20, 25, 36, and 72 h.p.i. with fungal conidia. Petioles were monitored for induction of coating by observation of KOH-stained freehand sections. Each sample consisted of a 5-cm segment of petiole severed 0.5 cm above the cut base. At each sample time, five petiole segments were pooled, and 3 g of this tissue pool was used for resistant and susceptible plants. At 36 h.p.i., water control samples were similarly taken and pooled. The experiment was repeated three times.

Petioles were ground in liquid nitrogen and total RNA was prepared as described by Prescott and Martin (16) with the following modifications. The extraction buffer was adjusted to pH 5.5. The RNA pellet was dissolved in 20 μL of 0.1% (v/v) diethylpyrocarbonate in water. The mRNA was fractionated on agarose gels containing formaldehyde (12), blotted onto APT paper or nylon membrane, and hybridized with pAPα, a potato anionic peroxidase cDNA clone (24) that was 32P-labeled with the random primer kit (Boehringer Mannheim Biochemicals). The blots were autoradiographed at -80°C. Results were compared with those obtained when the same membranes were hybridized with a 32P-labeled tomato actin genomic DNA probe (our unpublished data). The autoradiograms were scanned with a densitometer (Bio-Rad) to quantify the levels of peroxidase and actin mRNAs.

Tissue Blotting

Before blotting, all treated stems were monitored for induction of coating by observation of sudan III, IV or KOH-stained freehand sections. For hybridization, samples con-
Figure 1. Suberin histochemistry of tomato petioles induced by V. albo-atrum or ABA. Tomato petioles from resistant and susceptible plants were challenged by V. albo-atrum at a concentration of $9 \times 10^6$ conidia/mL or $10^{-6}$ M ABA. Suberin stains were applied and viewed by LM as described in "Materials and Methods." The figures were chosen to illustrate the staining reactions and have no bearing on quantification as previously discussed (12, 21, 26). a, Thick plastic section of a susceptible plant challenged with Verticillium for 18 h and stained with TBO. The conidia (C) within the trapping site (TS) vessel, indicated by red vinyl particles (RV), have germinated and the fungal hyphae (F) have grown through an uncoated pit membrane into the adjacent vessel. b, Thick plastic section of a resistant plant challenged with Verticillium for 18 h and stained with TBO. Note the heavy deposition of dark blue coating on the full border pit (large arrowhead) of the trapping site (TS) vessel, indicated...
sisted of a 2- to 3-mm segment of stem cut 2 to 4 cm above the base. The cut end was gently blotted onto 3-mm filter paper for several s, then placed onto a nylon (Nytran 45 μm) membrane wetted with sterile, distilled water. Gentle pressure was manually applied to the top of the sample for 30 s in order to blot the sample onto the nylon membrane. Samples of both resistant and susceptible plants were blotted for each of the three treatments 72 h after infusion. Blots were photographed with a Nikon microscope, then hybridized with the 32P-labeled potato peroxidase cDNA probe and autoradiographed as previously described for the Northern analysis.

RESULTS

Cytological Characterization of Wall Coating Induced by V. albo-atrum and ABA

The coating response induced in tomatoes by V. albo-atrum was investigated histochemically (LM) with stains known to react with suberin (Fig. 1, a–f). All tests were positive (Table I). With TBO, sudan black B, oil red O, and ammoniacal gentian violet, the color reactions were the same in the resistant and susceptible plants. However, sudan III, IV and 12 N KOH stained the two isolines differently. With the latter stain, coating in the susceptible plants was orange (Fig. 1e), whereas that in the resistant plants tended toward chocolate brown (Fig. 1d, f). In the water-infused controls, staining by sudan III, IV and 12 N KOH caused a pale orange reaction, but the color was diffuse in vascular bundles (Fig. 1g), and because a similar effect was not present with other suberin stains, it was assumed to be nonspecific. Intense, localized reactions, as seen in the intercellular spaces and pit membranes around conidial trapping sites (Fig. 1, a–f), were observed with all suberin stains but were not observed in controls. These localized depositions corresponded to the “coating” as defined by EM (25).

