

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 H₄, yields a family of mono- and di-alcohols with carbon chain lengths ranging from C₁₆ through C₃₀. 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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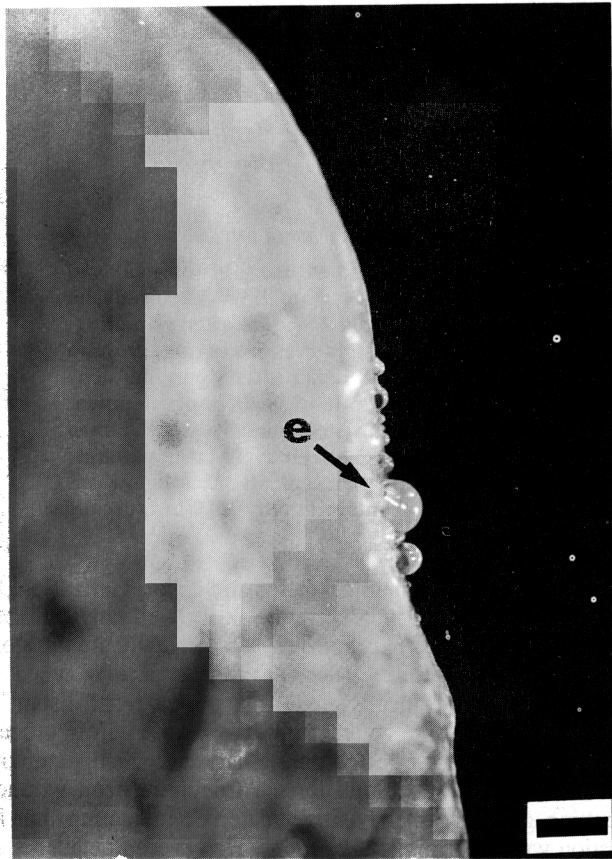


Fig. 1. Side view of exudate on stem end of 'Calypso' cucumber immediately after wounding. e = Exudate. Bar = 5 mm.

sue was sectioned at 12 to 14 μm 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

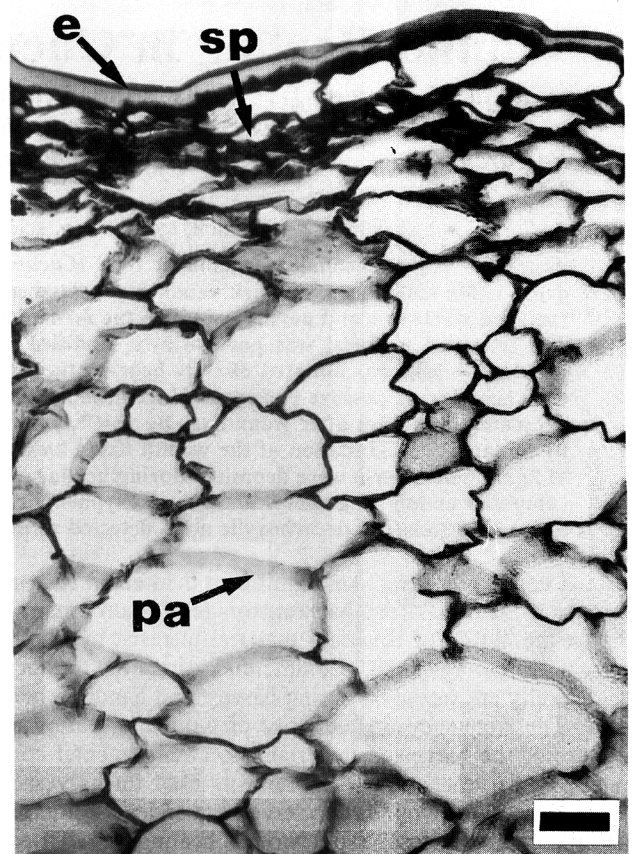


Fig. 2. Cross-section of wounded 'Calypso' cucumber hypodermis and outer mesocarp after 1 day at 26C and 85% to 90% RH. e = Exudate, sp = sclerified parenchyma (positive reaction with phloroglucinol-HCl reagent), pa = normal parenchyma. Bar = 50 μm .

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 ≤ 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)

Table 1. Comparison by day of cell layer scores combined from harvests I and II of sclerified parenchyma, phellem, and sclerenchyma cells on wounded 'Calypso' cucumbers.

