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Title: Anatomical Changes During Fruit Ripening in Two Lines of Tabasco Pepper (*Capsicum frutescens*, Solanaceae)

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Two lines of tabasco pepper (*Capsicum frutescens*) were previously selected from the McIlhenny seed production field, that differ in ease of fruit separation at the fruit-pedicel separation zone; the fruits of"McIlhenny Select," or easy pick (EP), separate readily from the pedicel, and hard pick fruits (HP), require more force to detach from the pedicel. Greenhouse grown plants were investigated to identify anatomical differences, between the two lines of tabasco pepper that may be associated with fruit ripening and thus ease of separation. Light microscopy and quantitative morphometry were used to examine cells and intercellular spaces, in the separation zone and in the fruit walls, at three day intervals from anthesis through the mature red-fruit condition. There was a significant difference in cell length, width, and area in the peripheral region of the separation zone between the two lines. There was a significant difference in cell length, width, and area in the midway region of the fruit wall, and cell length and area in the distal region. At maturity, easy pick cells were larger than hard pick cells and the cell walls and cell contents of easy pick cells appeared to be breaking down in the distal region. The length, width, and area of intercellular spaces was significantly different in the proximal, middle, and distal regions of the fruit. This suggests that a combination of

larger cells and more enzymatic hydrolysis of fruit cell walls during maturity in the easy pick line than hard pick is responsible for the ease of fruit detachment in the easy pick.

Anatomical Changes During Fruit Ripening in Two lines of Tabasco Pepper (*Capsicum frutescens*, Solanaceae)

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PREFACE

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Introduction

Peppers are an important horticultural crop both in the United States, with 50,585 ha. in production (Motsenbocker *et al.*, 1996), and in the world, with more than 3,000,000 ha. being grown (Bosland and Votava, 2000). *Capsicum frutescens*, the commercial tabasco pepper, is a member of the family *Solanaceae* that also includes other economically important crops: tomato, potato, tobacco, and petunia. The genus *Capsicum* consists of approximately 27 wild species and five domesticated species: *C. annum, C. chinese, C. baccatum, C. pubescens,* and *C. frutescens* (Andrews, 1995). Peppers are the second most valuable vegetable species in the United States next to tomatoes (Andrews, 1995). Tabasco pepper, the trademark variety of McIlhenny Co., New Iberia, Louisiana, is used in producing Tabasco Pepper Sauce®. Tabasco peppers are an important crop for sauce production and fresh market because of their pungency (60,000 to 80,000 Scoville heat units) (Andrews, 1995). In 1991 the hot pepper sauce market was estimated to be worth $70 million with a 10 to 15 percent annual increase (Petoseed Co., unpublished data).

Pepper fruits vary in size and shape. Mature fruits of tabasco pepper are typically 2.5-3.0 cm long and 1.0 cm wide; they have pointed shape at the apex and obtuse shape at the pedicle attachment (Fig. 1) (Bosland and Votava, 2000). The pedicle is erect and there is no visible annular constriction at the junction of the calyx and the pedicle; the calyx usually encloses the base of the flower with an intermediate margin (Bosland and Votava, 2000). Mature fruits of tabasco pepper normally separate with little force at the fruit-pedicel separation zone (Motsenbocker, 1996), the zone at the base of fruit that

contains the abscission layer (Esau, 1960). When the fruit is harvested it leaves the pedicel attached to the stem (Fig.2).

In the field many hard pick (HP) fruits are tenacious at the separation zone and go unpicked as workers move to plants with more easily detached fruits. This tenaciousness at the fruit separation zone negatively impacts sauce companies' profits because unpicked fruits are left in the field and this could be a significant fraction of total production. Two lines of tabasco pepper were previously selected from the McIlhenny seed production field (Motsenbocker, 1996) that differ in ease of fruit separation at the fruit-pedicel separation zone. The fruits of "McIlhenny Select" or easy pick (EP), separate readily from the pedicel but the fruits of the Hard Pick (HP) line requires more force to detach the fruit from the pedicel (Fig.2).

Easy fruit detachment at the pedicel/fruit junction is a dominant genetic character in wild type pepper plant, compared to fruit persistence (Sundberg *et al.*, in press). Through domestication, this trait has been selected against, thus producing fruit that tends to stay attached to the plant until it is picked by humans (Bosland and Voatava, 2000). This is unfortunate because the presence of pedicel and attached calyces is undesirable both for fresh produce and sauce production (Sundberg *et al.*, in press)

Fruit detachment, in general, is related to many anatomical changes in the fruit-pedicel junction (separation zone), such as breakage of tissues, senescence of tissues involved in separation process, and dissolution of cells, cell walls and middle lamella (Kozlowski, 1973).

A few studies have been conducted on fruit detachment in peppers. Motsenbocker (1996) reported that HP fruits are characterized by an increase in sclereid

cells, a structural component, across the separation zone during development. He examined peppers differing in ease of fruit detachment for differences in cell type where the fruit and the receptacle join. Scanning electron microscopy revealed that HP fruit exhibited a distinct group of sclereid cells that extended from the periphery of the fruit into the receptacle for at least 15 cell layers (Motsenbocker *et al.,* 1996). In contrast, fruit of more easily detachable EP peppers had fewer sclereid cells in the separation zone (Motsenbocker *et al.,* 1996). Sundberg *et al.*, (in press) reported that during ripening, sclerification proceeded centripetally in both lines but was more pronounced in the hard pick line. Also, at maturity there was a greater volume of intercellular space in the central zone than in the peripheral zone of both lines.

To date, most of the research on cell separation has dealt with ripening of fruits, especially of tomato (Bonghi *et al.,* 1992). The tomato fruit, *Lycopersicum esculentum,* has for some time been the favored model system for the study of fruit ripening, particularly as related to cell wall metabolism (Huysamer *et al.,* 1997).

The mechanical strength and texture of cell walls change dramatically during fruit softing process (Wakabayashi, 2000). In addition, ripe fruits contain large amounts of hydrolases that are involved in degradation of cell wall polymers. Thus, fruit ripening is a good model system to study the relationship between mechanical strength and structural features of cell walls and also the function and regulation of cell wall hydrolases in the degradation of cell walls (Wakabayashi, 2000).

As fruits ripen and soften, their cell walls undergo chemical and physical changes. Chemical changes that have been studied include: solubilization and degradation of pectin, a major component of the middle lamella that "glues" adjacent cells together; loss

of neutral sugars from pectinside-chains; and reduction in molecular weight of xyloglucan (hemicellulosic polysaccharide) (Redgwell *et al.*, 1997). Hydrolysis of pectic polysaccharides plays a major role in fruit ripening and is primarily responsible for the softing of fleshy fruits. Pectins account for up 60 % of cell wall mass in many fruits (Redgwell *et al.*, 1997).

Many other hydrolases are involved in tomato fruit ripening process such as cellulase (β-1,4-glucanase), polygalacturonases (PGs), endo β-mannase, pectin methylestrase (PME), and pectate lyases, which are thought to be involved in demethylation of the pectins and thus the breakdown of middle lamella (Patterson, 2001). All these enzymes can be present in a plant in a number of isoforms which are differentially expressed during the cell separation process (Bonghi *et al.*, 1992).

Patterson (2001) reported that increases in (PG) activity have been measured in several plant species, including tomato, during fruit ripening and three main (PG) isoforms were associated with fruit abscission in tomato. Also, increases in β-1,4-glucanase expression have been reported during the abscission of tomato flowers, and flowers and leaves of pepper (Roberts *et al.*, 2002). Blumer et al., (2000) found that (PME) activity in tomato fruit increased two to three-fold during ripening. In addition to cell wall degradation, Whitaker *et al.*, (2000) reported that the total phospholipid (PL) declined and phosphatidic acid increased in pericarp tissue during tomato fruit ripening, suggesting that increased Phospholipase activity alters membrane structure.

Immediately before and during cell separation, hydrolases for cell wall weakening are produced (Bonghi *et al.*, 1992). Also, pectic polymers are major constituents of the middle lamella and thus contribute to the cell adhesion mechanism. Degradation of

pectins, particularly that of polyuronides (Wakabayashi, 2000), may cause the collapse of cell adhesion and thereby decrease tissue strength. Finally, the number and the volume of the intercellular spaces between the adjacent cells are increased, and cells start to elongate in association with cell wall degradation process (Sundberg *et al.*, in press).

The objectives of this research were to identify any anatomical differences between the two lines of tabasco pepper that may be associated with fruit ripening and thus ease of separation. Light microscopy and quantitative morphometry were used to examine cells and intercellular spaces in the separation zone and in the fruit walls during different stages of fruit ripening and development. In this way we could determine if differences in fruit separation are due primarily to factors restricted to separation zone, or if distinctive changes occur throughout the entire fruit.

Materials and Methods

1. Sampling

Ten newly opened flowers each from easy and hard pick lines were tagged at (3 day) intervals in 1997 beginning as the first-tagged flowers reached anthesis and continuing until the fruits from those flowers were in the mature red stage. Fruits were randomly tagged on from one of four greenhouse-grown plants of each line. After 36 days all tagged fruits were collected. A total of thirteen collections were made of each line. The specimens can be described as belonging to one of 5 developmental stages: 3 mature red collections 1-3; 1 breaker, collection 4; 2 early breaker, collections 5 and 6; 1 mature green, collection7; and 6 immature green, collections 8-13 indexed by fruit size and color (Munsell book of color, 1976). The plants in this study were originally selected by Motsenbocker (1996) from a heterogeneous population of tabasco pepper in a

production field on Avery Island, Louisiana. All of the samples from each collection were prepared for microscopic examination as outlined below.

2. Slide preparation

Specimens were paraffin processed following standard procedures (Berlyn and Mischke, 1976); briefly, they were fixed in formalin-acitic-acid (FAA), dehydrated in a tert-butyl alcohol series and embedded in paraplast®. Serial longitudinal sections from at least 3 fruits from each collection were cut at 10 μm, mounted on slides and stained with safranin/fast green. Near-median sections were analyzed.

3. Analyses

For each specimen, analyses of cell length, width, area, perimeter, volume, and shape factor were made at 45 X, using morphometric analysis techniques (Toth, 1982). Similar determinations were made for intercellular spaces. Sigma Scan software (Jandel Scientific, San Rafael, CA.) was used to analyze digital images made with a Kodak UFX-DX Camera on a Nikon Y-FL 074806 light microscope. Analyses were made of cells in the center and peripheral regions of the separation zone, and in the fruit wall at the proximal end of the fruit, midway to the tip, and at the distal end of the fruit (Fig. 3). In a preliminary analysis pooled data from fruits of the same-staged line was used to examine trends for all characters. In the primary analysis length, width, area, and shape factor of both cells and intercellular spaces were compared at different developmental stages within a line using linear regression and runs test, and between lines using a two-sample t-test (Zar, 1999).

RESULTS

Six parameters (width, length, perimeter, volume, area, and shape factor) were measured for cell and intercellular spaces analyses. In the preliminary analysis, regression plots of average value for parameter vs marking day demonstrated that there was no difference between lines for two factors, perimeter and volume. Therefore, these factores are not included in the more detailed analysis that follows.

The regression statistics for the length, width, area, and shape factor of cells and intercellular spaces are summarized in tables 1 and 2 respectively.