If the fungus-induced coating was suberin, we reasoned that ABA, which is known to induce suberization (4, 24), might also induce the formation of a similar coating. To test this possibility, ABA was infused into the vascular system of tomato petioles, and the results were monitored by observation of stained fresh freehand sections (Fig. 1h) or thick (LM) and thin (TEM, Fig. 2a and b) plastic sections. ABA at 10^{-6} M elicited a coating response (Table I, Figs. 1h, 2a and b) identical to that previously observed in Verticillium-infected tomatoes (25). The response occurred in both isolines. Although no attempt was made to quantify amounts of coating in ABA-treated tissues, the reaction appeared to be further advanced in resistant than in susceptible plants at 72 h. Infusion of 10^{-6} M ABA produced a minimal response, and 10^{-4} M ABA produced none.

Chemical Composition of the Coating Material

Because the coating material cannot be isolated separately from the cell walls, we had to use entire petioles for chemical analyses. Because the major aliphatic components of suberin are ω-hydroxy C_{18} and ω-hydroxy C_{16} acids and their corresponding dicarboxylic acids (11), reductive cleavage of suberin should yield the corresponding α,ω-diols. However, such diols can also be generated from components of the cuticle. Therefore, we carefully and completely removed, by physical means, the cuticle-containing outer layer of cells from the petiole, and we thoroughly extracted the remaining tissue to remove soluble lipids. Upon exhaustive LiAlH₄ treatment, the final insoluble material yielded an alkane diol fraction that could be detected on the thin-layer chromatograms (data not shown). Combined capillary GLC-MS was used to analyze the depolymerization products.

When intact petioles, with the epidermis attached, were subjected to capillary GLC-MS analysis, C_{18} triol was found to be the major product (data not shown), indicating that dihydroxy C_{16} acid is the major component of the cutin of tomato petiole. This dihydroxy acid was composed of about 40% 9,16-dihydroxy isomer and 60% 10,16-dihydroxy isomer. In addition, smaller amounts of 18-hydroxy-9,10-epoxy C_{16} acids and 9,10,18-trihydroxy C_{16} acids were identified by mass spectrometry as described earlier (26). On the other hand, when the epidermal layer from the petiole was removed prior to analysis, none of these acids were detected. Because C_{16} triol, C_{18} triol, and C_{18} tetraol are easily detected by GLC-MS, it is clear that the cutin-containing layer was completely removed by the peeling process used. Alkane diols were detected in the depolymerization products of the internal tissue that contained the vascular coating material. Repeated experiments with selected ion monitoring indicated that C_{18} diol was the most consistently observed component, although C_{16} diol was also found. Therefore, we used C_{18} diol as a measure of suberin.

Infusion of petioles with V. albo-atrum conidia or ABA caused an increase in the C_{18} diol that was released by reductive cleavage of suberin (Fig. 3). Because the total ion peak area was used as the measure of the diol, the best way to compare the relative peak areas was to use the -fold increase over untreated controls. Most often, C_{18} peaks were very small, except in the case of the resistant line infused with...
conidia or ABA (Fig. 3). The treated susceptible line showed a lesser increase in the aliphatic components (Fig. 3).

**Northern Blot Analysis**

To test whether the induction of suberization in tomato is also reflected in the gene transcript level of the suberization-associated peroxidase, Northern blot analysis was done. Total RNA, extracted from tomato petioles infected with conidia of *V. albo-astrum* for time intervals varying from 0 to 72 h.p.i., was subjected to Northern blot analysis with a 32P-labeled potato peroxidase cDNA probe. Fungal-induced peroxidase transcript was detectable by 12 h.p.i. in resistant plants and by 20 h.p.i. in susceptible ones (Fig. 4). Peroxidase transcript levels continued to increase throughout the 72-h period (Fig. 5). Quantification of the amounts of anionic peroxidase mRNA in comparison with levels of actin mRNA showed that higher levels of peroxidase transcript were found consistently in the resistant plants during all of the intervals studied. Induction was not observed in untreated petioles nor in the water controls even after 72 h of treatment (Fig. 5).

To test whether ABA can induce the expression of the anionic peroxidase in the vascular tissue, total RNA isolated 72 h after infusion of ABA was subjected to Northern blot analysis. Infusion with ABA instead of the fungal conidia also caused substantial accumulation of the peroxidase mRNA (Fig. 5). Again, the level of transcript was consistently higher in resistant GCR 218 plants than in susceptible GCR 26. Northern blot analysis further showed that the fungal- and ABA-induced peroxidase transcript was identical in size (about 1.6 kilobase) to the anionic peroxidase transcript that accumulates in ABA-treated tomato callus (22) and in suberized potato tuber slices (23).