Day	Mean scores						
	Sclerified parenchyma ^z			Phellem ^z			
	Sudan IV	Phloroglucinol	Morphological	Sudan IV	Phloroglucinol	Morphological	Sclerenchyma ^y
1	1.0	2.0	2.0	1.0	1.0	1.0	1.0
2	2.3	2.2	2.3	1.0	1.0	2.0	1.0
3	3.0	2.4	3.0	2.2	1.2	3.5	1.0
4	3.0	2.9	3.0	2.3	2.0	3.5	1.8
5	3.0	3.0	3.0	3.2	2.8	4.5	2.2
6	3.0	3.0	3.0	4.7	4.4	5.7	3.5
7	3.0	3.0	3.0	4.8	4.6	5.7	3.2
Trend	**	**	**	**	**	**	**
SE ^x	0.03	0.04	0.09	0.08	0.06	0.03	0.10

^zScore values assigned to given cell layers: 1 = 0 cell layer; 2 = 0 to 1; 2.5 = 0 to 2; 3 = 1 to 2; 3.5 = 1 to 3; 4 = 2 to 3; 4.5 = 2 to 4; 5 = 3 to 4; 5.5 = 3 to 5; 6 = 4 to 5; 7 = 5 to 6.

^yScore values assigned to relative abundance of sclerenchyma cells: 1 = no sclerenchyma cells; 2 = a few scattered (one to two) layers present, but lightly; 3 = several scattered layers when present (or one to two layers for a large area, moderate); 4 = several layers (two to four) covering a large area (heavy).

^xSE \geq standard error of the mean.

**Significant at $P \leq 0.001$.

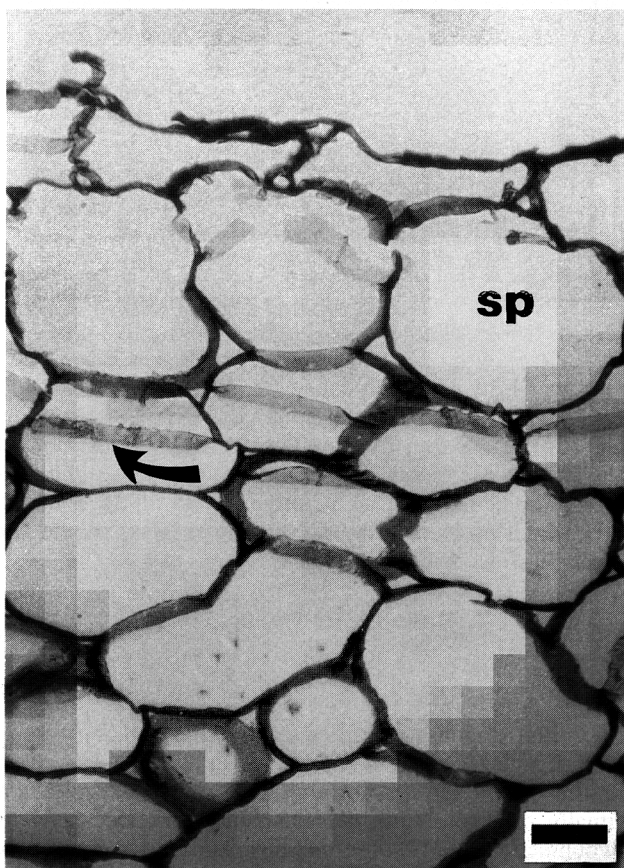


Fig. 3. Cross-section of wounded 'Calypso' cucumber outer mesocarp after 2 days at 26C and 85% to 90% RH. Note wound periderm initiation (curved arrow). Exudate was disrupted during sectioning. sp = Sclerified parenchyma. Bar = 50 μ m.

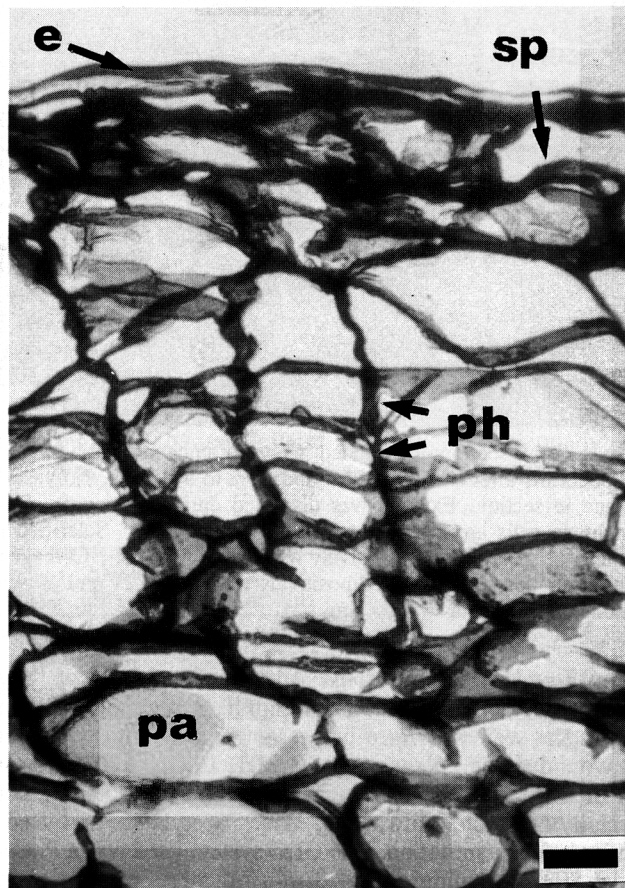


Fig. 4. Cross-section of wounded 'Calypso' cucumber hypodermis and outer mesocarp after 10 days at 26C and 85% to 90% RH with well-formed wound periderm. Note sclerenchyma absent. e = Exudate, sp = sclerified parenchyma, pa = normal parenchyma, ph = phellem (positive reaction with Sudan IV). Bar = 50 μ m.

analysis (Walter and Schadel, 1983). The internal standard, 7-hydroxy coumarin, was added before ether extraction.