The correlation coefficients of all regressions were low, but the linearity of these regressions was confirmed by subjecting the residuales of each regression to a runs test (Zar, 1999). None of the regressions tested positive for a non-random pattern.

I. Separation Zone

At early stages, the separation zone of both lines consisted of parenchyma cells with intact cytoplasm and large vacuoles. In some cells depostion of early secondary walls was indicted by faint red staining and increased wall thickness. The intercellular spaces were very distinct (Fig. 4). In the mature red stages cells had well developed secondary walls that were thick and dark red staining. No cytoplasm was evident in these sclereid cells (Fig.4). In both lines, sclereids were more pronoucced in HP than EP in both central and peripheral regions.

A. Easy Pick, Quantitative parameters

1. Central Region

a. Cells

Cell width, length, area, and shape factor (S.F) decreased during fruit ripening (Fig. 5, 6, 7, 8). The calculated "Y" value, from the regression line, for width in the mature red (MR) stage, marking day 1, was 25.04 while it was 33.92 in the immature green stage (IMG), marking 13. Corresponding values for length was 34.09 in (MR) and 46.51 in (IMG); for area was 614.66 in (MR) and 1176.46 in the (IMG); and for shape factor (S.F) was 0.73 in (MR) and 0.79 in (IMG). All parameters were significantly different from 0-slope (width, $F > 0.0019$; length, $F > 0.0023$; area, $F > 0.0028$; S.F, $F > 0.0128$).

b. Intercellular Spaces

Width, length, area, and shape factor of the intercellular spaces also decreased during fruit ripening (Fig. 9, 10, 11, 12). The calculated "Y" value for width was 6.37 in (MR) and 11.43 in (IMG). Corresponding values for length was 13.30 in (MR) and 17.53 in (IMG); for area in the (MR) was 61.52 and 141.93 in the (IMG); and for S.F in the (MR) was0.54 and 0.62 in the (IMG). All parameters were significantly different from 0-slope (width, $F > 0.0056$, length, $F > 0.189$; area, $F > 0.03$; S.F, $F > 0.17$).

2. Peripheral Region

a. Cells

As in the central region, the cell width, length, area, and S.F decreased during fruit ripening (Fig. 5, 6, 7, 8). The calculated "Y" value for width in (MR) was 22.66 and 25.72 in (IMG). Corresponding values for length in (MR) was 29.29 and 35.36 in (IMG); for area in (MR) was 480.60 and 674.80 in (IMG); and for S.F in (MR) was 0.72 and 0.82 in (IMG). All parameters were significantly different from 0-slope (width, $F > 0.09$; length, $F > 0.005$; area, $F > 0.0045$; S.F, $F > 0.0061$).

b. Intercellular Spaces

Width, area, and S.F of intercellular spaces decreased during fruit ripening, but length of intercellular spaces increased (Fig. 9, 10, 11, 12). The calculated "Y" value for width in (MR) was 5.52 and 6.64 in (IMG). Corresponding values for length in (MR) was 11.14 and 10.94 in (IMG); for area in (MR) was 46.65 and 53.05; and for S.F in (MR) was 0.59 and 0.67 in (IMG). Width ($F > 0.25$) and S.F ($F > 0.29$) were significantly different from 0-slope, while length ($F < 0.94$) and area ($F < 0.68$) were not.

B. Hard Pick, quantitative parameters

1. Central Region

a. Cells

Cell width, length, area, and S.F decreased during fruit ripening (Fig. 13, 14, 15, 16). The calculated "Y" value for width in (MR) was 24.92 and 36.33 in (IMG). Corresponding values for length in (MR) was 33.57 and 47.46 in (IMG); for area in (MR) was 622.91 and 1279.36 in (IMG); and for S.F in (MR) was 0.74 and 0.80 in (IMG). All parameters were significantly different from 0-slope (width, $F > 0.00003$; length, $F > 0.00003$; area, $F > 0.00001$; S.F, $F > 0.022$).

b. Intercellular Spaces

The width and length of intercellular spaces increased during fruit ripening, but area and S.F decreased (Fig. 17, 18, 19, 20). The calculated "Y" value for width in (MR) was 39.82 and 14.24 in (IMG). Corresponding values for length in (MR) was 14.44 and 13.65 in (IMG); for area in (MR) was 70.94 and 81.57 in (IMG); and average S.F in (MR) was

0.5 and 0.6 in (IMG). Width ($F > 0.32$) and S.F ($F > 0.07$) were significantly different from 0-slope, meanwhile length ($F < 0.73$) and area ($F < 0.57$) were not.

2. Peripheral Region

a. Cells

Cell width increased during fruit ripening, but length, area, and S.F decreased (Fig. 13, 14, 15, 16). The calculated "Y" value for width in (MR) was 26.17 and 25.70 in (IMG). Corresponding values for length in (MR) was 34.23 and 34.37 in (IMG); for area in (MR) was 667.27 and 672.25 in (IMG); and for S.F in (MR) was 0.71 and 0.80 in (IMG). Width($F > 0.08$), length($F > 0.014$), and area($F > 0.01$) were not significantly different from 0-slope, S.F($F > 0.01$) was significant.

b. Intercellular Spaces

Width, length, and area of intercellular spaces increased during ripening, but S.F decreased (Fig. 17, 18, 19, 20). The calculated "Y" value for width in (MR) was 7.44 and 5.65 in (IMG). Corresponding values for length in (MR) was 14.67 and 8.86 in (IMG); for area in (MR) was 80.34 and 28.57 in (IMG); and for S.F in (MR) was 0.52 and 0.63 in (IMG). All Parameters were significantly different from 0-slope (width, $F > 0.08$; length, $F > 0.014$; area, $F > 0.01$; S.F, $F > 0.01$).

C. Comparison of Easy Pick vs Hard Pick

There were no significant differences in cells at the separation zone, central region, between the two lines, although mature easy pick cells tended to be larger. However, width ($t = 2.46$, $0.02 > P > 0.01$, $n = 78$), length ($t = 3.38$, $0.002 > P > 0.001$, $n = 78$), and area ($t = 3.02$, $0.005 > P > 0.002$, $n = 78$) were significantly different in the peripheral region (Figs. 5, 13; 6, 14; and 7, 15). At maturity, easy pick cells were

smaller than their hard pick counter parts in all parameters although they were larger in the immature green stage. In the central region, intercellular space length ($t = 2.59$, $0.02 > P > 0.01$, $n = 83$) and area ($t = 3.56$, $P < 0.001$, $n = 83$) were significantly different between the two lines (Figs.10, 18 and 11, 19). At maturity intercellular spaces were smaller in all dimensions in the easy pick line than in their hard pick counter parts, while intercellular spaces were larger in immature easy pick in the peripheral region. Intercellular spaces width ($t = 3.99$, $P < 0.001$, $n = 78$), length ($t = 3.16$, $0.005 > P > 0.002$, $n = 78$), and area ($t = 4.19$, $P < 0.001$, $n = 78$) were significantly different between both lines (Figs.9, 17; 10, 18; and 11, 19). Again, the easy pick intercellular spaces were smaller in the mature fruit than similar spaces in the hard pick line while they were of comparable width, but longer than hard pick in the immature stage.

II. Fruit Wall

In EP line, cell size in the immature green stage was smaller than in the mature red stage. While in the HP line, cell size in the immature green was larger than the red mature stage (Fig. 21). At maturity, cell walls in the easy pick were less distinct and cytoplasm was more diffuse comparing to hard pick cells of the same stage. This was true for all three regions examined.

A. Easy Pick

1. Proximal Region

a. Cells

Cell width, length, and area increased during fruit ripening, while S.F decreased (Fig. 5, 6, 7, 8). The calculated "Y" value for width in (MR) was 45.86 and 43.66 in (IMG). Corresponding values for length in (MR) was 114.07 and 113.29 in (IMG); for

area in (MR) was 4018.71 and 3895.64 in (IMG); and for S.F in (MR) was 0.58 and 0.61 in (IMG). Width ($F < 0.59$), length ($F < 0.95$); and and area ($F < 0.87$) were not significantly different from 0-slope; S.F ($F > 0.33$) was significant.

b. Intercellular Spaces

Width, length, and area of intercellular spaces increased during fruit ripening, while S.F decreased (Fig. 9, 10, 11, 12). The calculated "Y" value for width in (MR) was 9.38 and 7.56 in (IMG). Corresponding values for length in (MR) was 17.82 and 16.51 in (IMG); for area in (MR) was 106.96 and 95.03 in (IMG); and for S.F in (MR) was 0.51 and 0.54 in (IMG). Width ($F > 0.12$) and S.F ($F > 0.37$) were significantly different from 0-slope; length ($F < 0.69$) and area ($F < 0.7$) were not significant.

2. Midway Region

a. Cells

Cell width, length, and area increased during fruit ripening, S.F was decreased (Fig. 5, 6, 7, 8). The calculated "Y" value for width in (MR) was 47.19 and 46.83 in (IMG). Corresponding values for length in (MR) was 101.45 and 91.68 in (IMG); for area in (MR) was 3478.47 and 294.68 in (IMG); and for S.F in (MR) was 0.62 and 0.69 in (IMG). Width ($F < 0.93$) and area ($F < 0.68$) were not significantly different from 0-slope, while length ($F > 0.27$) and area($F > 0.024$) were significant.

b. Intercellular Spaces

Width, length, area, and S.F of intercellular spaces decreased during fruit ripening (Fig. 9, 10, 11, 12). The calculated "Y" value for width in (MR) was 6.80 and 8.80 in (IMG). Corresponding values for length in (MR) was 14.56 and 14.96 in (IMG); for area in (MR) was 71.12 and 86.61 in (IMG); and for S.F in (MR) was 0.525 and 0.529 in

(IMG). Width ($F > 0.0.09$) was significantly different from 0-slope, meanwhile length ($F < 0.88$), area ($F < 0.53$), and S.F ($F < 0.9$) were not significant.

3. Distal Region

a. Cells

Cell width and S.F decreased during fruit ripening, but length and area increased (Fig. 5, 6, 7, 8). The calculated "Y" value for width in (MR) was 49.74 and 51.61 in (IMG). Corresponding values for length in (MR) was 104.39 and 83.01 in (IMG); for area in (MR) was 3802.71 and 3308.63 in (IMG); and for S.F in (MR) was 0.63 and 0.73 in (IMG). Width ($F < 0.67$) was not significant from 0-slope; length ($F > 0.008$), area ($F > 0.32$), and S.F ($F > 0.0007$) were significantly different.

b. Intercellular Spaces

Width, area, and S.F of intercellular spaces decreased during fruit ripening, but length increased (Fig. 9, 10, 11, 12). The calculated "Y" value for width in (MR) was 8.20 and 9.67 in (IMG). Corresponding values for length in (MR) was 59.04 and 3.78 in (IMG); for area in (MR) was 93.16 and 95.72 in (IMG); and for S.F in (MR) was 0.48 and 0.58 in (IMG). Width ($F > 0.33$), length ($F > 0.23$), and S.F ($F > 0.0004$) were significantly different from 0-slope, area ($F < 0.93$) was not significant.

