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Scanning Electron Microscopy of the Surface of Pickling Cucumber Fruit[1]

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Abstract. **Scanning electron microscopy revealed the presence of stomata, trichomes, scars left by detached trichomes, and epidermal cells on the surface of fresh, pickling cucumbers. Size, frequency and distribution of stomata were determined. Stomata, recessed several μm, were the only apparent, natural openings in the epidermis for gas exchange. Stomata were most numerous in the middle (20.2/mm^2), less in the blossom end (10.4/mm^2) and essentially absent in the stem end section of large (3.8–5.1 cm diameter), 'GY14' fruit. Stomatal frequency on large fruit was only about one-third that on small (1.9–2.7 cm diameter) fruit, but the stomatal index for the middle section of each size was similar (0.17–0.18). Large 'GY14' fruit were estimated to contain 130,000 stomata, with potential stomatal pore area (assuming open guard cells) representing 0.062% of the fruit surface area.**

Microscopic studies of the surface of fresh fruits and vegetables reveal structures which may be important in disease resistance and postharvest storage stability of the product. For example, transpiration, gas exchange, entry of microorganisms, loss of aroma, and temperature reception depend upon the nature and distribution of epidermal structures (11). A better understanding of the mechanism of gas exchange in cucumber fruits may help to explain how bloater formation (hollow cucumbers) occurs during brine fermentation. Bloater damage is a serious quality defect of brined cucumbers, and has been attributed to the accumulation of CO_2 in the brine (5, 8). Bloater damage can be prevented by purging CO_2 from the brine (4, 6, 7). Thus, factors affecting the exchange of CO_2 between brine and cucumber tissue may be related to the problem of bloater damage. Stomata are involved in gas exchange of many fruits and vegetables (11). Although Barber (1) reported the presence of stomata in various Cucurbitaceae, she stated that stomata were not present in the fruit of *Cucumis sativus* (cucumber). Others have made reference to the presence of stomata on cucumber fruit, but without details (10). The objectives of this study were to observe the general nature of the surface of pickling cucumbers and to determine the size, frequency, and distribution of structures in the epidermis which may be involved in gas exchange.

Materials and Methods

Cucumbers were grown in the field in North Carolina using standard cultural practices. Cultivars examined represented important ones grown commercially in the area and included 'Chipper,' 'Calypso,' 'Addis,' unidentified cultivars from pickle companies, and a gynoecious parental line, 'GY14.' 'Chipper'

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and 'GY14' were used more extensively due to their availability. Sizes of cucumbers referred to herein are: midget (<1.9 cm diameter); no. 1 (1.9–2.7 cm); no. 2 (2.7–3.8 cm); no. 3 (3.8–5.1 cm); and no. 4 (5.1–5.8 cm).

Initial attempts to study the surface features of cucumbers included light microscopic examination of epidermal strips, and of impressions obtained by the replica method described by Sampson (12). Primary replicas of the fruit surface were made by applying General Electric liquid silicone rubber (RTV-21) with Tenneco Nuodex Nuocure 28 catalyst. Secondary replicas were then made by applying clear nail varnish to the silicone replicas.

Sections (5 mm^2 × 1 mm thick) were dissected for scanning electron microscopy (SEM) from the surface of the fruit at 3 locations (stem, middle and blossom). On size no. 3 fruit, 3 samples were taken: 1.5 cm from the edge of the stem and blossom ends, and equidistant between the stem and blossom ends. On size no. 1 fruit, samples were taken only from the middle section. Samples were prepared for SEM by general procedures described for biological soft tissue (2, 3, 9), including fixing in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate, pH 7.2, dehydrating in ethanol and Freon 113, critical-point drying (Bomar SPC-50/Ex apparatus), and gold coating (Polaron E 5000 Diode Sputtering System). Samples were observed at various magnifications and zero tilt at 20 Kev with an Etec Autoscan Microscope operating in the secondary electron mode. Fresh, uncoated samples were also examined.