Reductive depolymerization. Samples of purified periderm, wound, and mesocarp tissues (60 mg) were refluxed overnight with Li Al H₄ in tetrahydrofuran. The sample work-up was as described previously (Walter and Schadel, 1983), except that

the internal standard used was 1,12-dihydroxydodecane. After isolation of the dihydroxy fragments by thin-layer chromatography, they were transformed into trimethylsilyl ethers before mass spectrometry and quantitation by GLC.

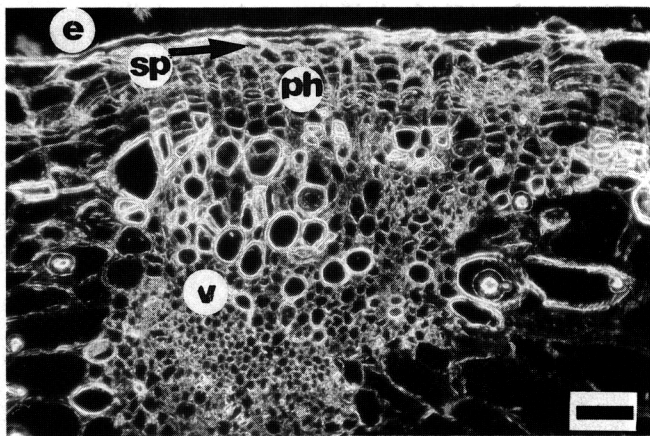


Fig. 5. Cross-section of wounded 'Calypso' cucumber epidermis and hypodermis after 6 days at 26C and 85% to 90% RH with vascular tissue and well-formed periderm above vascular tissue. e = Exudate; sp = sclerified parenchyma, ph = phellem cells (reacted positively with Sudan IV), v = vascular bundle. Bar = 100 μ m.

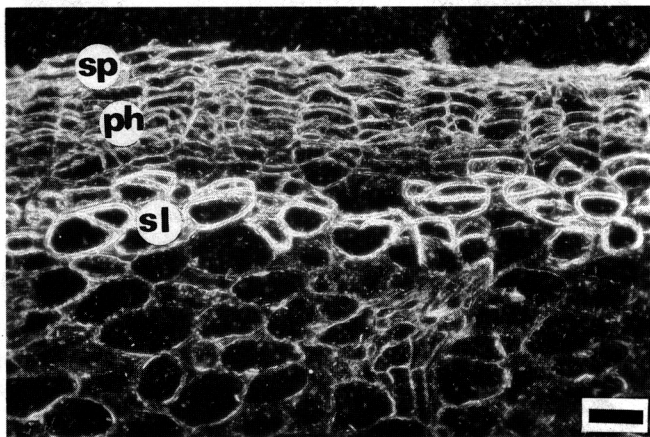


Fig. 6. Cross-section of wounded 'Calypso' cucumber epidermis and hypodermis after 7 days at 26C and 85% to 90% RH. No vascular tissue in section. Exudate was disrupted during sectioning. Sclerenchyma cells are prominent beneath wound. sp = Sclerified parenchyma cells (reacted positively with phloroglucinol-HCl reagent); ph = phellem cells (reacted positively with Sudan IV); sl = sclerenchyma cells. Bar = 100 μ m.

Gas-liquid chromatography. Acetylated products of the alkaline cupric oxide oxidation of lignin have been identified previously and were shown to be present in sweetpotato periderm and wound tissue (Walter and Schadel, 1983). Authentic samples of the expected breakdown products were obtained commercially (Aldrich, Milwaukee, Wis.), acetylated, and used as standards for quantitation. The GLC system used was a Packard model 803 containing a 3 \times 3 mm i.d. glass column packed with 3% OV101 on Supelcoport 80/100 mesh. The column temperature was programmed to increase 5C/min beginning at 140C and ending at 225C, with a final hold of 8 min. Individual components were quantitated by determination of each response factor relative to the internal standard 7-hydroxycoumarin. A Hewlett-Packard model 3390A integrator was used to measure peak areas.