B. Hard Pick

1. Proximal Region

a. Cells

Cell width, length, area, and S.F decreased during fruit ripening (Fig. 13, 14, 15, 16). The calculated "Y" value for width in (MR) was 45.53 and 47.01 in (IMG). Corresponding values for length in (MR) was 88.68 and 104.03 in (IMG); for area in

(MR) was 3012.97 and 3584.33 in (IMG); and for S.F in (MR) was 0.62 and 0.64 in (IMG). Width ($F < 0.75$) and S.F ($F < 0.61$) were not significantly different from 0-slope, while length ($F > 0.1$) and area ($F > 0.29$) were significant.

b. Intercellular Spaces

Width, length, and area of intercellular spaces increased during fruit ripening, but S.F decreased (Fig. 17, 18, 19, 20). The calculated "Y" value for width in (MR) was 7.18 and 4.94 in (IMG). Corresponding values for length in (MR) was 14.76 and 7.79 in (IMG); for area in (MR) was 70.64 and 20.24 in (IMG); and for S.F in (MR) was 0.50 and 0.54 in (IMG). Width ($F > 0.016$), length ($F > 0.008$), and area ($F > 0.004$) were significantly different from 0-slope; S.F ($F < 0.45$) was not significant.

2. Midway Region

a. Cells

Cell width, length, area, and S.F decreased during fuit ripening (Fig. 13, 14, 15, 16). The calculated "Y" value for width in (MR) was 43.27 and 50.14 in (IMG). Corresponding values for length in (MR) was 75.95 and 104.49 in (IMG); for area in (MR) was 2320.93 and 3986.38 in (IMG); and for S.F in (MR) was 0.64 and 0.66 in (IMG). Width ($F > 0.14$), length ($F > 0.002$), and area ($F > 0.001$) were significantly different from 0-slope, S.F ($F < 0.65$) was not significant.

b. Intercellular Spaces

Width, length, and area of intercellular spaces increased during fruit ripening; S.F decreased (Fig. 17, 18, 19, 20). The calculated "Y" for width in (MR) was 6.60 and 5.71 in (IMG); for length in (MR) was 14.29 and 7.00 in (IMG); for area in (MR) was 69.70 and 20.07 in (IMG); and for S.f in (MR) was 0.48 and 0.53 in (IMG). Width ($F > 0.44$),

length ($F > 0.01$), and area ($F > 0.02$) were significantly different from 0-slope; S.F ($F < 0.59$) was not significant.

3. Distal Region

Cell width, length, area, and S.F decreased during fruit ripening (Fig. 13, 14, 15, 16). The calculated "Y" value for width in (MR) was 47.83 and 51.73 in (IMG). Corresponding values for length in (MR) was 83.94 and 87.54 in (IMG); for area in (MR) was 2959.67 and 3434.19 in (IMG); and for S.F in (MR) was 0.63 and 0.72 in (IMG). Width ($F < 0.49$) and length ($F < 0.7$) were not significantly different from 0-slope, while area ($F > 0.39$) and S.F ($F > 0.01$) were significant.

b. Intercellular Spaces

Width, length, and area of intercellular spaces increased during fruit ripening, S.F decreased (Fig. 17, 18, 19, 20). The calculated "Y" value for width in (MR) was 6.99 and 4.88 in (IMG). Corresponding values for length in (MR) was 14.62 and 6.23 in (IMG); for area in (MR) was 73.49 and 15.30 in (IMG); and for S.F in (MR) was 0.51 and 0.56 in (IMG). Width ($F > 0.08$), length ($F > 0.006$), area ($F > 0.01$), and S.F ($F > 0.31$) were significantly different from 0-slope.

4. Comparison of Easy and Hard Pick Fruit Walls

In the Distal Region, hard pick cells were larger than their easy pick counterparts during early development. However, by the mature red stage easy pick cells were consistently larger. The differences in cell length ($t = 3.6$, $P < 0.001$, $n = 117$) and area ($t = 2.27$, $0.05 > P > 0.02$, $n = 117$) were significant between the two lines (Figs. 6, 14 and 7, 15). In the Midway Region, cell width was approximately the same between mature red fruits of the two lines (Figs. 5, 13). Cell length and area showed the same pattern as

in the Distal Region. At maturity the easy pick cells were larger but in the early immature green stage they were smaller than coresponding HP cells (Fig. 21). There were significant differences in cell width ($t = 2.17$, $0.05 > P > 0.02$, $n = 118$), length ($t = 5.56$, $P < 0.001$, $n = 118$), and area ($t = 5.09$, $P < 0.001$, $n = 118$) (Figs. 5, 13; 6, 14; and 7, 15). In the Proximal Region, mature red easy pick were larger than their HP counterparts. Immature easy pick cells also were consistently larger. However, none of these comparisons were significant.

In the Proximal Region, the intercellular spaces between easy pick cells were larger than between the HP in all dimensions. Intercellular space length ($t = 2.34$, $0.05 > P > 0.02$, $n = 125$) was significant between the two lines (Figs. 10, 18). In the Midway region, intercellular spaces were larger in easy pick than hard pick (all dimensions at all stages). The differences in intercellular space width ($t = 3.17$, $0.002 > P > 0.001$, $n = 120$), length ($t = 3.53$, $P < 0.001$, $n = 120$), and area ($t = 3.54$, $P < 0.001$, $n = 120$) were significant (Figs 9, 17; 10, 18; and 11, 19). In the Distal Zone, width and area of intercellular spaces were larger in EP than in HP at the immature green stage, but consistently larger at maturity. The intercellular spaces differences in width ($t = 3.07$, $0.005 > P > 0.002$, $n = 116$) and area ($t = 2.66$, $0.01 < P > 0.005$, $n = 116$) were significant between the two lines (Figs. 9, 17 and 11, 19). EP cells were larger than HP in mature red but smaller in the immature stage. Again, mature red easy pick cells were larger than HP but smaller in immature green stage. The differences in length ($t = 3.6$, $P < 0.001$, $n = 117$) and area ($t = 2.27$, $0.05 > P > 0.02$, $n = 117$) were significant (Figs. 6, 14 and 7, 15).

Discussion

Both in EP and HP fruits, sclereids differentiated in the separation zone (both central and peripheral regions) during the immature green stage, but they were more pronounced in HP line than EP. Thus, some similar physical and/or structural changes occured in the fruit separation zone in both lines that hardenned the tissue at the end of the peduncle. However, there were differences as well. In the central region, all measured parameters were negatively associated with ease of fruit separation at maturity. That is, smaller mature cells in EP were associated with easier separation. A similar pattern was observed in the peripheral region. Developing sclereids in the peripheral region were smaller in the immature green HP but mature sclereids were larger in the mature red stage compared to the EP line. This suggests that the increased overall lignification of larger sclereids in the mature hard pick is associated with greater tenacity of the fruit.

Intercellular spaces were more pronounced, particularly in EP, in the central region of the separation zone in both lines (Fig. 22). In the peripheral region, larger spaces were more pronounced in mature HP than EP, but at the immature green stage there were much smaller spaces in the HP compared to EP. In general, the tendency was for intercellular spaces to increase during early fruit development, when the cells are capable of enlarging and stretching, but then to decrease as cells matured. The larger intercellular spaces in the central region of the mature EP would explain why the fruit separate so cleanly from the peduncle. If most of the pectic compounds holding cells together was already digested, permitting larger spaces to form, parenchyma cells from

the fruit would not remain attached to the peduncle where the fruit detached. This pattern is similar to that described by Sundberg *et al*,. (in press) (Fig. 4).

Elongation of developing sclereid cells in the peripheral region during immature stages in the EP comparing to HP would create more spaces between the adjacent cells. This support the idea that a less force would be needed on the separation zone in the EP line than HP (Sundberg et al., in press).

Cell walls of developing fruits in immature EP were very uniform and distinct and the cytoplasm appeared "normal" compared to the mature red stage where walls were less distinct, more irregular, and the cytoplasm appeared degenerate. In HP the cell walls became even more clear and distinctive as they matured. This suggests that at maturity the cell walls of HP had greater integrity these of the EP line. One explanation could be that there was more enzymatic hydrolysis of cell walls during maturation in the EP line than HP. If some components of the cell walls were hydrolyzed, the remaining components would be held more loosely and the walls would appear fainter, thicker, and "fuzzier" when viewed microscopically. In tomato, there was a correlation between cell wall swelling and the degree of pectin solubilization. Cell walls were very distinct and thicker in unripe tomato comparing to ripe tomato, suggesting that wall swelling occurred as a result of changes to viscoelastic properties of the cell wall during pectin solubilization creating more intercellular spaces (Redgwell *et al.*, 1997). A similar process may occur in tabasco. This supports that (PGs) activity increase during fruit ripening (Patterson, 2001).

In both lines, there was a cell size gradient over the length of the fruit wall, starting from the proximal region (largest cell size) toward the distal region (smallest cell

size). This suggests that cell elongation may start from the proximal region and progresses toward distal end so that at maturity the proximal cells had elongated for longer time. Alternatively, elongation could have been uniform throughout the fruit but cell maturation and arrest of growth.

EP cells in immature green stage, in all regions, were smaller than their HP counterparts, but consistently they were larger in the mature red stage. Again, this implies a difference in growth rates between the two lines with either an increase in EP, or a decrease in HP. Mature EP cells were more elongated in all regions comparing to HP. This supports the acid growth theory of cell elongation; that there was greater cell wall loosing in EP line during development in which weakened cell walls would allow greater stretching than in HP. Intercellular spaces were more pronounced in EP line, in all regions of the fruit wall. This suggests an inverse association between thickness of cell wall and the size of intercellular spaces could play a major role in the ease of fruit separation.

Future studies should be conducted in fruit ripening in tabasco pepper, especially enzyme localization and changes in gene expression during fruit maturation.

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Table 1. Regression statistics for cells parameters (length (L), width (W), area (A), and shape factor (S.F) in all fruit locations (central region (A), peripheral region (B), proximal region (C), midway region (D), and distal region (E). Slope of EP cells (b1), slope of HP cells (b2); t.values between the two slopes at $\alpha = 0.05$; regression coefficient (R^2). The (*) represents the significant values of (t).

Location, Parameter	EP(Cells) b1	HP(Cells) b2	EP(Cells) intercept	HP(Cells) intercept	t. value	EP(Cells) R^2	HP(Cells) R^2	v. value
A, W	0.7399	0.95049	24.30095	23.97029	-1.5527	0.248725	0.442712	79
A, L	1.03429	1.15702	33.06455	32.42101	-0.6026	0.241452	0.323017	79
A, A	46.8171	54.7052	567.8428	568.2021	-0.9148	0.233818	0.412104	79
A, S.F	0.0056	0.00485	0.722052	0.732945	0.48733	0.168754	0.111539	79
B, W	0.25498	-0.03886	22.4032	26.20928	2.45881*	0.086456	0.001258	74
B, L	0.50592	0.01123	28.78691	34.22159	3.37819*	0.218981	6.74E-05	74
B, A	16.1838	0.41515	464.4146	666.8508	3.01762*	0.225667	6.79E-05	74
B, S.F	0.00794	0.00791	0.714015	0.698068	0.0194	0.211582	0.20112	74
C, W	-0.18271	0.12342	46.04469	45.40296	-1.0626	0.00414	0.00165	126
C, L	-0.06531	1.27873	114.1383	87.40501	-1.6248	4.96E-05	0.033684	126
C, A	-9.46631	47.6136	4028.173	2965.357	-1.3595	0.0004	0.018997	126
C. S.F	0.00289	0.00158	0.572969	0.617306	0.54848	0.013699	0.004421	126
D, W	-0.02994	0.57526	47.21833	42.64972	-2.1735*	0.000122	0.041604	114
D, L	-0.81436	2.37785	102.2674	73.57655	-5.5613*	0.019247	0.164386	114
D, A	-15.3141	138.787	3493.779	2182.145	-5.0973*	0.002692	0.176603	114
D, S.F	0.00626	0.0017	0.610078	0.637664	1.88317	0.077415	0.004032	114
E, W	0.15633	0.32152	49.58019	47.50702	-0.4889	0.002813	0.009044	113
E, L	-1.78192	0.30004	106.1721	83.63723	-3.6029*	0.109679	0.002767	113
E, A	-41.1738	39.5438	3843.886	2920.121	-2.2751*	0.016091	0.013793	113
E, S.F	0.00802	0.00792	0.624115	0.619391	0.04529	0.170016	0.113759	113

Table 2. Regression statistics for intercellular spaces parameters (length (L), width (W), area (A), and shape factor (S.F) in all fruit locations (central region (A), peripheral region (B), proximal region (C), midway region (D), and distal region (E). Slope of EP cells (b1), slope of HP cells (b2); t.values between the two slopes at $\alpha = 0.05$; regression coefficient (R^2). The (*) represents the significant values of (t).