Dimensions of surface features were determined from micrographs of the middle section of 'GY14' fruit. All micrographs were taken with the SEM at 2000× magnification, zero degree tilt, and a constant working distance. Measurements were averaged for 10 no. 1 and 10 no. 3 fruit. Guard cells of stomata were measured from center ridges. Stomatal pores, elliptical in shape, were measured from the inside wall of open guard cells. Pore area was calculated from the formula for an ellipse (14):

$$\text{Area} = \pi ab,$$

where a and b are the major and minor axes.

For determination of stomatal and trichomal frequencies, ten random fields per section were counted on the viewing screen of the SEM at 200× magnification. Epidermal cell frequencies were determined from SEM micrographs taken at 200× magnification at zero degree tilt and a constant working distance.

Determination of the stomatal index (I) for pickling cucumbers was calculated using the formula suggested by Salisbury (13):

$$I = [S/E + S]\ 100,$$

where S = stomata per unit area and E = epidermal cells per unit area.

The surface area of the fruit was estimated by the formula for a prolate spheroid (14):

$$\text{Surface area} = 2\pi b^2 + 2\pi \frac{ab}{e} \sin^{-1} e, \text{ where } e = \frac{a^2 + b^2}{a},$$

and a and b are the major and minor semi-axes, respectively.

Results

SEM photomicrographs of cucumber surfaces. Surface features of cucumber fruit as viewed by SEM, including stomata, trichomes, scars left by detached trichomes, epidermal cells, and warts with spines attached, are shown in Fig. 1 and 2. Fig. 1A, 1B and 1C illustrate differences in the surfaces of 4.5, 1.3 and 0.7 cm diam fruit, respectively. Stomata were observed with opened (Fig. 1D) and closed (Fig. 1E) guard cells, and with various degrees of opening. No attempt was made to determine percentages of open and closed stomata, as samples varied in this regard due to unknown factors. Factors affecting stomatal action were beyond the scope of the present study. Stomata were recessed several microns in the epidermis (Fig. 1A, 1F) of fruit, which contrasts with protruding stomata in cucumber leaf (16). Trichomes appeared collapsed or shrunken in Fig. 2A and 1B, but we have no evidence to indicate that they appear otherwise on the fruit. Samples which had been fixed and critically-point dried as well as unfixed and uncoated gave similar appearances. Likewise, we have no evidence to indicate that the sunken appearance of epidermal cells (Fig. 1F, 2A) is an artifact.

Stomatal, trichomal and epidermal cell frequencies were greater on small- than large-sized fruit (Fig. 1A, 1B). Also, stomatal and epidermal cells appeared smaller on the small-sized fruit. Trichomes (Fig. 2A, 1B), when detached, left scars which were especially noticeable on large fruit (Fig. 2B, 1B).

Stomata were the only natural openings observed in the epidermis; no lenticels or other natural openings were seen. We have been unable to determine when stomata are first formed because of the close spacing of trichomes on small fruit (Fig. 1C).

Warts, which may be seen with the unaided eye, were viewed by SEM with spines attached (Fig. 2C). A hair appendage (not shown), 600-800 μm and 3-4 cells in length, occasionally was observed atop spines of small fruit, but always appeared to have been broken or distorted. These appendages apparently are very fragile and were broken during preparation for SEM. Barber (1) referred to such appendages on cucumber spines.

General characteristics of stomata, trichomes and the epidermis as shown have been observed repeatedly by SEM, using fixed and critical-point dried as well as fresh, unfixed and undried samples. Surface features of 'Chipper,' 'Addis' and 'GY14' cucumbers were typical of those illustrated; 'Calypso' differed in that the epidermis was less deeply ridged and stomata were less recessed.

Stomata also were observed by light microscopy in epidermal strips and replicas of fruit surfaces, but only after extensive searching. When observed they were poorly defined. The recessed position of stomata and the presence of trichomes on cucumber fruit apparently limit the usefulness of these simple methods.

Dimension, frequency, and distribution of surfaces structures. Dimensions of stomata and trichomes for 2 sizes of 'GY14' fruit are given in Table 1. Stomatal dimensions were significantly greater (P <1%) in size no. 3 than in size no. 1 fruit, but trichomal dimensions were not significantly different. Stomatal and trichomal dimensions of 'Chipper' fruit, although not determined statistically, were similar to those of 'GY14' fruit. Other general observations indicated that the warts were larger in size no. 3 than in no. 1 cucumbers, but the spines were similar in size.