Compounds released from cucumber tissue by Li Al H₄ reduction were quantitated by the same GLC system as above, except that the separation was conducted isothermally at 250C and the internal standard used for calculation of the response

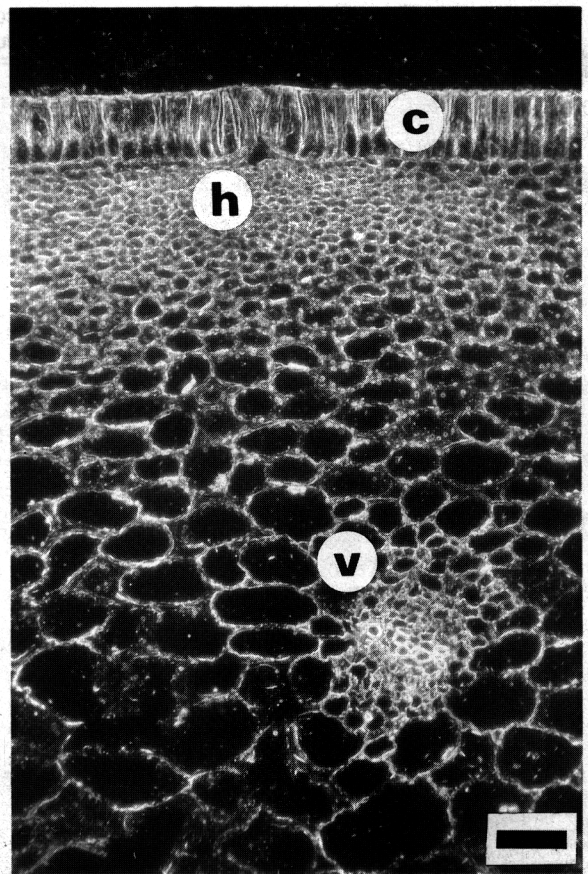


Fig. 7. Cross-section of unwounded 'Calypso' cucumber epidermis and hypodermis. c = Cuticle, h = hypodermis, v = vascular bundle. Bar = 100 μ m.

factors was 1,12-dihydroxydodecane. Identification was done with a Hewlett-Packard model 5985 GC/MS. The column used was a 30 \times 0.32 mm i.d. capillary column coated with DB 1701. The initial column temperature was 200C (1-min hold), then programmed to 275C at 5C/min (20-min hold). The carrier gas (He) inlet pressure was 0.77 kg \cdot cm⁻². Mass spectra were obtained at 70 eV ionizing voltage.

Results and Discussion

Histology

The initial response of the cucumber to wounding was production of a clear, sticky exudate (Fig. 1). The exudate covered the entire wound surface, forming thick droplets in places and eventually drying to form a presumably protective layer over the wound. Behr (1949) observed this exudate and found a negative relationship between depth of wound and intensity of exudate production, suggesting the exudate was not initiated in the pulp. He did note a relationship between maturity of the fruit and intensity of exudate production: the younger the fruit, the greater the intensity of exudate production. He attributed this relationship to the higher fluid contents, etc. of young cells.

No desiccation was observed for uninjured parenchyma cells at the wound surface; the exudate appears to provide a moisture barrier and to facilitate rapid cork initiation. Wounded sweetpotatoes that do not produce a copious exudate exhibit varying degrees of desiccation of the uninjured parenchyma cells at the wound surface. The degree of desiccation varies with relative

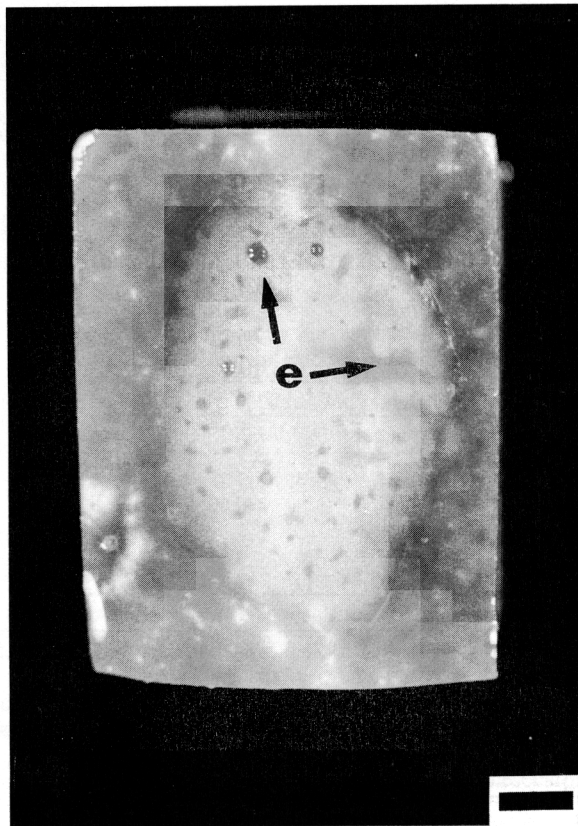


Fig. 8. Excised 'Calypso' cucumber wounds after fixation in 3% glutaraldehyde in 0.15 M NaOAc buffer, pH 5.5. Note dark coloration of exudate droplets and clear coloration of exudate between droplets. Exudate droplets were red, which appeared dark in this photograph. e = Exudate. Bar = 3 mm.

humidity and temperature, and desiccation was associated with thickening of the cell walls (lignification or suberization) be-

neath the desiccated area. This thickening continues to sub- tending cells until the wound cork is initiated (Walter and Schadel, 1982). Poor wound cork formation has been observed in sweet- potatoes where no desiccation occurred (Weimer, 1921). This pattern leads to speculation as to whether the exudate on cu- cumbers provides protection by preventing desiccation or by providing a barrier that is represented by desiccated cell layers in the sweetpotato.