Location, Parameter	EP(I.S) b1	HP(I.S) b2	EP(I.S) intercept	HP(I.S) intercept	t. value	EP(I.S) R^2	HP(I.S) R^2	v. value
A, W	0.426834	-4.50548	5.938956	44.32456	1.806283	0.204522	0.022126	79
A, L	0.353215	-0.06588	12.94182	14.50449	2.587455*	0.050112	0.002684	79
A, A	6.70707	0.886407	54.81886	70.05071	3.652911*	0.130542	0.00708	79
A, S.F	0.00634	0.007782	0.538695	0.502205	-0.45118	0.05408	0.07289	79
B, W	0.092878	-0.1378	5.430164	7.442331	3.889565*	0.041031	0.070589	74
B, L	-0.01671	-0.48382	11.15489	15.15318	3.160319*	0.000209	0.134419	74
B, A	0.533041	-4.31395	46.12147	84.65178	4.194696*	0.00546	0.146909	74
B, S.F	0.006471	0.00949	0.586053	0.507458	-0.89929	0.034834	0.145729	74
C, W	-0.15152	-0.18671	9.527575	7.365287	0.496505	0.034508	0.097266	125
C, L	-0.10921	-0.57991	17.92838	15.33162	2.339954*	0.002301	0.114264	125
C, A	-0.9944	-4.19996	107.9509	74.84218	1.826398	0.002186	0.135585	125
C, S.F	0.002929	0.003052	0.503839	0.493985	-0.04373	0.011674	0.009865	125
D, W	0.167273	-0.07418	6.629242	6.669825	3.168019*	0.04321	0.011194	116
D, L	0.032977	-0.60783	14.52749	14.90276	3.5292*	0.000326	0.108698	116
D, A	1.290636	-4.13572	69.83312	73.83301	3.535446*	0.006183	0.090122	116
D, S.F	0.000307	0.001674	0.524973	0.487312	-0.55342	0.000146	0.005379	116
E, W	0.122182	-0.17647	8.077863	7.169802	3.074258*	0.014973	0.059073	112
E, L	-4.6046	-0.69923	63.6423	15.32134	-1.35315	0.018455	0.141226	112
E, A	0.212618	-4.84906	92.95204	78.3381	2.657703*	0.000121	0.106153	112
E, S.F	0.008856	0.004645	0.466984	0.502557	1.420777	0.123253	0.020026	112

Figure 1. Tabasco pepper fruits at different stages of maturity. From left: 3 immature green; breaker; mature green; mature red; early breaker.

Figure 2. Pedicels and detached red-matured fruits of "McIlhenny Select" (right) and hard pick (HP) tabasco pepper (left). Note the "clean" (white) peduncle on right where fruit has separated cleanly. On left, peduncle is dark where fruit tissue remains attached after separation.

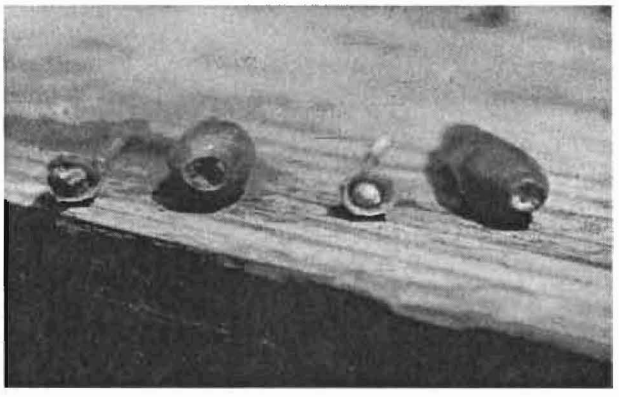


Figure 3. Longitudinal section in a pepper fruit showing all the regions: separation zone, central region (S.Z.C); separation zone, peripheral region (S.Z.P); proximal region (P.R); midway region (M.R); and distal region (D.R).

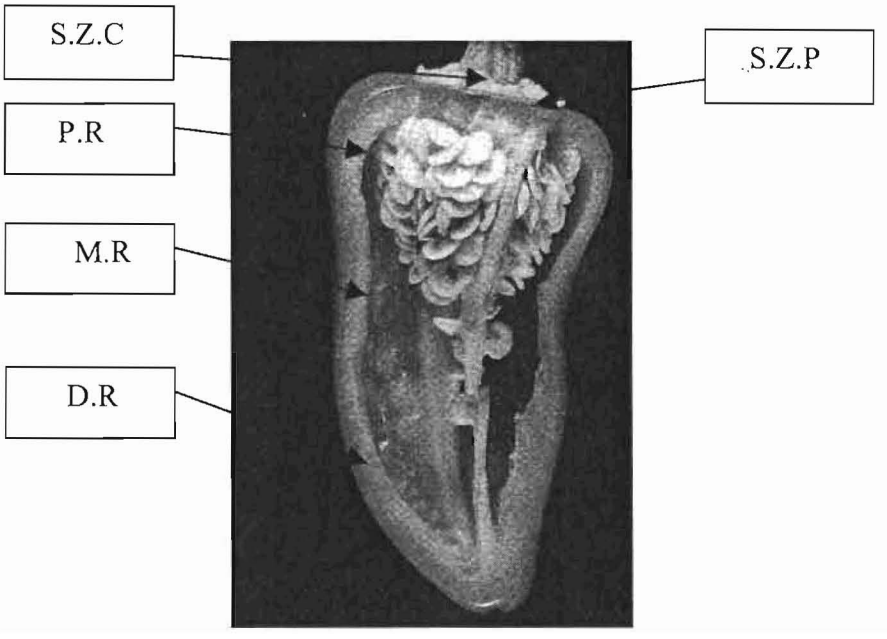

S.Z.C
P.R
M.R
D.R
S.Z.P

Figure 4. Longitudinal sections through separation zone of EP and HP lines at different stages of maturity (immature green (bottom) and mature red (top). Magnification: 45X.

Figure 5. Regression lines in EP line for cell width in all regions of the fruit based on parameters listed in Table 1: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

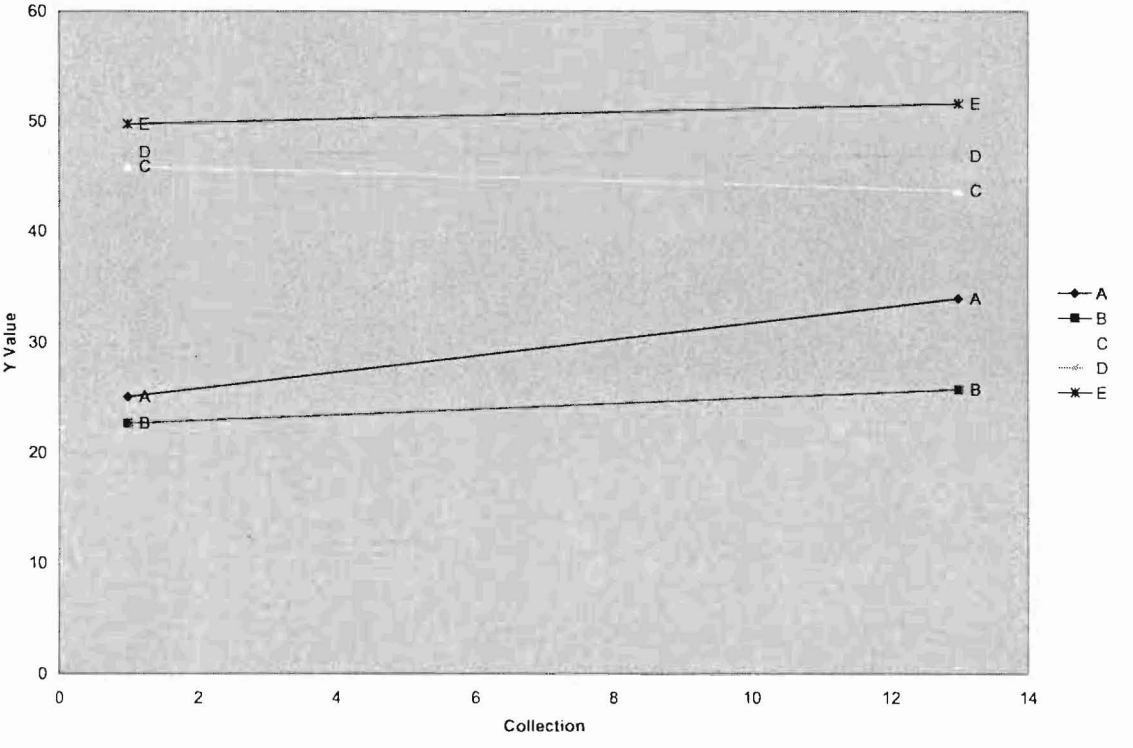

60
50
40
30
20
10
0
Y Value
0
2
4
6
8
10
12
14
Collection
E
D
C
A
B
A
B
C
D
E

Figure 6. Regression lines in EP line for cell length in all regions of the fruit based on parameters listed in Table 1: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