The frequencies of epidermal cells, trichomes and stomata were greater for no. 1 than no. 3 cucumbers (Table 1). These frequencies were determined in the middle section of the cucumbers. Trichomal scars, especially noticeable on no. 3 fruit (Fig. 1A, 2B), suggest that at least part of the difference for the lower frequency of trichomes on larger fruit may be due to greater breakage.

The frequency of epidermal cells and stomata varied significantly by section (stem, middle and blossom) on 'Chipper' and 'GY14' cucumbers (Table 2). The order of frequency was: for epidermal cells, blossom > middle > stem; and for stomata, middle > blossom > stem (Table 2). There was no significant cultivar × section interaction (P = 84%) for the 2 cultivars by ANOVA (15). Also, there was no significant difference between cultivars when stomatal frequencies were averaged over all sections (P = 57%). The frequency distribution for

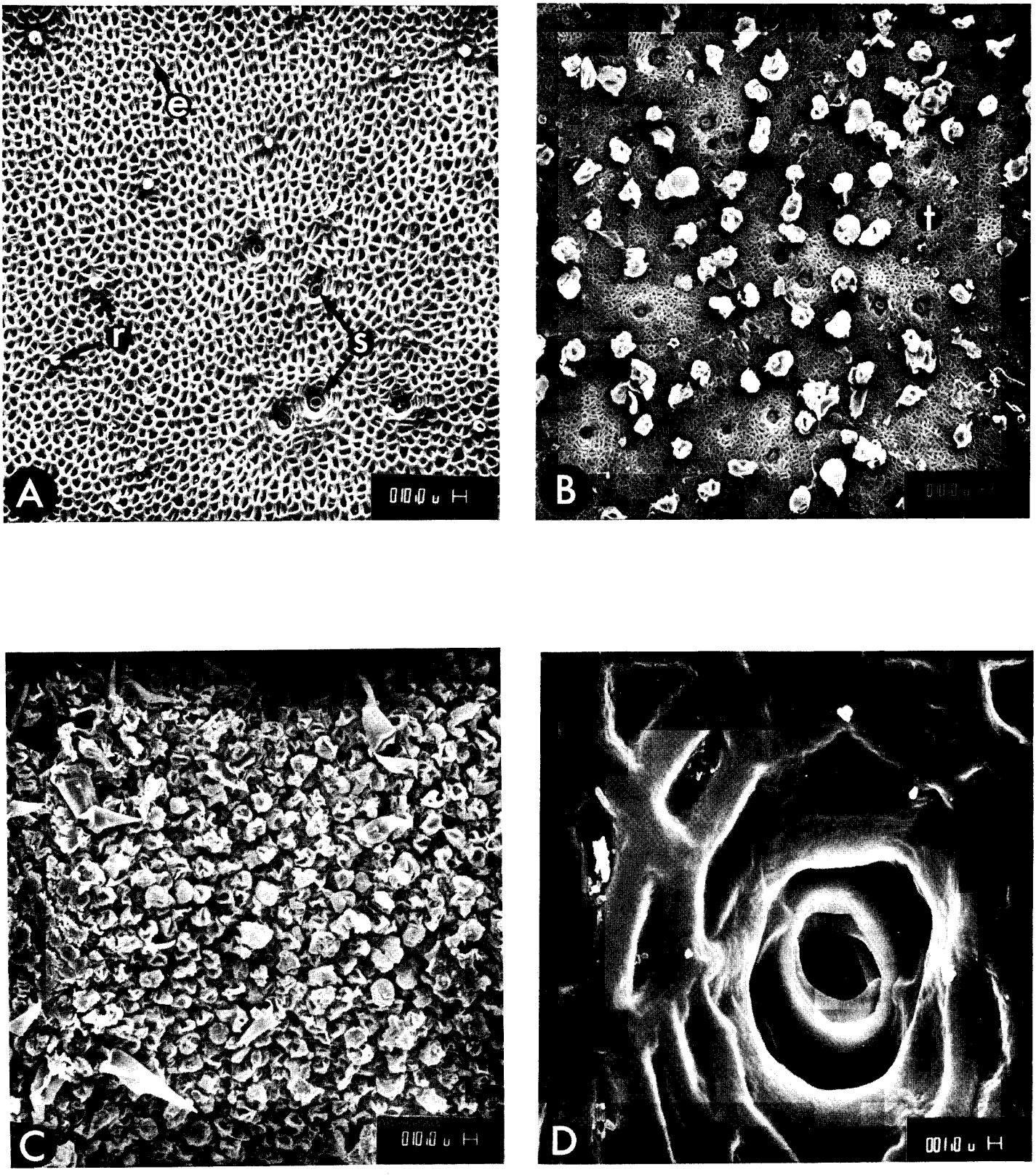
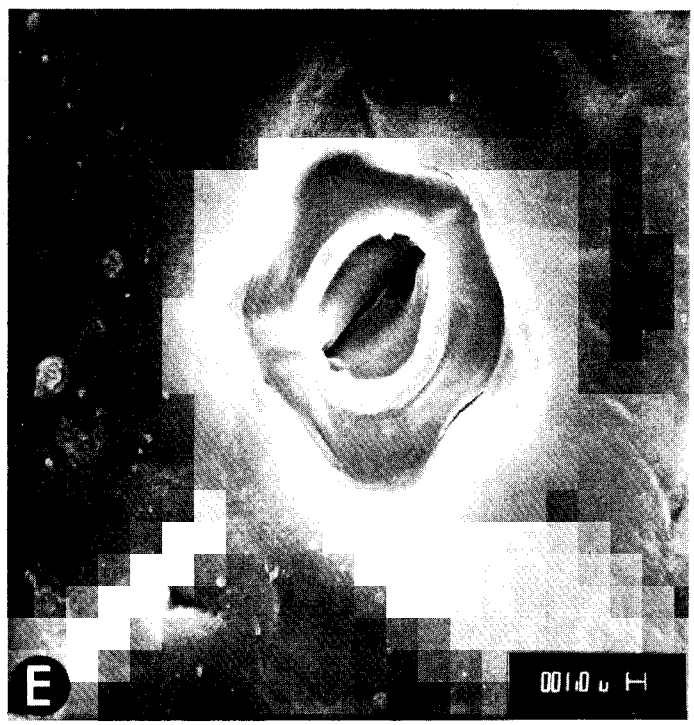