**Tissue Blot Analysis**

Tissue blot analysis (Fig. 6a) confirmed the induction of anionic peroxidase mRNA in tomatoes infused with *V. albo-astrum* or ABA and the absence of induction in water controls (Fig. 6b). Further comparison of the autoradiographs with the photographed blots (Fig. 6c) showed that the induction of the transcript was confined to the vascular tissues. Light microscopic observation of a sudan III, IV-stained section of stem above the blotted cut surface from the fungus-infused resistant plant showed the orange suberin response in the corresponding xylem (Fig. 6d).

**Table I. Histochemical Reactions of Vascular Coating in ABA- and Verticillium-Induced Tomatoes**

<table>
<thead>
<tr>
<th>Tissue Preparation</th>
<th>Stain</th>
<th>GCR</th>
<th>GCS</th>
<th>Expected Reaction of Suberin</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed, plastic</td>
<td>TBO</td>
<td>Turquoise a</td>
<td>Turquoise a</td>
<td>Dark blue b</td>
<td>19</td>
</tr>
<tr>
<td>Fresh material</td>
<td>Sudan III, IV</td>
<td>Dark blue b</td>
<td>Dark blue b</td>
<td>Dark blue</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Sudan black B</td>
<td>Red brown</td>
<td>Orange</td>
<td>Orange-red</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>12 N KOH</td>
<td>Black</td>
<td>Black</td>
<td>Black</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Oil red O</td>
<td>Dark orange</td>
<td>Pale orange</td>
<td>Dark orange</td>
<td>6, 16</td>
</tr>
<tr>
<td></td>
<td>Gentian violet</td>
<td>Cherry red</td>
<td>Cherry red</td>
<td>Red</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Purple</td>
<td>Purple</td>
<td>Violet</td>
<td>6, 16</td>
</tr>
</tbody>
</table>

a Type A. b Type B.

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**Figure 2.** TEM of coated vessel in an ABA-induced tomato plant. Tomato petiole from a resistant plant was infused with 10⁻⁶ M ABA. Samples were prepared for EM as described in "Materials and Methods." a. Low magnification of vessel cross-section showing coating (C) on secondary vessel walls and pit membranes. Note the vacuolar fragmentation (V) in the neighboring xylem parenchyma cell. (Magnification about ×8600.) b. Enlargement of bracketed area in panel a showing lamellar coating of the full border pit. (Magnification about ×9750.)
CHEMICAL CHARACTERIZATION OF VASCULAR COATING

Figure 3. Effect of infusion of V. albo-atrum conidia and ABA on the level of vascular suberin in resistant and susceptible tomato petioles. Results of one experiment in which tomato petioles from resistant (Res) and susceptible (Sus) plants were challenged by V. albo-atrum at a concentration of $9 \times 10^9$ conidia/mL or $10^{-6}$ M ABA for 72 h. Suberin in the petioles was analyzed as described in "Materials and Methods." The amount of octadecene-1,18-diol was used as a measure of suberin and the fold increases in resistant and susceptible petioles were calculated on the basis of the amount of the diol generated from petioles infused with water for 72 h.

Figure 4. Effect of infusion of V. albo-atrum conidia on the time course of induction anionic peroxidase mRNA in susceptible and resistant tomato petioles. Results of one experiment in which tomato petioles from susceptible (a) and resistant (b) plants were challenged by V. albo-atrum at a concentration of $9 \times 10^9$ conidia/mL. Total RNA was probed with $^{32}$P-pAP$_3$ at 12, 15, 20, 25, and 36 h after infusion.

Figure 5. Effect of infusion of V. albo-atrum conidia and ABA on the level of anionic peroxidase mRNA in susceptible and resistant tomato petioles. Results of one experiment in which tomato petioles from susceptible (S) and resistant (R) plants were challenged by V. albo-atrum (Vaa) at a concentration of 9 to $10^9$ conidia/mL or $10^{-6}$ M ABA for 72 h. Control petioles (con) were removed from the plant and mRNA was extracted at 0 h. In a second set of controls, petioles were infused with water (H$_2$O) for 72 h. Total RNA was probed with $^{32}$P-pAP$_3$.