Cork formation

Daily development of cork formation in wounded cucumbers held at 26C and 85% to 90% RH was observed microscopically (Table 1). After 1 day, zero to one layer of parenchyma cells directly beneath the exudate reacted positively with the phlo- roglucinol-HCl reagent (PG), indicating that aldehydes associ- ated with lignin were being deposited (Fig. 2). Up to two parenchyma cell layers reacted positively with PG by day 3. The cells in these two layers (henceforth referred to as sclerified parenchyma) gave no morphological indication of desiccation. Rarely were more than two cell layers of the parenchyma thus sclerified. The positive PG results concur with those observed by Behr (1949) on the walls of dead cells and of several healthy cells beneath wounds on cucumber petioles. El-Din-Fouad (1956) did not observe staining by PG of healthy cells adjacent to the wound area in any *Cladosporium cucumerinum*-susceptible cu- cumber tissue examined (cotyledons, leaves, petioles, and stems), although dead cells beneath the wounds did not react positively with PG.

Cork was initiated directly beneath sclerified parenchyma. Sudan IV, a test for fatty acids associated with suberin, yielded positive results as early as day 3 for the phellem. Staining usu- ally intensified, and the number of cell layers increased over time. By day 6, the Sudan IV was staining the last initiated phellem cells. Phellem was often initiated by day 2 (Fig. 3). When it was initiated by day 2, the phellem was patchy and only one to two cell layers thick. By day 5, the phellem thick- ened to three to four cell layers. The phellem occasionally thick- ened to as many as eight cells by day 10, although the four to

Table 2. Response of cucumber tissue to histological stains.

Tissue	Sudan IV	Phloroglucinol	Orcinol	Ruthenium red- methylene blue	Maule reaction	Aniline sulfate- methylene blue	Toluidine blue	Periodic acid-Schiff
Epidermis	+ (red)	-	-	+ (walls pink to red)	-	+ (dark blue)	+ (deep blue)	+ (blue)
Normal parenchyma	-	-	-	+ (dark smoky purple)	-	+ (light blue)	+ (purple)	+ (reddish purple)
Sclerified parenchyma	+ (red)	+ (red)	+ (red)	+ (pink to red)	-	+ (dark blue)	+ (purple)	+ (reddish purple)
Wound phellem	+ (red)	± (pinkish red)		(only two cell layers +; green)	-	+ (turquoise)	+ (purple)	+ (reddish purple)
Sclerenchyma	-	+ (purplish)		+ (pink)	-	(0 in section)	+ (blue)	+ (blue)
Xylem elements	+			± (greenish hue)		+ (soft green to bluish green)	+ (bluish green)	+ (purple)
Exudate	-	-	-	-	-	-	+ (blue)	+ (pale brown)
Cuticle	+ (red)	-	-	-	-	+ (pale/light blue)		
Stain specificity	Fatty acids (suberin and cutin)	Aromatic aldehydes (lignin and gums)	Oleoresinous substances	To differentiate suberin, lignin, sclerenchyma, and parenchyma	Lignin	To differentiate lignin, sclerenchyma, pectin, and cellulose	Cellulose	Insoluble polysaccharides

Table 3. Comparison by day of cell layer scores from harvests I and II of sclerified parenchyma, phellem, and sclerenchyma cells on wounded 'Calypto' cucumbers.

Harvest	Day	Mean scores						
		Sclerified parenchyma ^z			Phellem ^z			
		Sudan IV	Phloroglucinol	Morphological	Sudan IV	Phloroglucinol	Morphological	Sclerenchyma ^y
I	1	1.0	2.0	2.0	1.0	1.0	1.0	1.0
	2	2.3	2.3	2.3	1.0	1.0	2.0	1.0
	3	3.0	2.5	3.0	2.0	1.3	3.3	1.0
	4	3.0	2.8	3.0	2.0	2.0	3.3	2.0
	5	3.0	3.1	3.0	3.0	2.7	5.0	2.7
	6	3.0	3.0	3.0	5.0	4.0	5.7	3.7
	7	3.0	3.0	3.0	5.0	4.7	6.0	3.0
	Trend	**	**	**	**	**	**	**
	SE ^x	0.06	0.07	0.06	0.14	0.10	0.14	0.16
II	1	1.0	2.0	2.0	1.0	1.0	1.0	1.0
	2	2.3	2.0	2.3	1.0	1.0	2.0	1.0
	3	3.0	2.3	3.0	2.3	1.0	3.7	1.0
	4	3.0	3.0	3.0	2.7	2.0	3.7	1.7
	5	3.0	2.8	3.0	3.3	2.8	4.0	1.7
	6	3.0	3.0	3.0	4.3	4.8	5.7	3.3
	7	3.0	3.0	3.0	4.7	4.5	5.3	3.3
	Trend	**	**	**	**	**	**	**
	SE ^x	0.06	0.07	0.06	0.13	0.10	0.14	0.15