Fig. 1. Scanning electron micrographs of the surface of 'GY14' pickling cucumber fruit of various sizes showing general characteristics and stomata. A) 4.5 cm diameter fruit showing stomata (s), trichomal scars (r) and epidermal cells (e). B) 1.3 cm diameter fruit showing trichomes (t). C) 0.7 cm diameter fruit covered with trichomes. D) Open stoma on a 5.0 cm diameter fruit. E) Closed stoma on a 'Chipper' fruit of 5.0 cm diameter. F) Cross section of a stoma exposed by cutting through the cucumber fruit surface.



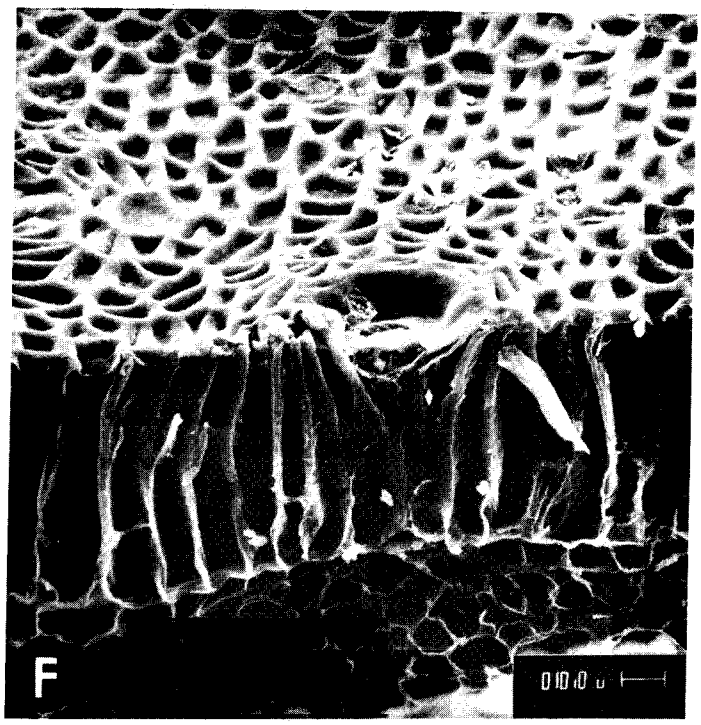


Table 1. Dimensions, frequency, and index of surface features on size no. 1 and no. 3 'GY14' fruit.[z]

Variable	Size of fruit		t value[y]
	no. 1, 2.0 cm diam	no. 3, 5.0 cm diam	
Dimensions			
Stomatal guard cells	8.5 x 5.0 μm	9.7 x 7.9 μm	1.86 4.16**
Area of stomatal pore[x]	33.6 μm^2	59.9 μm^2	7.52**
Trichomes	22.3 μm	23 μm	0.26
Trichomal scars	8.3 μm	8.7 μm	0.18
Frequency			
Epidermal cells/mm^2	45,109.9	13,707.4	20.38**
Stomata/mm^2	78.8	24.7	12.80**
Trichomes/mm^2	315.1	54.2[w]	47.00**
Index			
Stomatal	0.174	0.179	
Trichomal	0.694	0.394[w]	

[z]All measurements were made at the middle section of 10 fruit of each size. These fruit were harvested in June, 1977.

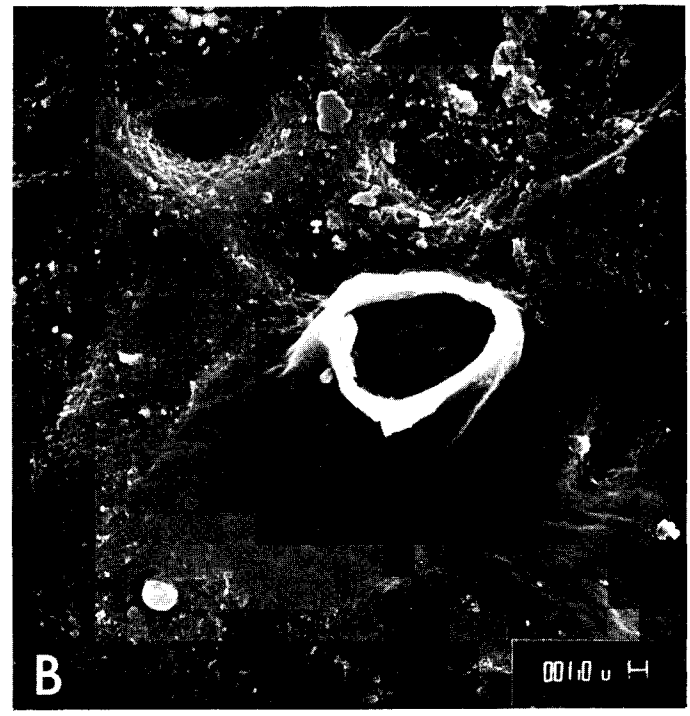
[y]Significant at the 1% probability level (**) with 9 degrees of freedom, using the 2-tailed "t" test (15).

[x]Area is calculated, assuming all stomatal guard cells are open. This assumption likely deviates from reality as stomatal opening is probably influenced by environmental factors as has been noted for stomata of leaves (10).

[w]Trichomal scars, particularly noticeable on no. 3 fruit, suggest that these values may be low due to breakage of trichomes on the larger fruit.

stomata averaged over 'GY14' and 'Chipper' cucumbers by section is given in Fig. 3. The occasional occurrence of microscopic fields with numerous stomata, but more frequent fields with 0 to 2 stomata, suggests a tendency for stomatal clumping on the fruit.

