

# Wound Healing in Cucumber Fruit

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**Abstract.** Wound healing in cucumber fruit (*Cucumis sativus* L., cv. Calypso) was studied using histological and degradative techniques. A thick exudate appeared at the wounded surface shortly after wounding. This material retarded water loss and possibly aided in the formation of sclerified parenchyma observed 24 hours after wounding. The sclerified material was positive to a modified Weisner stain, indicating lignification was occurring. Wound periderm (cork) was initiated directly beneath the sclerified parenchyma cells within 48 hours after wounding. The cork layers were positive to Sudan IV stain, indicating suberin was being formed. The rate of phellem development decreased by 6 days after wounding. By day 7, younger phellem cells and sclerified parenchyma cells were stained by Sudan IV. Degradation of the wound tissue by chemical procedures demonstrated that relatively large amounts of lignin and suberin were deposited during healing. Fragments from the lignin degradation indicated that lignin was composed mainly of guaiacyl and *p*-hydroxyphenyl residues. Suberin was found to contain mainly 1,16-hexadecane and 1,18-octadecene decarboxylic acids detected as the silylated diol derivatives.

Wound healing by plants has significant theoretical and practical impact. Successful postharvest storage of plant organs depends on the ability of the plant material to resist invasion by pathogenic microorganisms. Commercially important plant organs generally are wounded during harvest and handling before storage. The frequency and severity of wounding varies with the nature of the harvested material, the environmental conditions during harvest, and the implements used for harvest and transportation. Since wounds can serve as avenues for microbial attack and moisture loss, rapid wound healing is required if organ decay and/or desiccation are to be avoided.

Wound responses differ with regard to plant species and organ (El Hadidi, 1969; Rittinger et al., 1987). In many dicotyledons, wounds are closed by deposition of biopolymers in cells adjacent to the wounded area, followed by formation of a wound periderm or cork beneath the sealed area. The deposited material has been shown, in some cases, to be suberin (Cottle and Kolattukudy, 1982) and lignin-like in others (Walter and Schadel, 1983). Suberin is believed to contain an aromatic domain, lignin-like in nature, that is covalently bonded to the cell wall and an aliphatic domain bonded to the aromatic regions (Kolattukudy, 1984). Suberized tissue generally has an affinity for lipid stains such as Sudan IV and, upon reductive depolymerization with Li Al H<sub>4</sub>, yields a family of mono- and di-alcohols with carbon chain lengths ranging from C<sub>16</sub> through C<sub>30</sub>. Lignified tissue has little affinity for Sudan IV stain, but is highly reactive with Weisner stain (Walter and Schadel, 1983). Reductive depolymerization yields only small amounts of mono- and di-alcohols, while alkaline oxidation gives relatively large amounts of *p*-hydroxybenzaldehyde and vanillin. Alkaline oxidation of suberized tissue gives similar degradation products. Thus, using

a combination of histochemical stains and depolymerization, the nature of the wound response can be fairly well characterized.

Cucumbers destined for processing uses generally are not stored for long periods of time. However, during periods when the fruit is not available locally, it is transported from distant locations and, as a result, several days may elapse before processing is begun. Wounds inflicted during harvest and shipping could result in serious raw material losses. Cucumber fruit wounded by cutting gave Weisner positive material, indicating that lignification was occurring (Behr, 1949). However, a detailed study of the wound healing process for cucumber fruit has not been published. This investigation was initiated to study wound healing in cucumber fruit using a combination of histological and chemical techniques.

