Structure and Composition of Normal Skin (Periderm) and Wound Tissue from Cured Sweet Potatoes

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Abstract. The initial response of the sweet potato [Ipomoea batatas (L.) Lam] to wounding involves deposition of a polymeric material on the inside of several layers of parenchyma cells at the site where the root’s protective periderm has been removed. Analysis of normal periderm and wound tissue indicate that the wound tissue is similar to the normal periderm with respect to aromatic components, but contains only 14% as much suberin.

Sweet potatoes are held under curing conditions for 4–7 days after harvest for optimum storage stability (21). The primary purpose of the curing environment is to heal rapidly wounds inflicted during harvest. An unhealed wound provides an entrance for microorganisms which cause root loss through decay. Experiments have shown that recommended curing conditions expeditiously heal wounds and that healed wounds are resistant to infection (13, 15).

The chronology of the wound-healing process in sweet potatoes is well-documented (1, 15, 19). Microscopic examination of wounded tissue has shown that the first observable change is the desiccation of several layers of the outermost parenchyma cells exposed to the air after wounding, followed by a progressive “suberization” of the parenchyma cells beneath the desiccated cells. Suberization, the initial observable morphological response to wounding in sweet potato, provides a barrier to further moisture loss and impedes microbial invasion. The final stage in the wound-healing process is the formation of a wound periderm beneath the “suberized” parenchyma cells. The wound is healed when the wound periderm has attained a thickness of 3 to 7 layers of cells (20).

The composition of the “suberized” parenchyma cells above the wound periderm is unknown. McClure (14) observed in 1960 that the so-called “suberized” parenchyma cells contained 10 times as much lignin as normal parenchyma cells and had a much stronger affinity for an acidic solution of phloroglucinol than for lipid stains such as Sudan IV. Since suberin has been shown to have aliphatic polyester and aromatic regions (8), it was surprising that the so-called “suberized” parenchyma cells did not strongly absorb lipid stains. A recently published study (20) confirmed the observation that acidic phloroglucinol (PG) reacts with fully “suberized” parenchyma cells to give a deep red-purple color. This study used the color intensity of wound tissue after treatment with PG to predict when wound periderm formation had begun. Their data showed that when the root had “suberized” enough layers of cells to give a red-purple color with PG, wound periderm formation was sufficiently far advanced that wound healing would continue to completion even if the roots were placed in a 13° to 18°C chamber. On this basis, they used the color intensity to determine when to end curing.

The PG reaction is one of the most widely used tests for lignin (7). It appears that the sweet potato’s first response to wounding is to deposit a lignin-like polymer with a high degree of aromatic aldehyde character in the several layers of cells beneath the outermost desiccated cells prior to formation of the wound periderm beneath the PG-positive cells. Lignification has been reported to occur when certain wounded plant tissues are infected by microorganisms (5) and in response to wounding of cucumber fruit and various Brassica species (17).
Kolattukudy and coworkers (8, 9) have extensively investigated the composition of suberin from numerous plant sources and have shown that suberin can be quantitated by reductive depolymerization with lithium aluminum hydride, followed by measurement of the amount of 1,18-octadecadiene diol (ODD) formed. Kolattukudy and Dean (10) have shown that suberization occurred in white potato tubers in response to wounding and that the suberin has a very similar composition to that of the natural periderm (skin). Kolattukudy et al. (12) reported the suberin composition of the normal periderm of sweet potatoes and other roots and tubers of economic importance. Our study (20) and that of McClure (14) indicated that the initial response to wounding in sweet potatoes might be lignification rather than suberization of parenchyma cells at the site where the root’s protective periderm was removed. The purpose of this research was to compare the suberin and lignin content of both normal periderm (skin) and healed wounds from sweet potatoes and to examine the microstructure of sweet potato wound tissue.

Materials and Methods

Culture. This study was conducted using ‘Jewel’ sweet potatoes harvested on 4 dates from 2 locations near Raleigh, N.C. The roots were grown in Norfolk sandy loam soil and were harvested from 100 to 140 days after transplanting. At each harvest date, 50 kg of U.S. no. 1 roots were wounded with a spoon or razor blade by scraping each root along the entire length. The wounds (2 per root) were 5- to 7-mm-wide, and 0.5- to 2-mm-deep. After wounding, the roots were placed in an environmental chamber and held at 30°C and 84-88% relative humidity for 7 days. The wound tissue was excised from each root and collected for purification. Normal skin (periderm) was removed from a 50-kg batch of roots by scraping the surface with a spoon.