DISCUSSION

Normally, Verticillium invades the roots of plants. It colonizes the xylem vessels and subsequently spreads systemically into the stem and leaves. Unfortunately, natural infection is not suitable for many studies of the early host-parasite interactions in wilt disorders. The time of entrance of the fungus into the stele cannot be monitored, and initial sites of host response are few and difficult to localize. This combination of factors renders the natural system intractable to experiments that require chronological, quantitative, and chemical analyses. The method of inoculation used in this paper has been utilized for the study of fungal wilt interactions for many years (1, 15, 25), and the process of disease development is known to mimic natural infection (13, 14). In the present study, the conidial concentration was adjusted to provide the maximum number of trapping sites (i.e. the highest proportion of responding cells) without breaking down the normal host resistance (our unpublished data). All petioles were monitored histochemically before use to ensure that this was the case.

At present, no convenient method can separate the vascular cylinder from surrounding plant tissues. Use of whole petioles, therefore, is requisite for biochemical analysis. Because the xylem tissues represent a small proportion of the total mass, it is important to maximize the response in this way. When spores are infused into a petiole, the number of trapping sites per petiole cross-section is highest at the point of infusion and decreases toward the top, although some trapping sites occur in virtually all sections. If the spore concentration and conditions of infusion are closely monitored, the percentage of responding cells at any distance above the cut base remains constant (13). Hence, in the present experiments, care was taken to ensure that where quantitative comparisons were
Figure 6. Tissue blots of *V. albo-atrum* and ABA-induced resistant tomato petioles probed for anionic peroxidase message. Two tomato petioles from resistant plants were challenged by *V. albo-atrum* conidia at a concentration of $9 \times 10^6$ conidia/mL (I) or $10^{-6}$ M ABA (A) for 72 h. Two control petioles (C) were infused with water. a, Low magnification photograph of the set of six tissue blots (i.e. two each of I, C, and A) visualized by autoradiography in panel b. Star indicates the cross-section shown at higher magnification in panel c. b, Autoradiograph of six tissue blots showing that pAP$_3$ mRNA was strongly induced in some of the vascular bundles (e.g. arrowheads) in the *V. albo-atrum* or ABA-treated petioles (magnification about $\times 1$). c, Photograph of tissue blot of one (star) of the *V. albo-atrum*-infused petioles, prior to probing with $^{32}$P pAP$_3$, showing the vascular bundles that were indicated by arrowheads in the corresponding positions in panel a. d, Freehand section, taken from same petiole illustrated in 6b, stained with sudan III, IV to reveal the orange-brown suberin reaction in vascular bundles. (Arrowheads show corresponding positions.)
required, samples were taken of a specific length beginning at a set height above the initial cut.

The possible induction of wound responses must be considered in any excised system. This is particularly important when investigating stress or host-parasite interactions in which the processes being investigated (i.e. suberization, coating formation) can be induced by wounding itself. Previous studies with the tomato model system indicated that no wound responses were detectable cytologically more than 1 mm above the cut base (20). In the present study, the sampling technique was designed to eliminate potential artifacts resulting from wounding. That this goal was achieved is confirmed by the absence of induction of anionic peroxidase transcript, suberin deposition, or coating formation in water-infused controls. The occurrence of these phenomena in ABA- or conidia-infused petioles, therefore, can be attributed entirely to the presence of the plant hormone or the pathogen.

All of the experimental evidence indicates that the vascular coating is the cytological manifestation of the process of suberization. First, ABA, which induces coating formation, is known to induce suberization (4, 24). The concentration of ABA required to stimulate the coating response was $10^{-6} \text{ M}$, whereas previous work had indicated that a concentration of $10^{-4}$ m was necessary to induce suberization in potato callus cultures (4). The latter experiments involved a tissue mass resting on agar containing ABA, and thus, the actual concentration of the hormone in the tissue was probably much lower. On the other hand, infusion of ABA into the vascular tissue provides a very effective way to deliver ABA to the hormone-responsive cells and, therefore, a lower concentration may be effective.