## Materials and Methods

### Histology

Cucumber fruit were harvested on three dates from one spring planting. Twenty-one size no. 3 cucumbers (3.8 to 5.1 cm in diameter) were collected on each of the first two dates; 21 fruit of size no. 2 (2.7 to 3.8 cm in diameter), 21 of size no. 3, and 21 of size no. 4 (>5.1 cm in diameter, oversized) cucumbers were collected on the third harvest date. Cucumbers were washed, dried, and wounded by removing a 12- to 19-mm-diameter and 2- to 3-mm-deep patch of tissue with a razor blade. The wounding technique was a reproducible simulation of a moderately severe slice wound. Three wounds were cut on each cucumber from the first harvest date; four wounds were cut per cucumber from the second and third harvest dates. Wounds were cut in a longitudinal row from blossom end to stem end. Wounded cucumbers were placed in a Forma Scientific environmental chamber (model 39412-1; Marietta, Ohio) at ≈26C and 85% to 90% RH. Three cucumbers were removed on days 1 through 7 for the first and second harvests, and on days 1 through 7 and day 10 for the third harvest. Wounds were excised in a block of tissue, fixed in 3% glutaraldehyde in 0.1 M sodium acetate buffer (pH 5.5) and stored at 4C. After 5 to 6 days at 4C, wounded tissue was vacuum-infiltrated with fixative at 10 to 15 psi and 21C for three or four 2-hr periods. Infiltrated tissue was stored at 4C for 1 to 2 days, rinsed overnight in 0.1 M sodium acetate buffer (pH 5.5), dehydrated in an ethanol-tertiary butyl alcohol series (Johansen, 1940), vacuum-infiltrated, and embedded in Paraplast Plus (Monoject Scientific, St. Louis). Embedded tis-

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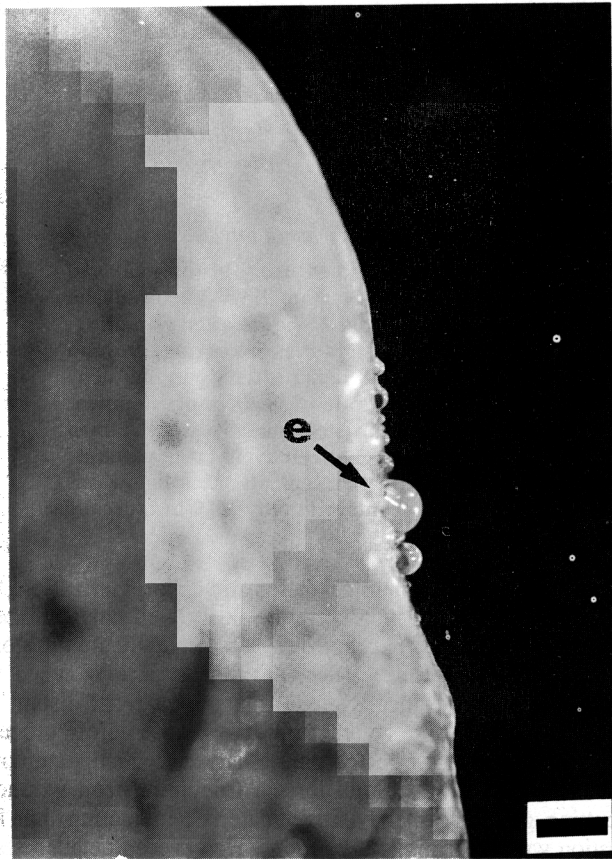


Fig. 1. Side view of exudate on stem end of 'Calypso' cucumber immediately after wounding. e = Exudate. Bar = 5 mm.

sue was sectioned at 12 to 14  $\mu\text{m}$  on a rotary microtome, mounted using Haupt's adhesive (Johansen, 1940), and air-dried.

Embedded sections were deparaffinized and rehydrated for anatomical and histochemical examination of the wound tissue. Wound tissue anatomy was examined using darkfield microscopy. Stains included were phloroglucinol-HCl (PG) reagent (Walter and Schadel, 1982) for detecting aldehydes associated with lignin formation in sclerified tissues and Sudan IV (Bradbury, 1973) for detecting suberized and cutinized tissue. Other tests and stains used on deparaffinized sections included Periodic Acid-Schiff (PAS) reaction for insoluble polysaccharides (Bradbury, 1973); orcinol for oleoresinous substances (Johansen, 1940); ruthenium red-methylene blue for differentiating suberin, lignin, sclerenchyma, and parenchyma (Gray, 1954); aniline sulfate-methylene blue for differentiating lignin, sclerenchyma, pectin, and cellulose (Garr, 1965); toluidine blue for cellulose (Feder and O'Brien, 1968) and lignin (O'Brien et al., 1964); and the Maule reaction for lignin (Bradbury, 1973). Catechol was applied directly to fresh wounds and exudates from fresh wounds to detect polyphenol oxidase (Schadel and Walter, 1981). PG reagent and orcinol were also applied directly to fresh wound tissue. Fresh tissue was hand-sectioned and examined for starch with a weak iodine solution and heat (Johansen, 1940). Fresh wounds were excised and fixed in ferrous sulfate (Johansen, 1940) to detect tannins.