Tissue purification. The purification described below removed adhering normal parenchyma tissue and noncovalently bonded monomers and waxes. Each batch of tissue (about 2–5 g skin or wound tissue) was heated for 30 min in 500 ml of a solution containing 2 g oxalic acid and 8 g ammonium oxalate. After washing with water, the residue was partially dehydrated with 100 ml ethanol and extracted (soxhlet) for 48 hr with 200 ml CHCl₃ and then for 24 hr with 200 ml methanol. The dried, extracted residue was shaken for 24 hr at room temperature in 500 ml of 0.05 M acetate buffer (pH 4.0) containing 5 mg cellulase (Aspergillus niger) and 0.5 ml pectinase (A. niger) (12). The tissue was then washed with water for 24 hr in 500 ml 0.05 M acetate buffer (pH 4.0) containing 25 mg of α-amylase (Barb孑llus subtilis) and 5 mg amyloglucosidase (Rhizopus spp.). The tissue was washed with 500 ml water and dehydrated with 200 ml ethanol. The soxhlet extraction (ethanol and methanol) and enzyme digestion (pectinase-cellulase) was repeated. The residue was washed thoroughly with water, followed by 95% ethanol. After air-drying, the residue was ground to ≤60 mesh particles. The powder was dried overnight in a vacuum oven at 115°C and 50°C and stored in a desiccator at room temperature until analysis.

Microscopy. Tissue blocks (2 × 1.5 × 0.75 cm thick) of normal skin and wound tissue were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer for ≥1 week. The fixed tissue was embedded in paraffin and sectioned at 15 μm on a rotary microtome (20). The sections were deparaffinized in a xylene-ethanol series and rehydrated in water prior to staining. Some sections were stained with acidic phloroglucinol and immediately examined with a light microscope. The number of layers of red-stained cells was enumerated and recorded. Other deparaffinized sections were stained with a saturated solution of Sudan IV in 95% ethanol applied directly to the section. Cover slips were placed on the sections and sealed with vaspar. After at least 24 hr, the sections were examined under a light microscope and the number of layers of red-stained cells was counted and tabulated.

Alkaline cupric oxide oxidation. Powdered, purified samples of skin and wound tissue (0.125 g) were mixed with 1.7 g powdered CuSO₄·5H₂O and 10 ml 3n NaOH in a stainless steel tube, sealed in a bomb, and heated at 180°C for 2.5 hr. The bomb was cooled and the tube was removed and centrifuged at 10^3 x g for 10 min. The supernatant was removed and stored under nitrogen and the pellet was suspended in water and centrifuged twice. All supernatants were combined and adjusted to a pH of 2.5 with HCl and rock salt added to saturation. One ml of a solution of 7-hydroxy coumarin (1 mg/ml) was added as internal standard and the mixture extracted twice with 40-ml portions of ethyl ether. The ether was dried and evaporated. The residue was derivatized overnight with acetic anhydride-pyridine (0.2–0.4 ml). Excess reagent was evaporated with a stream of nitrogen gas and the residue taken up with methanol to a volume of 0.4 ml for gas-liquid chromatography (GLC) analysis.

Reductive depolymerization. Powdered, purified samples of skin and wound tissue (0.125 g) were refluxed in 30 ml of tetrahydrofuran containing 0.2 g LiAlH₄ for 24 hr. One ml of 1,16-hexadecane diol (3 mg/ml) was added as internal standard to the cooled reaction mixture. The reaction mixture was stirred and 0.2 ml water, 0.2 ml of 15% (w/v) NaOH, and 0.6 ml water were added successively. This reaction workup resulted in a dry, granular precipitate (4). The precipitate was removed by filtration and extracted twice with 5 ml of ether. Twenty ml of water was added to the liquid phase, followed by extraction with 20-ml portions of ethyl ether. The ether layers were combined, dried, and evaporated. The residue was taken up in ether–methanol (1-1) and purified by thin-layer chromatography (TLC) prior to mass spectroscopy and quantitation by GLC (10).