Second, the material secreted by responding xylem parenchyma cells reacts positively with stains that are thought to indicate suberin. Chemical analysis of induced petiolar samples confirmed the presence of aliphatic components characteristic of suberin. Because the cuticle of the petioles was completely removed prior to the chemical analysis, the alkane-α,ω-diols could not have originated from the cuticle. This conclusion was corroborated by analysis of the depolymerization products derived from the cuticle of the petiole. The dominant component of the cuticular polymer was dihydroxy C$_{16}$ acid, with smaller amounts of 18-hydroxy,9,10-epoxy C$_{18}$ acid and 9,10,18-trihydroxy C$_{18}$ acid. The depolymerization products from the vascular tissue-containing material did not include these polyhydroxy acids that would have been readily detected by the α-cleavage ions resulting from the mid-chain hydroxyl group(s) (26). Because the alkane-α,ω-diols did not originate from the cuticle, they most likely were generated by the reduction of the ω-hydroxy fatty acids and dicarboxylic acids of suberin from the vascular coating material. Confirming this conclusion, the diols were obtained in significant quantities only from the tissue samples that cytologically showed vascular coating to be present.

Third, according to the current working hypothesis concerning the structure of suberin (11), the vascular coating would be expected to have a phenolic domain even though the nature of the aromatic components of this suberin remains unclear. Thus, the vascular coating process should require the expression of the gene for the peroxidase involved in the polymerization of the aromatic components of suberin. Northern blot analysis confirmed that the formation of vascular coating, due to infusion with *Verticillium* spores, also was associated with an increase in the levels of mRNA for the anionic peroxidase. The time-course of accumulation of the mRNA correlated with the previously established time-course for the quantitative deposition of coating (20). Further, tissue blot analysis showed that the elevated levels of peroxidase transcript were localized to the vascular bundles in which suberin was histochemically detectable. ABA treatment had similar effects.

Biochemical quantification of suberin and Northern blot analysis of the anionic peroxidase transcript indicated that the levels of both were higher in the resistant plants challenged by *Verticillium* than in the susceptible plants at 72 h.p.i. These observations support the suggestion that suberization is important to host defense (10). In previous studies, the relative increases in coating material in infected resistant and susceptible tomatoes have been documented thoroughly by three different methods (20, 25), and further quantitative analysis of the same pathosystem has provided convincing evidence that coating formation does play a major role in host resistance (21). Host colonization by *Verticillium* is discontinuous. Hyphae enter the root xylem and sporulate. The conidia are released and carried upwards in the transpiration stream until they are stopped by vessel endings (i.e. trapping sites). Before colonization can continue, the conidia must germinate to produce hyphae that grow through xylem walls (i.e. laterally or vertically) into adjacent vessel members. Conidia are again produced to begin a new cycle. The process continues until the pathogen enters the leaves. If the plant is susceptible, disease symptoms will develop and death may ensue (17). Earlier suberization (i.e. coating formation) in resistant plants prevents the escape of *Verticillium* from trapping sites (21), thereby curtailing host colonization.

Suberized walls can provide an impermeable physical barrier, because they are, apparently, not degraded by the extracellular enzymes that pathogens use to invade through normal wall barriers. Therefore, the pathogens are confined. Such physical confinement of pathogens could be a significant defense reaction in other pathosystems.

The fact that more suberin is apparently elicited in resistant or susceptible plants by ABA than by *Verticillium* (Fig. 3) is probably, in large measure, an artifact resulting from the method of treatment. When conidia are infused into the xylem, defense responses are stimulated only in a very localized fashion around actual trapping sites. On the other hand, ABA is delivered directly to all cells of the xylem, resulting in a massive production of suberin.

The fact that more suberin is elicited in resistant than in susceptible isolines by either ABA or *Verticillium* (Fig. 3) is more difficult to explain. The near-isolines simply may have a different capacity to respond regardless of the inducer. In fact, other published evidence suggests that this is the case in cv Craigella (2, 20). Recent studies in a different host-parasite system also indicate that ABA may be a mediator involved in the natural induction of host defense mechanisms (8).

The present study provides chemical evidence that vascular coatings are suberin and supports the hypothesis that suberization plays a role in plant defense. As such, these conclusions provide the justification for further molecular studies of the
structure, expression, and regulation of genes in the suberin biosynthetic pathway, particularly regarding their role in the *Verticillium*-tomato interaction.

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**LITERATURE CITED**

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