No. 3 'Chipper' and 'GY 14' cucumbers were estimated to contain 105,000 and 130,000 stomata per fruit, respectively, based on stomatal frequencies averaged over stem end, middle and blossom end sections (Table 2), and calculated surface areas for each fruit. Dimensions used to calculate surface areas were averaged for 10 fruit of each cultivar and applied to the formula for surface area given earlier. These surface areas for the no. 3 size fruit averaged 128 cm^2 for 'Chipper' and 126 cm^2 for 'GY14.' Stomatal pore areas were estimated at 0.118% and 0.062% of the surface of size no. 1 and 3 'GY14' fruit, respectively. These estimations were based on 130,000 stomata per fruit with average pore areas for no. 1 and 3 fruit as given in Table 1, assuming opened guard cells.



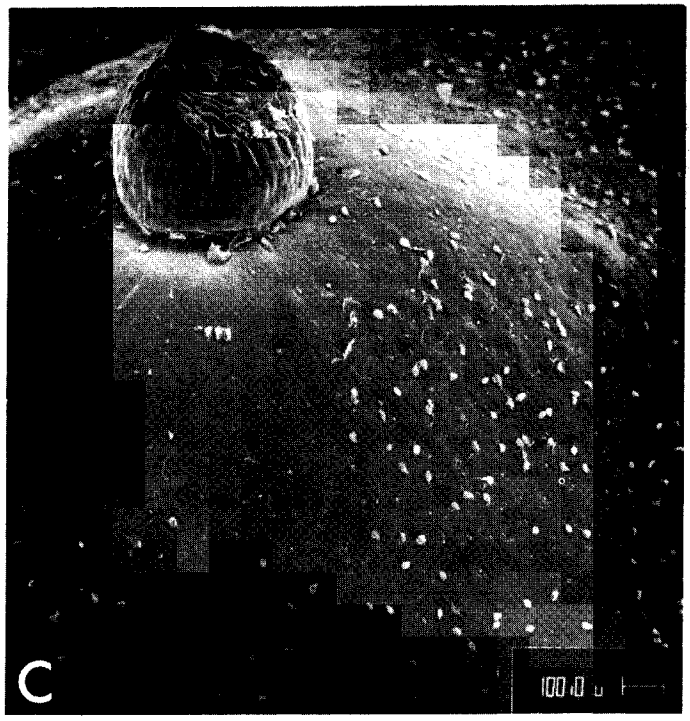


Fig. 2. Scanning electron micrographs showing a trichome, a trichomal scar and a wart on pickling cucumber fruit. A) Trichome on a 'GY14' fruit of 3.2 cm diameter. B) Scar left by removal of a trichome. C) Wart with spine attached on a 'Calypso' fruit of 2.8 cm diameter.

Table 2. Frequencies of epidermal cells and stomata by section on no. 3, 'Chipper' and 'GY14' fruit.[z]

Variable	Mean frequency per mm^2	t values[y]		Stomatal index
Epidermal cells				
'Chipper'				
Stem	7498.00	18.14		
Middle	9866.00	9.91	12.22	
Blossom	11543.00			
'GY14'				
Stem	7301.00	12.39		
Middle	9668.00	6.40	21.38	
Blossom	11247.00			
Stomata				
'Chipper'				
Stem	0.00	10.84		0.00
Middle	17.59	5.65	6.84	0.18
Blossom	6.83			0.06
'GY14'				
Stem	0.39	7.90		0.01
Middle	20.22	3.54	7.53	0.20
Blossom	10.36			0.09

[z]Epidermal cell frequencies were determined by averaging the cells in 10 random 1 cm^2 fields from a micrograph from each of 10 fruit. Stomatal frequencies were averaged from duplicate samples of 10 fruit; 10 random microscopic fields were counted from each sample. These fruit were harvested in September, 1977, and averaged 4.4 cm diam.

[y]All comparisons were significant at the 0.001 probability level with 9 degrees of freedom, using the two-tailed "t" test (15). First column refers to stem end-middle and middle-blossom end comparisons; second column refers to stem end-blossom end comparisons.

The stomatal indexes for size no. 1 and 3 'GY14' cucumbers were essentially the same (Table 1), confirming the constancy of this index for fruit within this size range. Stomatal indexes for the middle section of 'Chipper' (0.18) and 'GY14' (0.20) cucumbers were similar (Table 2).