Gas-liquid chromatograph. The retention times of the acetylated reaction products from the alkaline cupric oxide oxidation were measured on several columns. Samples of compounds expected from lignin degradation (6), p-hydroxy benzoaldehyde (PHB), p-hydroxyacetophenone (PHA), vanillin (VA), aceto-vanillone (AV), syringaldehyde (SA), and acetylsyringone (AS) were purchased from Aldrich Chemical Co. and derivatized with acetic anhydride-pyridine (2:1) prior to the GLC. Individual compounds were used to determine retention times. These retention times were compared to the retention times of unknown compounds obtained from the alkaline oxidation. A mixture of the standards was co-chromatographed with the unknown and the areas for individual compounds compared to the areas of the same compounds chromatographed without addition of the standard mixture. The retention times of samples and standards were identical in all cases. The GLC systems were as follows: A) 3 m × 3 mm i.d. column packed with OV-101 (3%) on chromosorb G (100/200 mesh). The temperature program began at 140°C and increased at 5°C/min until 225°C was reached. This temperature was held for 5 min. The carrier gas (N₂) flow rate was 35 ml/min; B) Same as A except 3% OV-17 was the liquid phase and the program started at 200°C (hold 3 min), followed by 5°C/min increase until a final temperature of 240°C. The instrument used in both A and B was a Packard model 803 system; and C) 30 m × 0.319 i.d. fused, silica column capillary-coated (1 μm film thickness) with DB-5. The temperature program was the same as with A. The carrier gas (H₂) inlet pressure was 0.77 kg/cm². A 5-liter sample split was used. The instrument was a
Varian model 3700. All 3 systems employed flame ionization detection.

The individual components then were quantitated by determination of their response factors relative to the internal standard (7-hydroxy coumarin). The amount of each component was computed. A Hewlett-Packard model 3390A integrator operating in the internal standard mode was used to measure the peak areas and perform the computations.

Since a standard of 1,18-octadecenol diol was unavailable, identification of the principal product from the LiAlH₄ reduction was performed on a GLC-mass spectrometer. The reaction mixture from the reduction was purified on the TLC system described by Kolattukudy and Agrawal (10). The isolated diol fraction was derivatized with bis-N,O-trimethyl silyl acetamide (BSA) and injected into a Hewlett-Packard model 5985B GC/MS. The column used was a 30 m × 0.32 mm i.d. capillary column coated with DB-5 (thick film). The carrier gas (He) inlet pressure was 0.77 kg/cm². The initial column temperature was 100°C (2 min hold), then programmed at 10°C/min to 175°C. At 175°C, the rate was decreased to 5°C/min until a final temperature of 240°C was attained. The mass spectrum was obtained at 70 eV ionizing voltage. Quantitation of TLC-purified, 1,18-dihydroxy octadecene was performed with system A. The internal standard procedure was used with 1,16-hexadecane diol as the added internal standard.

Results and Discussion

Microscopy. Microscopic examination of the wounds showed that significant healing (1, 15, 19) occurred during the 7-day curing treatment (Fig. 1). There were several layers of desiccated cells, followed by 4–5 layers of PG-positive cells, followed by several layers of rectangular, stacked cells which make up the wound periderm. Differential staining with the PG reagent and Sudan IV provided evidence as to the composition of PG-positive cells and wound periderm. The PG-positive cells referred to in past literature as the “suberized” cells are very weakly stained by Sudan IV and react strongly with PG reagent to give a red-purple color. The wound periderm is only light colored with PG reagent and strongly stained with Sudan IV. These histochemical tests indicate that the PG-positive cells have deposited polymeric material which has a large amount of aromatic aldehyde character (7) and very little lipid character. On the other hand, the wound periderm, which begins to form when the deposition of PG-positive material in the cells above has almost ceased, has a large amount of lipid character and very little aromatic aldehyde character. When normal periderm (skin) sections were treated with the same reagents, the results were very similar to those with the wound periderm. That is, the cell walls had a large degree of lipid character and smaller degree of aromatic aldehyde character.

Identification. Alkaline cupric oxide oxidation is a classical lignin degradation method (6) and provides information concerning the composition of the lignin. Cottle and Kolattukudy (3) recently have applied a similar alkaline nitrobenzene degradation procedure to study the phenolic regions of white potato suberin. The reaction products from either degradation are identical (6).