^zScore values assigned to given cell layer: 1 = 0 cell layer; 2 = 0 to 1; 2.5 = 0 to 2; 3 = 1 to 2; 3.5 = 1 to 3; 4 = 2 to 3; 4.5 = 2 to 4; 5 = 3 to 4; 5.5 = 3 to 5; 6 = 4 to 5; 7 = 5 to 6.

^yScore values assigned to relative abundance of sclerenchyma cells: 1 = no sclerenchyma cells; 2 = a few scattered (one to two) layers present, but lightly; 3 = several scattered layers when present (or one to two layers for a large area, moderate); 4 = several layers (two to four) covering a large area (heavy).

^xSE = standard error of the mean.

**Significant at $P \leq 0.001$.

Table 4. Mean scores for macroscopic color reactions of phloroglucinol-HCl reagent on excised 'Calypto' cucumber wounds from harvests II and III.

Day	Mean		
	Harvest II No. 3	Harvest III	
		Small (no. 2 and 3)	Large (no. 4, oversized)
1	2.0	2.1	2.8
2	2.9	2.3	2.9
3	3.2	2.7	3.5
4	3.7	---	---
5	3.8	3.8	3.8
6	3.9	4.0	4.0
7	4.0	4.0	4.0
10	---	4.0	4.0
	Trend	**	**
	SE ^z	0.02	0.08

^zSE = standard error of the mean.

**Significant at $P \leq 0.01$.

six layers of phellem were a more typical thickness (Fig. 4). The patchy distribution of the phellem could still be observed as late as day 10. Phellem was most rapidly and heavily produced next to the vascular tissue (Fig. 5). One to two layers of phelloderm were usually produced by day 7 (Fig. 6).

Sclerenchyma cells were occasionally observed beneath the phelloderm beginning with day 4. These cells were usually patchy, but, in some samples, extended over much of the wounded area (Fig. 6). The morphology of the sclerenchyma cells resembles the hypodermal cells at the base of the emergence or wart described by Barber (1909). These cells are sclerenchymatized, thickened, and pitted. Barber (1909) suggests the thickening of

these cells may serve to prevent evaporation or entrance of a pathogen after the emergence is removed. Esau (1977) refers to scattered sclerids present beneath the hypoderm in cucurbits in general, but no reference was made specifically to sclerids in *Cucumis sativus*. The sclerenchyma cells noted here reacted positively with PG, but did not stain with Sudan IV. Tissue adjacent to the wound appeared unaffected (Fig. 7).

Thickened portions of the exudate on the wound surface slowly turned bright red when tissue was placed in glutaraldehyde fixative. The thickened exudate appeared in Fig. 8 as dark-colored droplets. The thin, smooth areas of the exudate remained opaque. Only two histochemical tests or stains reacted positively with the exudate on deparaffinized sections and fresh wound tissue—these were toluidine blue (Table 2) and PAS counterstained with toluidine blue. Other areas, however, had positive results with several stains or tests. Orcinol yielded results similar to PG, again indicating the presence of aldehydes associated with lignin in the outermost cell layers of the wound. The Maule reaction, specific for syringyl groups (Vance et al., 1980), yielded negative results on these outermost cell layers. PG (a modified Wiesner test) reacts predominantly with substituted cinnamaldehyde groups and only weakly with syringyl groups (Vance et al., 1980).

General trends in significant differences for combined data from harvests I and II existed among the number of layers of sclerified, phellem, and sclerenchyma cells over time, regardless if determination was based on morphological features or stains (Table 1). The groupings were generally distinct. When the values were analyzed separately for harvests I and II (Table 3), groupings were very similar for all characteristics except two. These were morphological determinations of sclerenchyma cells and phellem, which varied slightly.

Table 5. Comparison on days 7 and 10 of cell color scores and microscopic color scores between different-sized, wounded 'Calypso' cucumbers.

Cucumber size	Day	Sclerified parenchyma cells			Phellem			Sclerenchyma	Wounds
		Sudan IV	Phloroglucinol	Morphological	Sudan IV	Phloroglucinol	Morphological		
Small	7	3.0	3.0	3.0	5.0	4.3	6.0	2.0	4.0
	10	3.0	3.0	3.0	7.0	6.3	7.6	3.3	4.0
Significance		NS	NS	NS	*	*	*	NS	NS
Large	7	3.0	3.3	3.0	6.0	4.8	6.5	3.3	4.0
	10	3.0	3.3	3.0	6.5	5.0	7.3	2.3	4.0
Significance		NS	NS	NS	NS	NS	NS	NS	NS

NS,*Not significant or significant at $P \leq 0.05$, respectively, using *t* statistic.