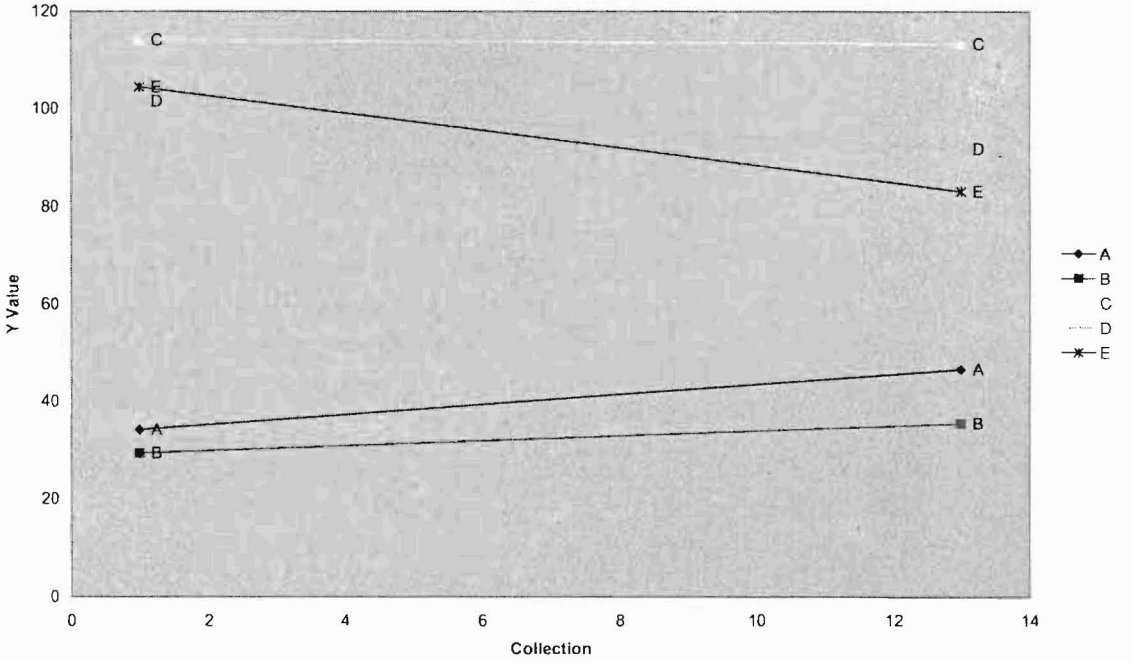

120
100
80
60
40
20
0
Y Value
0
2
4
6
8
10
12
14
Collection
A
B
C
D
E

Figure 7. Regression lines in EP line for cell area in all regions of the fruit based on parameters listed in Table 1: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

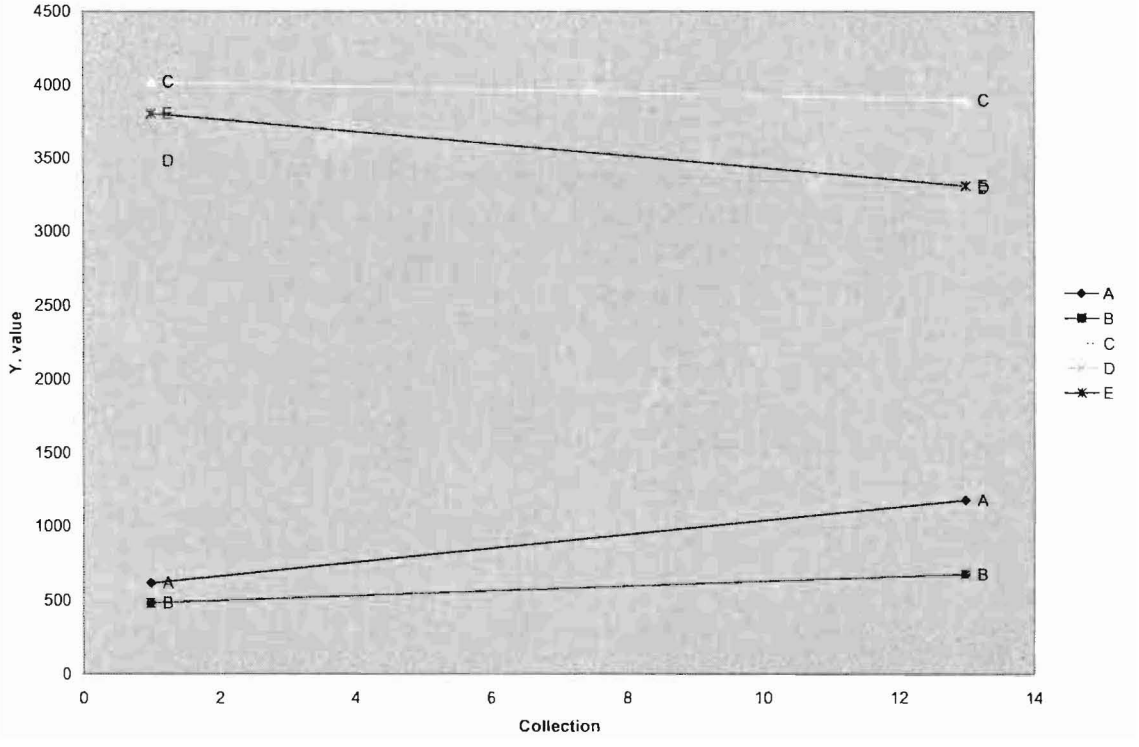

4500
4000
3500
3000
2500
2000
1500
1000
500
0
Y. value
0
2
4
6
8
10
12
14
Collection
A
B
C
D
E

Figure 8. Regression lines in EP line for cell shape factor in all regions of the fruit based on parameters listed in Table 1: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

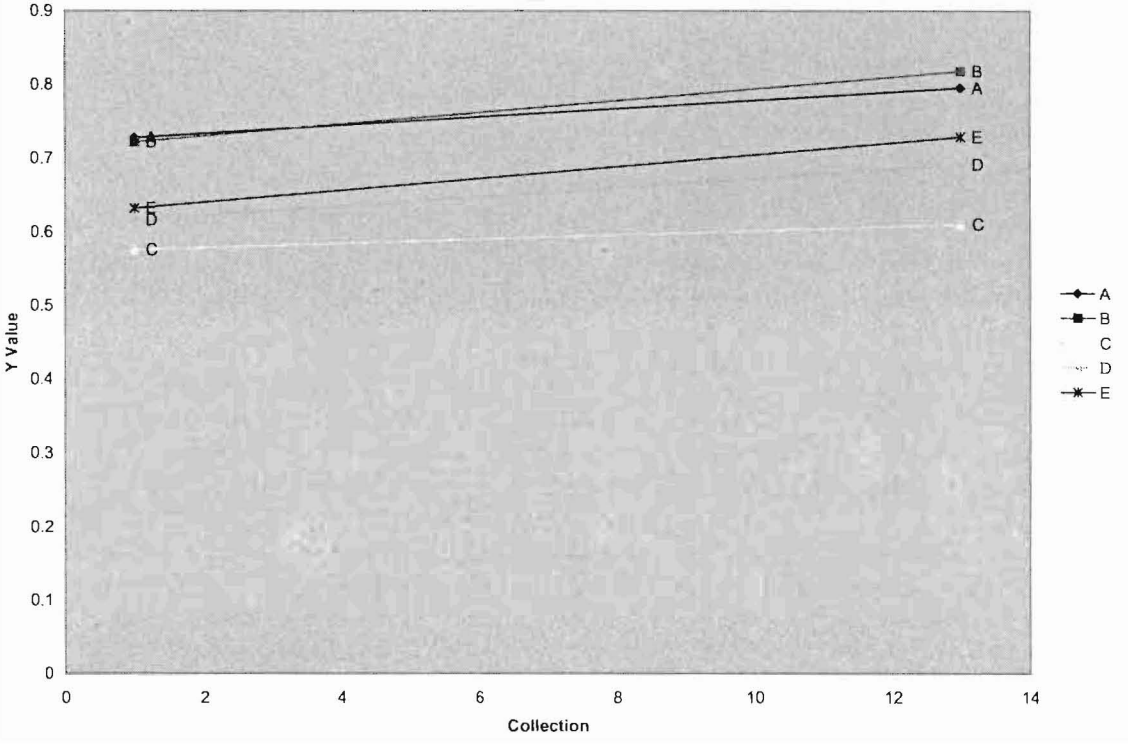

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Y Value
0
2
4
6
8
10
12
14
Collection
B
A
E
D
C
A
B
C
D
E

Figure 9. Regression lines in EP line for intercellular spaces width in all regions of the fruit based on parameters listed in Table 2: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

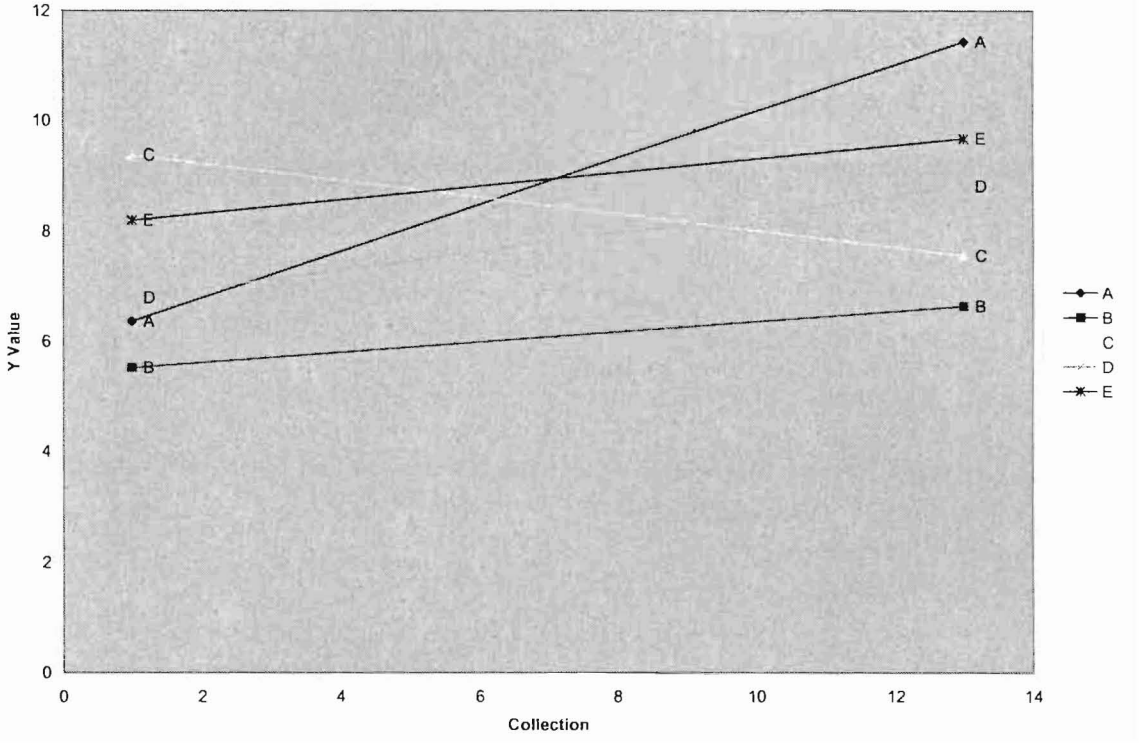

12
10
8
6
4
2
0
Y Value
0
2
4
6
8
10
12
14
Collection
A
E
D
C
D
A
B
C
E
B
A
B
C
D
E

Figure 10. Regression lines in EP line for intercellular spaces length in all regions of the fruit based on parameters listed in Table 2: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