A typical chromatogram is shown in Fig. 2. There are numerous peaks but the 6 standards were located with 3 columns described in the preceding section. The retention times relative (RRT) to vanillin on OV-17 are greater than are the RRT values on both OV-101 and DB-5 capillary columns (Table 1). Because all 6 standards were located in the reaction mixture on each of the 3 columns with sufficiently different RRT values and resolution (capillary column) and because the reaction products from this type oxidation have been investigated so thoroughly (6), no further attempts were made to prove the structures. Examination of Table 1 shows that with the exception of PHA on OV-17 and VA on OV-17, the relative area percentages are quite similar. These differences indicate that PHA on OV-17 and VA on OV-101 and the capillary may have extraneous compounds co-eluting.

Reductive cleavage of potato (Solanum tuberosum) skin with LiAlH₄ has been shown to yield 1,18-octadecene diol as the major product (10). This compound is formed from C-18-ene type compounds such as the ω-hydroxy carboxylic esters and the ω-dicarboxylic esters. The aliphatic portion of suberin consists mainly of this type of compound (8). Analysis of sweet potato skin has shown that C-18-ene compounds of the type listed above predominate in the aliphatic portion (12). LiAlH₄ reduction of sweet potato skin and GLC of the diol band from TLC purification gave a chromatogram with one major peak. When this peak was examined via GLC-MS, we found it to be identical to that previously reported for 1,18-octadecene diol Me₃ silyl diether (10). There was a molecular ion at m/e 428 and fragments at 413 (M-15), 397 (M-31), 338 (M-90), 323 (M-15-90), and 199 (Fig. 3). A peak with the same RRT was found when wound tissue was reduced (Fig. 4).

Chemical analyses. Newly formed wound periderm cells are fragile and are the weakest point of attachment to the healthy parenchyma cells below. Consequently, when the wound tissue was excised prior to purification, separation occurred at the wound parenchyma (20). Thus, the purified wound tissue which we chemically degraded was comprised mainly of those cells in which the PG-positive polymer was formed, but with a small percentage of wound periderm cells. Alkaline cupric oxide oxidation of skin and wound tissue released varying amounts of aldehydes and ketones derived from lignin or lignin-like aromatic polymers. PHB and PHA are derived from a p-hydroxy-phenyl type residues. VA and AV are derived from guaiacyl residues, and SA and AS are derived from syringyl residues. Both skin and wound tissues contained large amounts of guaiacyl residues, moderate amounts of p-hydroxy-phenyl residues and small amounts of syringyl residues (Table 2, Fig. 2).
Table 1. Gas chromatographic analysis of the reaction mixture from alkaline cupric oxide oxidation of sweet potato periderm.

<table>
<thead>
<tr>
<th>Compound</th>
<th>OV-17</th>
<th></th>
<th>OV-101</th>
<th></th>
<th>DB-5 coated capillary</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRT</td>
<td>Area (%)</td>
<td>RRT</td>
<td>Area (%)</td>
<td>RRT</td>
<td>Area (%)</td>
</tr>
<tr>
<td>p-Hydroxybenzaldehyde (PHB)</td>
<td>0.53</td>
<td>13.8</td>
<td>0.67</td>
<td>15.6</td>
<td>0.72</td>
<td>13.0</td>
</tr>
<tr>
<td>p-Hydroxyacetophenone (PHA)</td>
<td>0.72</td>
<td>15.3</td>
<td>0.88</td>
<td>4.1</td>
<td>0.89</td>
<td>3.1</td>
</tr>
<tr>
<td>Vanillin (VA)</td>
<td>1.00</td>
<td>35.9</td>
<td>1.00</td>
<td>49.2</td>
<td>1.00</td>
<td>54.1</td>
</tr>
<tr>
<td>Acetovanillone (AV)</td>
<td>1.34</td>
<td>26.4</td>
<td>1.21</td>
<td>21.3</td>
<td>1.16</td>
<td>20.9</td>
</tr>
<tr>
<td>Syringaldehyde (SA)</td>
<td>1.84</td>
<td>6.5</td>
<td>1.39</td>
<td>5.8</td>
<td>1.32</td>
<td>5.9</td>
</tr>
<tr>
<td>Acetosyringone (AS)</td>
<td>2.19</td>
<td>2.1</td>
<td>1.57</td>
<td>4.1</td>
<td>1.49</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*aRetention time relative to that of vanillin.