Table 6. Comparison by harvest for day 7 of cell layer scores^z of sclerified parenchyma, phellem, and sclerenchyma cells on wounded 'Calypso' cucumbers.

Harvest ^y	Sclerified parenchyma ^x			Phellem ^x			Sclerenchyma ^x
	Sudan IV	Phloroglucinol	Morphological	Sudan IV	Phloroglucinol	Morphological	
I	3.0	3.0	3.0	5.0 A	4.7	6.0	3.0
II	3.0	3.0	3.0	4.7 A	4.5	5.3	3.3
III S	3.0	3.0	3.0	5.0 AB	4.3	6.0	2.0
III L	3.0	3.3	3.0	6.0 B	4.8	6.5	3.3
MSD	NS	NS	NS	1.2	NS	NS	NS

^zMeans separated by Waller-Duncan multiple range test. Within a column, means with the same letter are not significantly different ($P \leq 0.05$).

^yS = small; L = large.

^xSee Table 1 for explanation of score values.

Table 7. Components from cupric oxide oxidation of cucumber, purified wound tissue, periderm, and mesocarp.

Component	Quantity (mg·g ⁻¹ of dry tissue)		
	Wound ^z	Periderm ^z	Mesocarp ^y
<i>p</i> -Hydroxybenzaldehyde (HB)	13.2 (± 0.4)	2.70 (± 0.04)	ND ^x
<i>p</i> -Hydroxyacetophenone (HA)	2.78 (± 0.25)	1.21 (± 0.21)	ND
Vanillin (VA)	21.6 (± 1.3)	2.51 (± 0.14)	0.77
Acetovanillone (AV)	7.35 (± 0.55)	0.60 (± 0.03)	ND
Syringaldehyde (SA)	0.22 (± 0.05)	ND	ND

^zTissue from two harvests. Each value is mean of duplicate analyses (± SD).

^yTissue from one harvest. Each value is mean of duplicate analyses.

^xND = not detectable.

Table 8. Components released by Li Al H₄ reduction of purified cucumber wound, skin, and mesocarp tissue.

Compound	Quantity (mg·g ⁻¹ of dry tissue)		
	Wound ^z	Periderm ^z	Mesocarp ^y
1,16-Hexadecane diol ^x	18.13 (± 0.10)	11.42 (± 2.38)	0.50
1,18-Octadecene diol ^x	29.88 (± 0.76)	10.01 (± 1.86)	0.46

^zTissue from two harvests. Each value is mean of duplicate analyses (± SD).

^yTissue from one harvest. Each value is mean of duplicate analyses.

^xIdentity established from mass spectrum.

Color tests

Wound color test scores for harvest II increased rapidly up to day 4, with smaller increases after that (Table 4). This result is similar to the relationship seen microscopically in harvests I and II, with morphological distinctions between days 1 and 2, and no differences among days 3-7 (Table 1). Although the morphological distinctions had disappeared by day 3, the microscopic PG reactions remained distinct until day 4, supporting a direct correlation between the number of layers of sclerified parenchyma reacting to PG with the macroscopic color intensity of PG, as was demonstrated in wounded sweetpotato (Walter and Schadel, 1982).

Cucumber size

Slight differences occurred in the PG macroscopic color test between large and small cucumbers for days 1, 2, and 3, but, by day 5, test scores were not significantly different (Table 4). No differences occurred between days or cucumber sizes in number of sclerified parenchyma or sclerenchyma cell layers. Distinct differences did occur, however, between days 7 and 10 for small cucumbers in the phellem, whether determination was made on morphological features or staining reactions (Table 5). Phellem values for large cucumbers on days 7 and 10 were not significantly different. Phellem values were also higher for small cucumbers than for large cucumbers for day 10, and the reverse was true for day 7. These differences, although not significantly different when viewed with the macroscopic color tests (Table 4), suggest a trend of slower formation of a sclerified parenchyma cell barrier in small cucumbers, but that small cucumbers can catch up and possibly surpass the larger cucumbers in phellem development.

Scattered clusters of sclerenchyma cells were present in most fruit examined. Two small fruit lacked sclerenchyma cells at day 7, but the other small fruit at day 7 had four or more layers of sclerenchyma cells present. These layers were usually not continuous across the entire wound.

When all three harvests were compared for day 7 values (Table 6), no significant differences were found among sclerified parenchyma or sclerenchyma cell layer values, or among PG and morphological determinations of phellem values. The only significantly different values observed were between the Sudan IV-staining phellem cell layers for harvest I and for large fruit from harvest III. Although different rates may occur in the early stages of wound cork formation, by day 7, in similar environmental conditions, fruit from all harvests and of all sizes were at a similar stage of development.