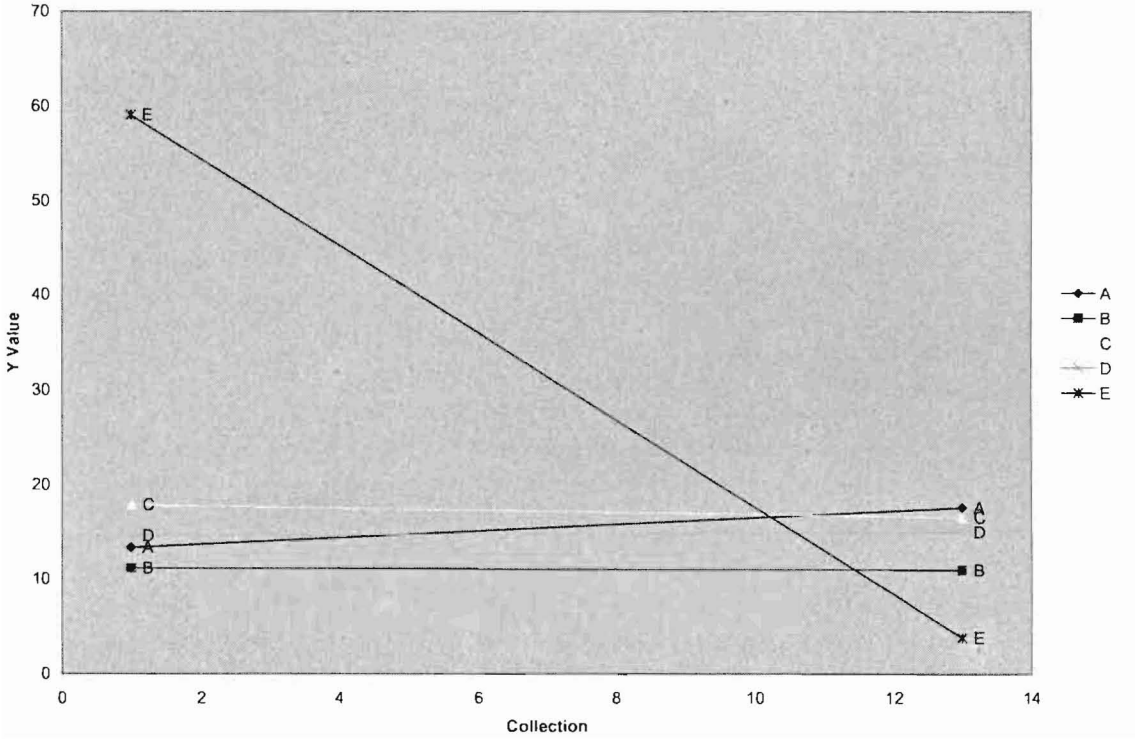

70
60
50
40
30
20
10
0
Y Value
0
2
4
6
8
10
12
14
Collection
E
C
D
B
A
C
D
B
E
A
B
C
D
E

Figure 11. Regression lines in EP line for intercellular spaces area in all regions of the fruit based on parameters listed in Table 2: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

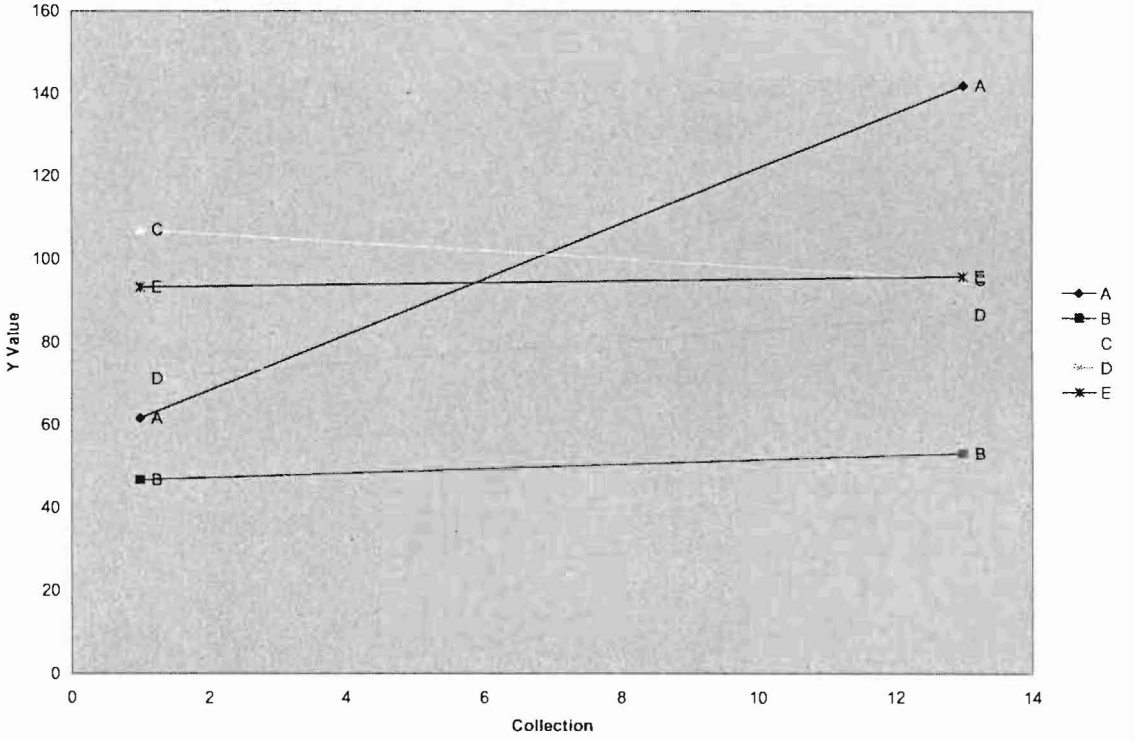
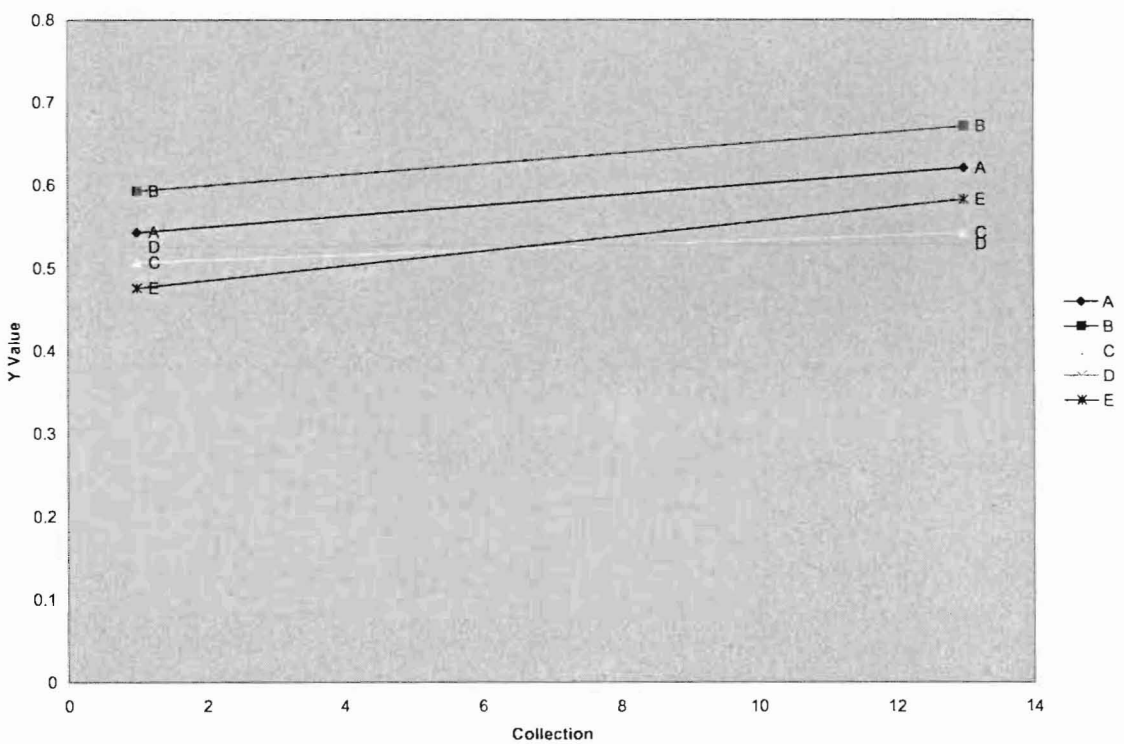

160
140
120
100
80
60
40
20
0
Y Value
0
2
4
6
8
10
12
14
Collection
A
B
C
D
E

Figure 12. Regression lines in EP line for intercellular spaces shape factor in all regions of the fruit based on parameters listed in Table 2: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).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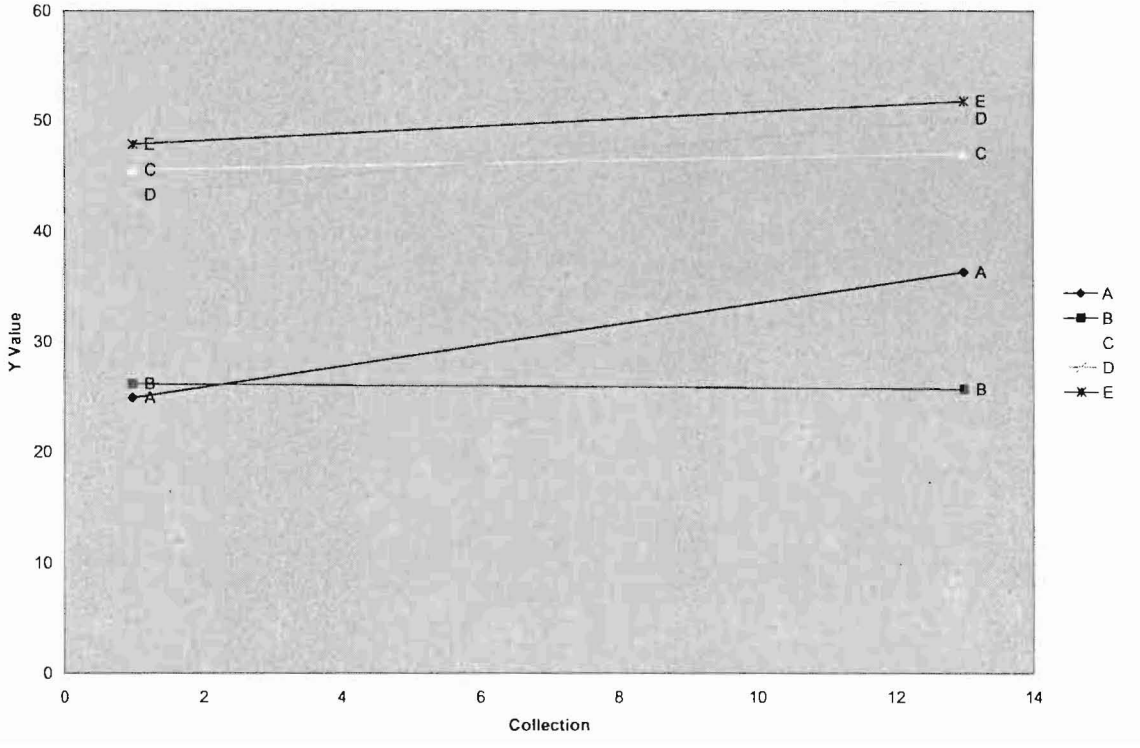
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Y Value
0
2
4
6
8
10
12
14
Collection
B
A
E
C
D
B
A
D
C
E
A
B
C
D
E

Figure 13. Regression lines in HP line for cell width in all regions of the fruit based on parameters listed in Table 1: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).