#### Color tests

Wound tissue from each sample day was excised by slipping a razor blade directly under the wound and then placing the excised wound tissue directly into PG reagent for 10 min. Tissue

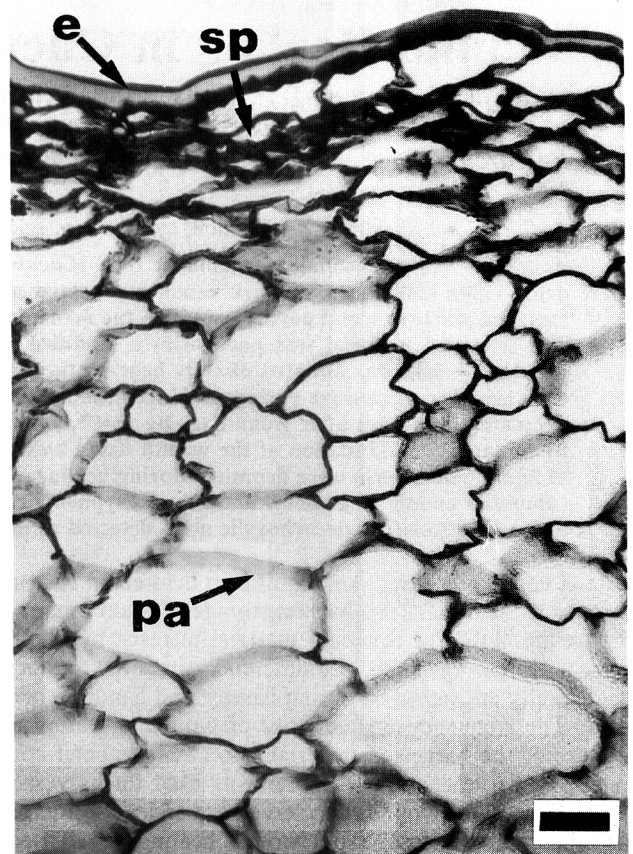


Fig. 2. Cross-section of wounded 'Calypso' cucumber hypodermis and outer mesocarp after 1 day at 26C and 85% to 90% RH. e = Exudate, sp = sclerified parenchyma (positive reaction with phloroglucinol-HCl reagent), pa = normal parenchyma. Bar = 50  $\mu\text{m}$ .

was removed from the reagent, blotted dry, and macroscopic color reactions were scored visually using a scale of 1 = no color change, 2 = pale pink, 2.5 = patchy pink, 3 = medium to deep pink, and 4 = purple.

#### Data analysis

Analysis of variance using the General Linear Models procedure (SAS Institute, 1985) served to identify statistically significant trends. Where appropriate, mean separation was done by the Waller-Duncan K ratio *t* test and the *t* statistic (SAS Institute, 1985).

#### Biopolymer analysis

**Tissue purification.** Fruit from harvests 1 and 2 were wounded and placed in the environmental chambers described above. After 7 days, the fruit were removed and wound tissue excised. In addition, samples of periderm and mesocarp were also removed. The tissue samples were purified as previously described for sweetpotato tissue (Walter and Schadel, 1983). After air-drying, the purified tissue was ground to  $\leq 60$  mesh, vacuum-dried (50C), and stored in a desiccator at room temperature.

**Alkaline cupric oxide oxidation.** Powdered samples of periderm, wound, and mesocarp tissue (60 mg) were mixed with 1.7 g  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  and 10 ml of 3 N NaOH and heated at 180C for 3 hr in a sealed tube. The residue was neutralized, extracted with ether, and the resulting alcohols derivitized with acetic anhydride prior to gas-liquid chromatographic (GLC)