Biopolymer analysis

Two chemical approaches were taken in the analysis of the biopolymers from wound, periderm, and mesocarp tissue. Alkaline cupric oxide oxidation is a commonly used degradation method to determine the composition of lignified tissue (Vance et al., 1980; Hammerschmidt, 1985). Reductive depolymerization with Li Al H_4 has been used to study the aliphatic regions of suberin and cutin (Kolattukudy and Agrawal, 1974; Kolattukudy, 1984). Histochemical studies described in the previous section indicated that lignification was initiated within 24 hr of wounding, and suberization was observed 48 hr after wounding. Since wound tissue for analysis was collected 7 days after wounding, it was expected that significant amounts of lignin and suberin biopolymer constituents would be found. Degradation of lignified wound tissue by alkaline cupric oxides resulted in the formation of vanillin (VA) and *p*-hydroxybenzaldehyde (HB) as the major components, with smaller amounts of syringaldehyde (SA), *p*-hydroxyacetophenone (HA), and acetovanillone (AV). HB and HA are derived from *p*-hydroxyphenyl residues, VA and AV from guaiacyl residues, and SA from syringyl residues. We found that for cucumber wound tissue, VA was the most abundant oxidation product, followed by HB, AV, and HA. SA was present in very small amounts (Table 7). Oxidation products from cucumber periderm lignins were much less abundant than from wound lignins, and their relative distributions were different. Cucumber mesocarp tissue contained almost no lignin. These data also indicated that the lignin in wound and periderm tissues are comprised mainly of *p*-hydroxyphenyl and guaiacyl residues, but in different ratios. The small amount of SA present in wound tissue is consistent with the negative Maule reaction (Table 2). Asada and Matsumoto (1972) found similar amounts of HB and VA in wounded radish root inoculated with downy mildew. Walter and Schadel (1983) found that, in sweetpotato roots, VA and HB levels were 5.01 and 1.99 $\text{mg}\cdot\text{g}^{-1}$ of wound tissue, respectively, and that periderm and wound tissue were similar in composition. Hammerschmidt (1985) reported that, for two white potato cultivars, HA was greater than VA in wound tissue, while the reverse was true for normal periderm. Absolute amounts of the oxidation products were of the same magnitude as reported for sweetpotato (Walter and Schadel, 1983).

Reductive depolymerization of cucumber tissue gave two major products. These were identified by their mass spectra as 1,16-hexadecanediolsilyl ether and 1,18-octadecanediolsilyl ether. The hexadecane compound had a barely discernible molecular ion at 402 and fragment ions at m/e 387 (M^+-CH_3), m/e 371 ($\text{M}^+-\text{CH}_3-\text{CH}_2$), and m/e 312 [$\text{M}^+-\text{HO Si}(\text{CH}_3)$] (Walton and Kolattukudy, 1972). The C_{18} enediol had a mass spectrum identical to that reported for sweetpotato wound tissue (Walter and Schadel, 1983). Hydrolysis products recovered from the three types of tissue declined in the order of wound, periderm, and mesocarp (Table 8). The C_{18} diol made up 62% of the total

hydrolysate in wound tissue, as compared to approximately equal amounts of C_{16} and C_{18} diols from the periderm and mesocarp. These findings differ from the study on sweetpotato, which showed that the C_{18} diol was the major hydrolysis product and that more than seven times as much of this diol was released from the skin as from the wound tissue.

The wound healing process in cucumber is similar to that reported for other plant organs in that the cucumber is protected after wounding from dehydration and to some extent from infection by deposition of biopolymers, such as lignin and suberin. However, in cucumber, a viscous exudate that was released within minutes of wounding effectively sealed the wound and prevented moisture loss so that no visible cellular desiccation occurred. In sweetpotato (Walter and Schadel, 1983), and carrot and potato (Rittinger et al., 1987), several layers of desiccated cells were observed above the healed wound. Within 1 day of wounding cucumbers, PG-positive sclerified parenchyma cells were observed. By the 2nd day, these cells were also positive to Sudan IV, indicating that lignification occurred first, followed immediately by suberization. Usually, this phase of wound healing was completed after two layers of cells beneath the wound had been sclerified. This pattern contrasts with that in sweetpotatoes in which three or more layers of sclerified cells were formed (Walter and Schadel, 1983). Additionally, for sweetpotato wounds, both histological and degradative studies showed that lignin was the predominant biopolymer of sclerified cells. In cucumber, meristematic wound phellem, which was initiated 3 days after wounding, was positive to both PG and Sudan IV, indicating that both lignin and suberin were being deposited. Phellem formation was usually complete within 10 days and consisted of four to six cell layers. In view of the effect of temperature and humidity on wound healing rates, it is improper to compare the results of cucumber with other plants unless similar conditions were used. Chemical analysis of the wound tissue showed that fairly large amounts of lignin and suberin were deposited during the healing process. Both suberized and lignified plant tissues are resistant to attack by fungi and bacteria (Lauritzen, 1935; Brown et al., 1978) and should provide an effective barrier to pathogen invasion.

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