60
50
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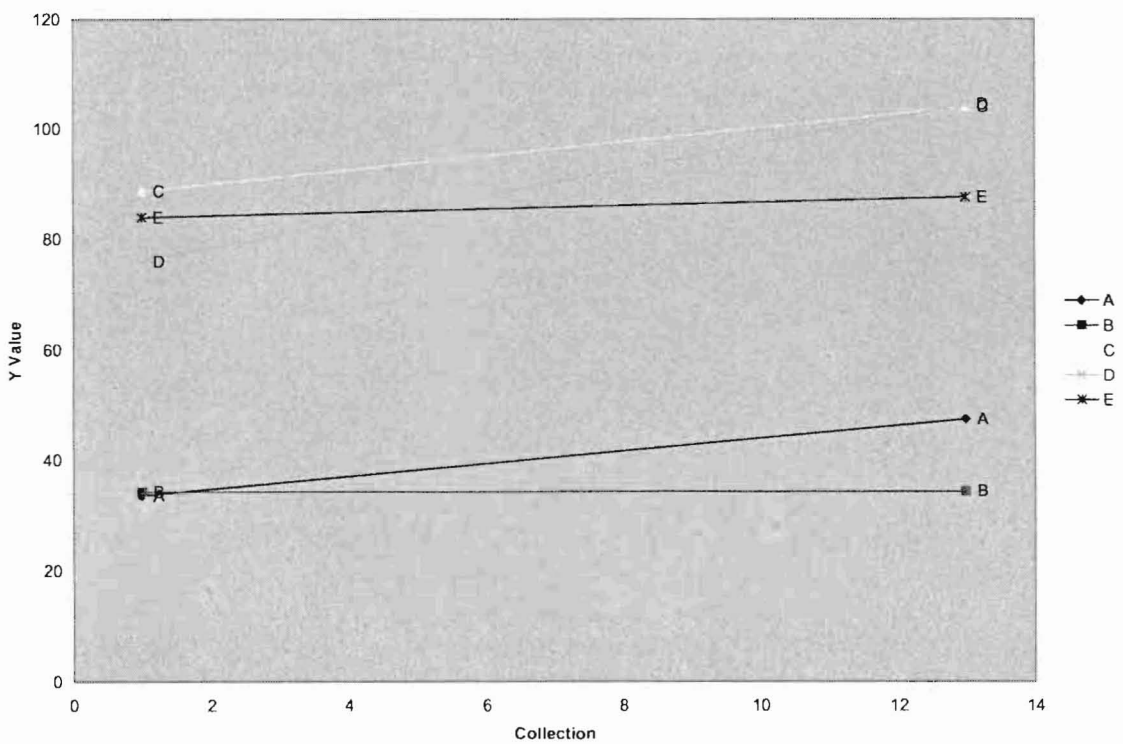
20
10
0
Y Value
0
2
4
6
8
10
12
14
Collection
A
B
C
D
E

Figure 14. Regression lines in HP line for cell length in all regions of the fruit based on parameters listed in Table 1: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).


120
100
80
60
40
20
0
Y Value
0
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4
6
8
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12
14
Collection
A
B
C
D
E

Figure 15. Regression lines in HP line for cell area in all regions of the fruit based on parameters listed in Table 1: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

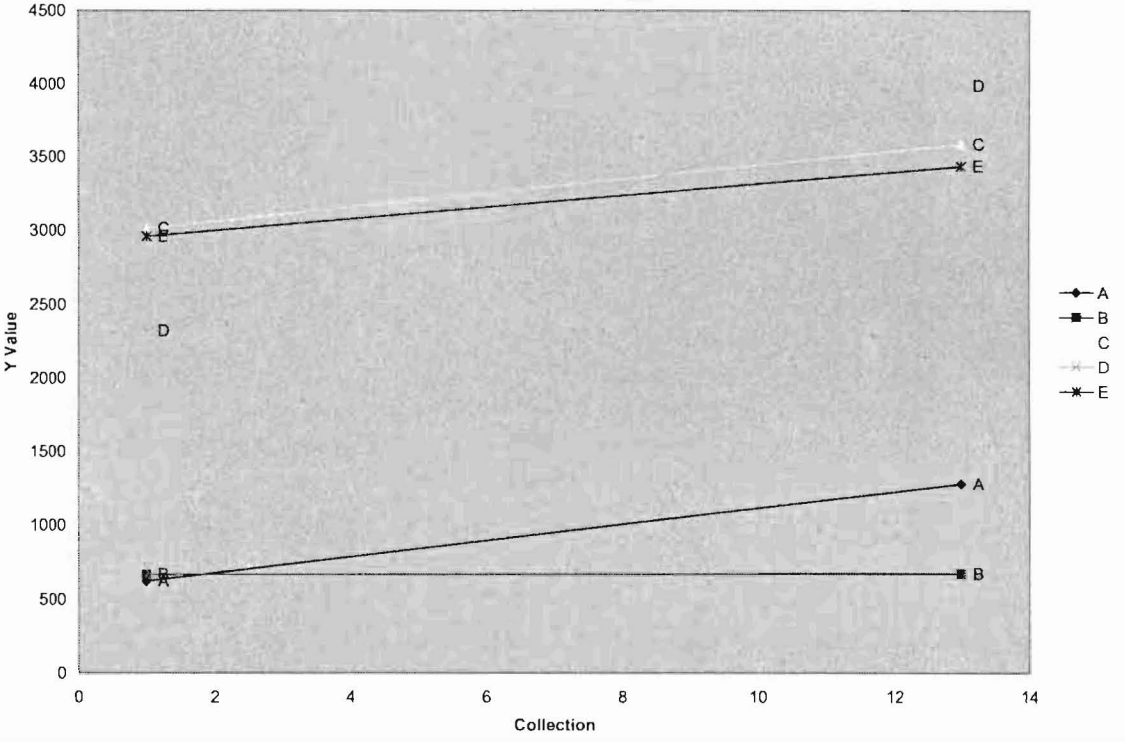

4500
4000
3500
3000
2500
2000
1500
1000
500
0
Y Value
0
2
4
6
8
10
12
14
Collection
A
B
C
D
E

Figure 16. Regression lines in HP line for cell shape factor in all regions of the fruit based on parameters listed in Table 1: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

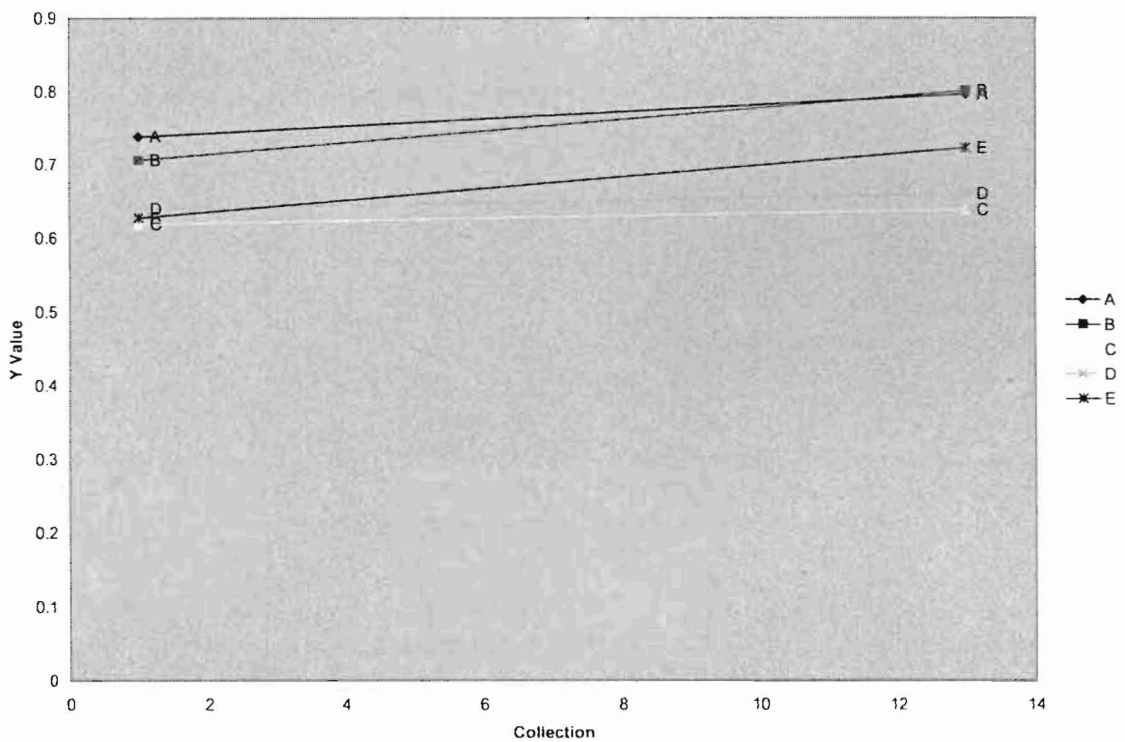

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Y Value
0
2
4
6
8
10
12
14
Collection
A
B
C
D
E

Figure 17. Regression lines in HP line for intercellular spaces width in all regions of the fruit based on parameters listed in Table 2: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

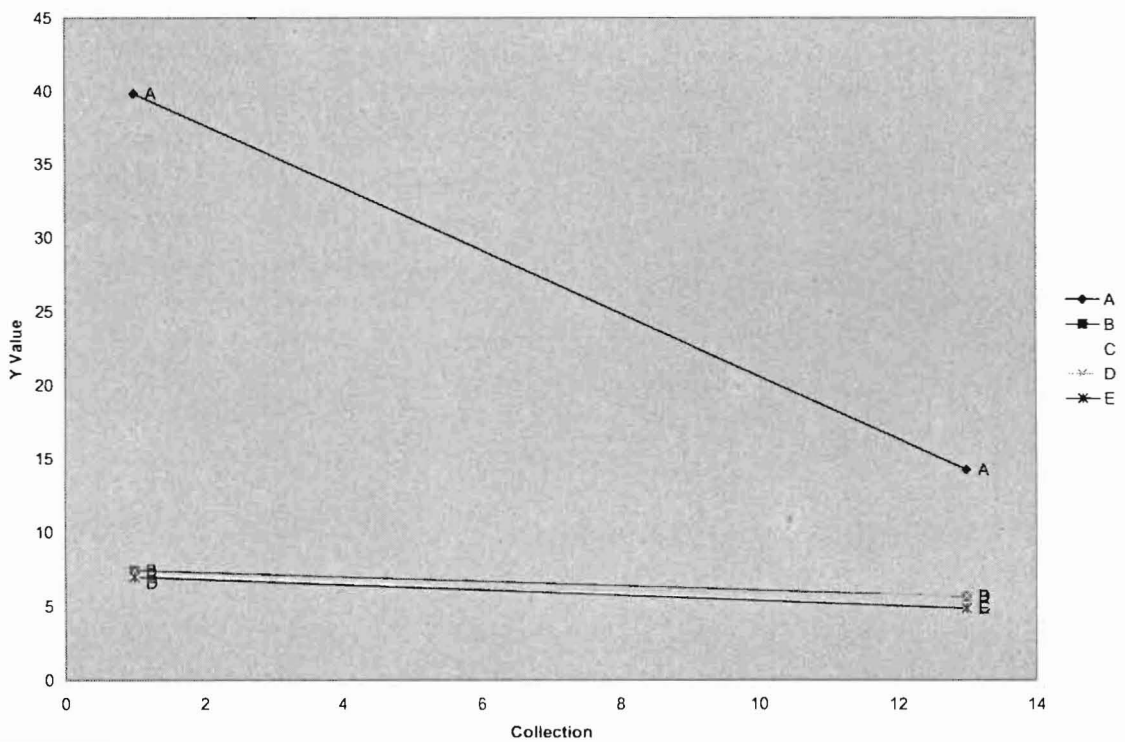

45
40
35
30
25
20
15
10
5
0
Y Value
0
2
4
6
8
10
12
14
Collection
A
B
C
D
E

Figure 18. Regression lines in HP line for intercellular spaces length in all regions of the fruit based on parameters listed in Table 2: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