Discussion

Since stomata were the only natural openings observed in cucumber fruit, it seems reasonable that they may be important

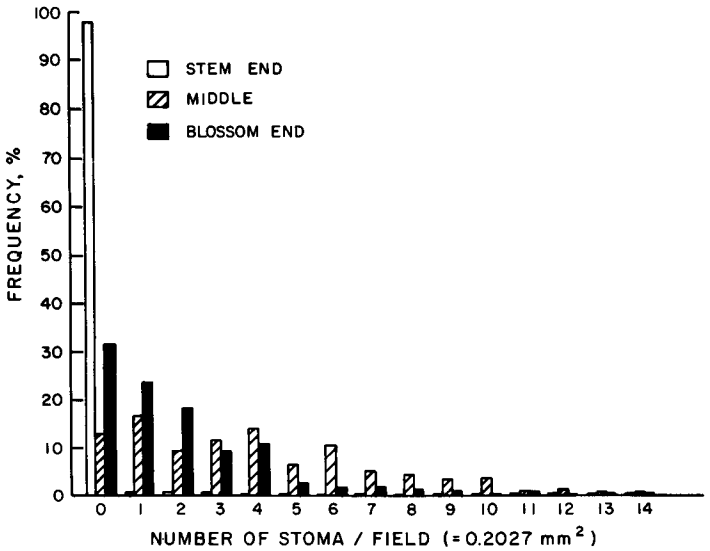


Fig. 3. Frequency distribution of stomata by location on cucumber fruit. Data averaged for 10 'Chipper' and 10 'GY14' fruit.

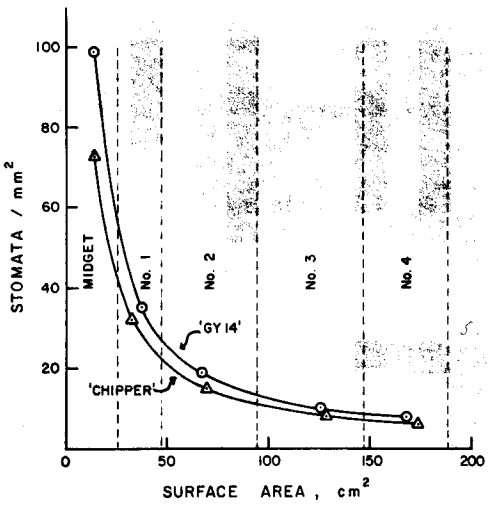


Fig. 4. Relationship between surface area and stomatal frequency of various sizes of 'Chipper' and 'GY14' cucumbers. Points for size no. 3 cucumbers were based on stomatal frequencies averaged over all sections (Table 2), from which other points were extrapolated.

in regulating moisture loss and gas exchange in the fruit. The rate of CO_2 exchange in brined cucumbers may be related to susceptibility of the fruit to bloater damage. Bloater damage apparently is caused by a buildup of CO_2 inside the fruit, although the mechanism is not fully understood.

The lower frequency of stomata in the epidermis of large, as compared to small, cucumbers may be an important factor contributing to greater susceptibility to bloater damage in large fruit. Lower stomatal frequency in the large fruit may result in lower rate of CO_2 exchange. Stomatal index relates stomatal frequency to total epidermal cell frequency. This index takes into account epidermal cell enlargement as the plant grows (10), the index remaining essentially constant after differentiation of stomata and through subsequent plant growth. The similarity of stomatal indexes for size no. 1 and 3 'GY14' cucumbers indicates that stomata were differentiated by the time the fruit reached the no. 1 size, or earlier. Thus, stomatal frequency can be estimated for any size of 'GY14' fruit equal to or larger than size no. 1 by determining the surface area, since the fruit contained a total of about 130,000 stomata (Fig. 4).

Differences in stomatal frequency among cultivars may contribute to variations in storage and brining properties of pickling cucumbers. Further studies on stomatal frequency and distribution, and factors which affect stomatal action, may prove useful in assessing the significance of stomata in relation to storage, bloater formation and other factors associated with pickling cucumbers.

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