Fig. 2. Gas-liquid chromatogram (GLC) of products from alkaline cupric oxide oxidation of normal periderm (periderm) and wound tissue (wound). PHB = p-hydroxybenzaldehyde; PHA = p-hydroxyacetophenone; VA = vanillin; AV = acetovanillone; SA = syringaldehyde; AS = acetosyringone; IS = internal standard (7 hydroxy coumarin). All components were transformed into acetate esters prior to GLC.

Fig. 3. Mass spectrum of compound (as trimethyl silyl ether) released by LiAlH₄ hydrolysis of normal periderm and wound tissue.

Asada and Matsumoto (2) reported a similar distribution of degradation products from mildew-infected Japanese radish roots. These workers did not find syringyl residues. Rhodes and Wooltorton (18) found in "lignified" swede root disks that PHB was present in greater quantity than was VA. No SA was found. In both cases, lignified tissue gave a strong positive test with PG reagent. Cottle and Kolattukudy (3) found more VA than PHB and traces of SA in white potato wound periderm.

Our results are in general agreement with the above workers in that polymers containing p-hydroxyphenyl and guaiacyl residues were released by alkaline hydrolysis from cells adjacent to tissue injured either by cutting or by microbial attack. However, we did find that small amounts of syringyl residues were present in sweet potato. Both sweet potato periderm and wound tissue had very similar amounts of the 2 major residues, but wound tissue contained only ½ as many syringyl residues, indicating that the aromatic portion of the polymeric material formed in response to injury is not identical to that of the naturally

Table 2. Quantitation of components from alkaline cupric oxide oxidation of 'Jewel' sweet potato skin and wound tissue.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Periderm (skin)</th>
<th>Periderm (wound)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Hydroxybenzaldehyde</td>
<td>1.95</td>
<td>1.99</td>
</tr>
<tr>
<td>p-Hydroxyacetophenone</td>
<td>0.39</td>
<td>0.25</td>
</tr>
<tr>
<td>Vanillin</td>
<td>5.03</td>
<td>5.01</td>
</tr>
<tr>
<td>Acetovanillone</td>
<td>1.55</td>
<td>1.28</td>
</tr>
<tr>
<td>Syringaldehyde</td>
<td>0.68</td>
<td>0.09</td>
</tr>
<tr>
<td>Acetosyringone</td>
<td>0.29</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*Skin and wound samples collected from 50 kg of 'Jewel' sweet potatoes. Each value is the mean of duplicate analyses from 4 harvest dates.

Amount present in skin and wound tissue different at $P \leq 5\%$.

Amount present in skin and wound tissue different at $P \leq 0.1\%$. 

observed when normal periderm was treated with PG resulted because the lipid portion of the polymer present in the walls of normal periderm impeded penetration of the reagent into aromatic aldehyde regions of the polymer. Reeve et al. (16) emphasized that histochemical and analytical results may be quite different because the intensity of a histochemical color reaction is dependent upon cellular structure.

Products from the reductive hydrolysis of the 2 tissue types gave a different pattern. The amount of 1,18-octadecene diol released from periderm was about 7 times greater than the amount released from wound tissue (Table 3, Fig. 4). Since the amount of this compound released by LiAlH₄ has been used to estimate the amount of suberin present (11), it appears that sweet potato wound tissue is much less suberized than is the normal periderm.

**Summary and Conclusions**

Wound healing in sweet potatoes involves the deposition of a polymeric material in the parenchyma cells at the site where the root’s protective periderm has been removed. The polymeric material has more aromatic aldehyde character than lipid character as indicated by a strong positive PG test, a weak Sudan IV test, and degradation products indicative of a lignin-like polymer with very little suberin character. The wound tissue and normal periderm (suberin) have similar aromatic sections but very dissimilar amounts of suberin. A wound periderm forms in sweet potatoes after deposition of the lignin-like polymer has slowed which, on a histochemical basis, is identical to normal periderm.

**Literature Cited**