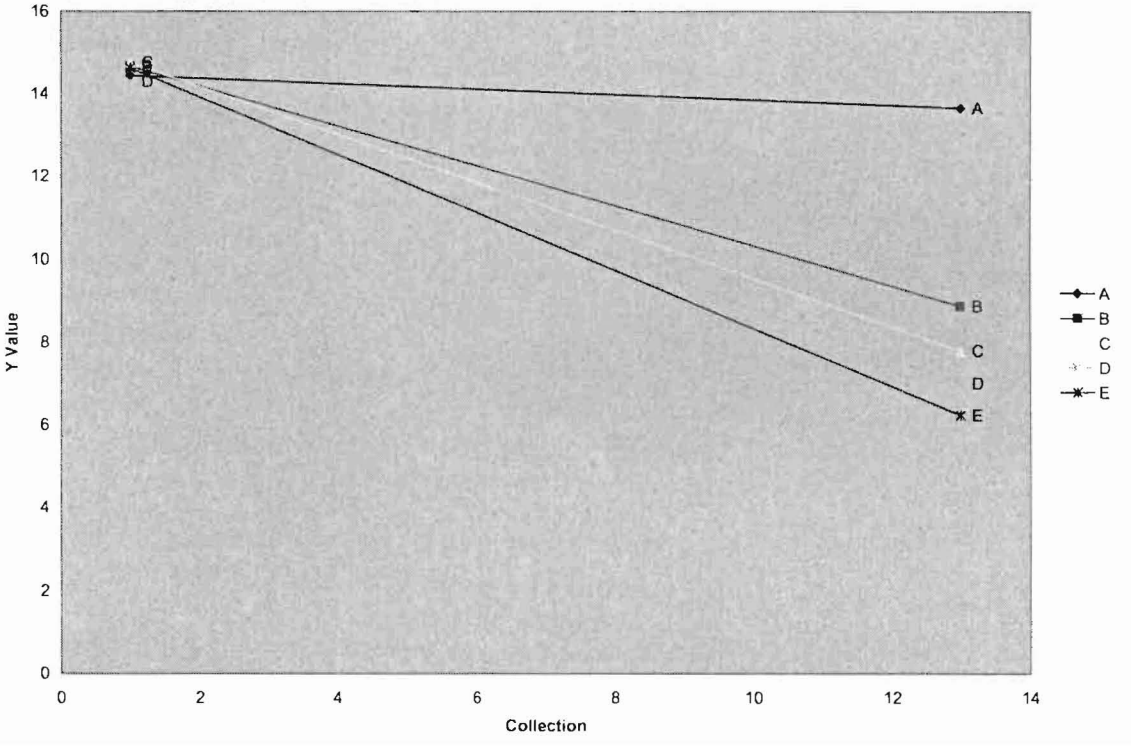

16
14
12
10
8
6
4
2
0
Y Value
0
2
4
6
8
10
12
14
Collection
A
B
C
D
E
A
B
C
D
E

Figure 19. Regression lines in HP line for intercellular spaces area in all regions of the fruit based on parameters listed in Table 2: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E).

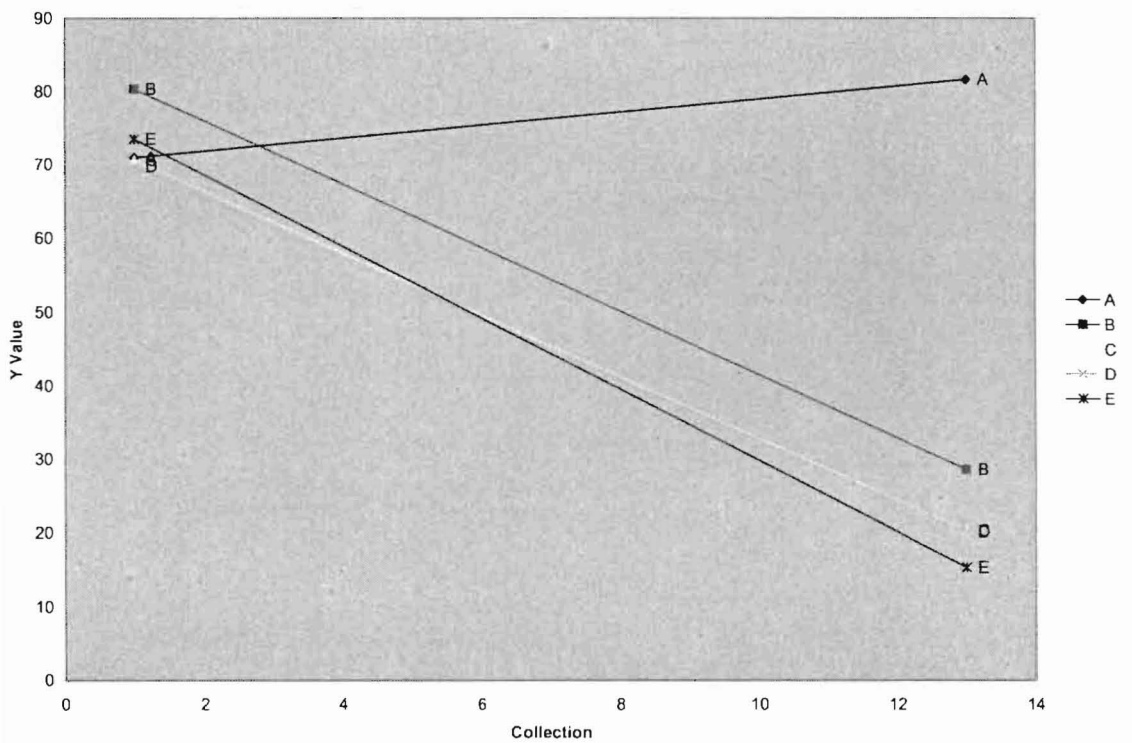

90
80
70
60
50
40
30
20
10
0
Y Value
0
2
4
6
8
10
12
14
Collection
A
B
C
D
E
A
B
E
B
D
E
A
B
C
D
E

Figure 20. Regression lines in HP line for intercellular spaces shape factor in all regions of the fruit based on parameters listed in Table 2: separation zone, central regions (A); separation zone, peripheral region (B); proximal region (C); midway region (D); and distal region (E)

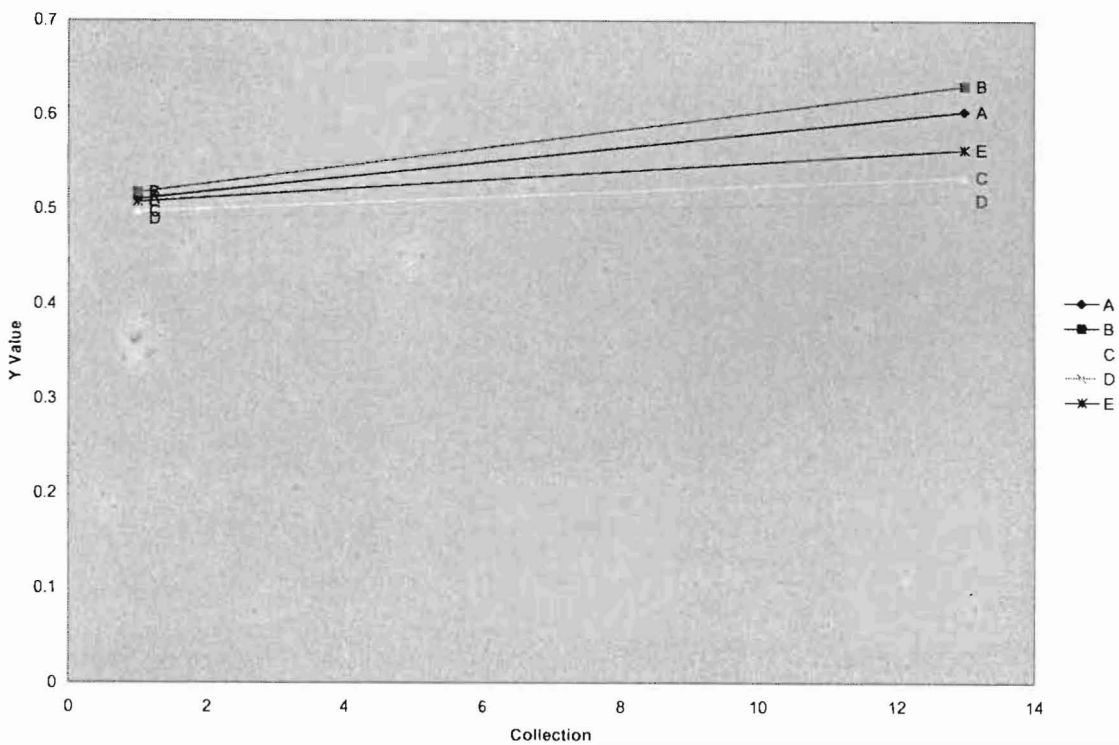
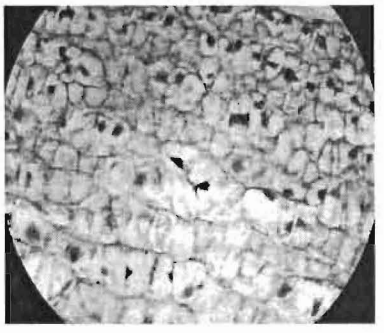
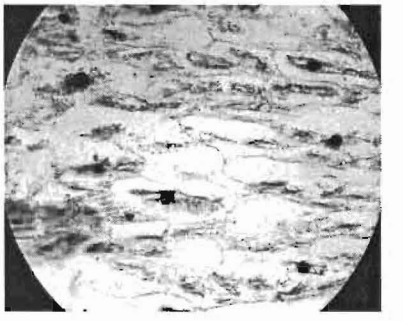

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Y Value
0
2
4
6
8
10
12
14
Collection
B
A
E
C
D
A
B
C
D
E

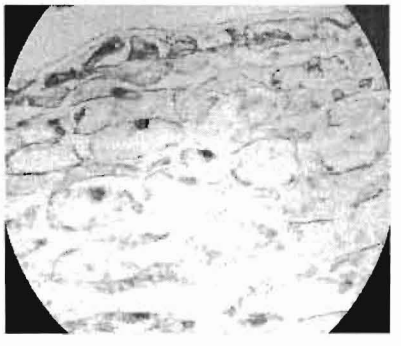
Figure 21. Longitudinal sections of Tabasco pepper fruit (Proximal region) showing two different stages of maturity (immature green and mature-red) in EP line and HP line.



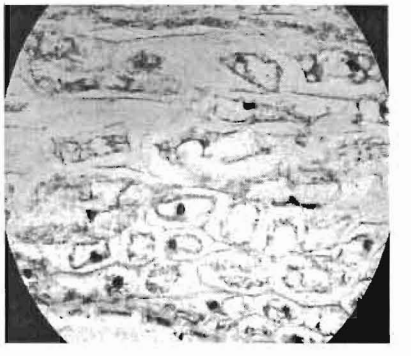
Immature green EP



Mature red EP



Immature green HP



Mature red HP

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