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Physiological Factors Associated with Yellow Shoulder Expression in Tomato Fruit

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Abstract. **Yellow shoulder (YS) expression was associated with differences in composition, respiration rate, and ethylene evolution between the yellow and normal red tissue in tomato (*Lycopersicon esculentum* Mill.) fruit. The YS tissue was higher in pH, dry matter, alcohol-insoluble solids (AIS), calcium content, respiration rate, and ethylene evolution than the red blossom-end tissue. The YS tissue had less phosphorus, potassium, citric acid, and a lower titratable acidity than the red tissue. Expression of YS was not associated with imbalances in fructose, glucose, malic acid, nitrogen, or magnesium concentration. Substantial YS development occurred in fruit of nonuniform ripening gene cultivars allowed to vine-ripen. Yellow shoulder was alleviated by harvesting fruit in the mature-green stage and room-ripening.**

Yellow shoulder is a tomato fruit disorder visually expressed as a yellow or yellow-orange shoulder region separated from the normal red tissue by a distinct line of demarcation. The yellow tissue never turns a normal red color, even after prolonged storage. It is a form of blotchy ripening (nonuniformly colored fruit) primarily affecting fruit from genotypes that contain the nonuniform ripening gene (12, 15). Severity of YS symptoms may vary from discoloration in only several millimeters of tissue next to the stem scar, to discoloration of almost the entire stem half of the fruit. Internal symptoms of YS are expressed as white pericarp and septa tissue.

Environmental conditions influence the amount of YS expression. All forms of blotchy ripening, including YS, were especially severe in fruit from plants of low K fertility (10, 12, 18) and during the spring growing season (12). A high pericarp temperature in the shoulder region of fruit from nonuniform ripening gene cultivars was associated with the incidence of YS (15). YS was increased under high relative humidity conditions, which was attributed to both short-wave radiation and an increased effective radiation load (9).

Uneven pigmentation of tomato fruit can take many forms. Previous compositional studies (5, 19) used fruit that did not have a sharp line of demarcation between the abnormal and red tissues, indicating the fruit did not have the characteristic YS form of blotchy ripening. No information exists on the physiological similarities and/or differences between YS and normal red tissue. The objective of this study, therefore, was to compare the respiration rate, C_2H_4 evolution, and concentrations of major minerals, sugars, and organic acids in YS vs. red tissue. Effect of fruit maturity at harvest on expression of YS also was determined.

Materials and Methods

Fruit source. Fruit from four large-fruited fresh market tomato cultivars ('Celebrity', 'Duke', 'Flora-Dade', and 'Walter') were obtained from staked plants grown under full-bed plastic mulch at the LSU Hill Farm, Baton Rouge, La. during Spring 1984. All cultivars had the nonuniform ripening gene characteristic.

Analyses. Compositional and physiological measurements were made on fruit harvested at the table-ripe stage of maturity, with and without YS symptoms. Ten replications, consisting of three fruit per replication, from each of the four cultivars were analyzed. Three fruit per replication were needed to obtain a sufficient sample of YS tissue for analyses. Paired-comparison analyses were made of the outer pericarp tissue (including the skin) from YS areas and normal red areas of the same fruit. Comparisons also were made between red shoulder (RS) tissue and red blossom-end (RB) tissue from fruit free of YS symptoms.

Fresh pericarp tissue from each replication was homogenized at high speed for 1 min in a Virtis 45 blender. A portion of the homogenate was centrifuged at 14,500× *g*, 1°C, for 15 min. Titratable acidity was determined by titrating 10 ml of the supernatant and 140 ml of preboiled CO_2-free deionized H_2O to pH 8.1 with 0.1 N NaOH. The pH of the supernatant–H_2O mixture was measured with a Cole–Parmer digital pH meter before titrating. Soluble solids content of the supernatant was determined with a Bausch and Lomb table-top refractometer. Dry matter was determined by drying 20 g of the homogenate at 70° for 48 hr.

Another 20-g portion of the pericarp homogenate was boiled in 80% ethanol for 15 min, cooled, filtered through Whatman #4 paper, and made up to 100 ml in 80% ethanol. Alcohol-insoluble solids content was determined by the weight of the powder remaining on the filter paper after 24 hr under vacuum at 45°C. Sugars and organic acids in the filtrate were determined by high-performance liquid chromatography as previously described (11).

Pericarp mineral concentration was determined on finely pulverized freeze-dried tissue. Potassium, Ca, and Mg were determined by flame atomic absorption spectrophotometry; P by colorimetry; and N by the Kjeldahl method, all according to AOAC procedures (1).

Carbon dioxide and C_2H_4 evolution of 1-cm-diameter disks were determined by gas chromatography. Each pair of disks (yellow vs. red, red vs. red) was excised with a cork borer from the same longitudinal position on the fruit. Each disk was blotted on tissue paper to remove adhering cell sap, weighed, and placed in a 25-ml Erlenmeyer flask that then was sealed with a silicone stopper. Carbon dioxide and C_2H_4 analyses of the head space were made after 1 hr at 23°C.

Oxygen consumption of 1 g (fresh weight) outer pericarp tissue slices was determined by a Gilson respirometer. The tis-

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sue slices were placed in respiratory flasks that contained in the center well a fluted filter paper wick and 0.4 ml of 10% KOH to absorb CO_2 during O_2 uptake measurements. The flasks and contents were equilibrated for 30 min at 23°C before respiratory measurements were started.

Statistics. Statistically significant differences ($P < 0.05$) between YS and RB tissue (and between RS and RB tissue) in the amount of each component were determined by the Student's *t* test for paired comparisons (13). Analyses of the percentage data were based on arcsin transformations.

Harvest maturity. Two-hundred mature-green fruit from 100 different plants (two fruit/plant) of both 'Flora-Dade' and Walter' were harvested on 7 June. Fruit were selected at random from different positions on the plant and all were 5 × 6 in size. Fruit were placed in a dark room and ripened for 2 weeks at 23°C, 75% RH. An additional 200 mature-green fruit were randomly tagged and allowed to ripen on the vine for 2 weeks. The amount of YS development after 2 weeks of room or vine-ripening was determined. The severity of symptoms was classified on the basis of amount of external surface area with YS as follows: none = YS free; slight = 0–5% YS; moderate = 5–25% YS; severe = 25–50%YS.

Results and Discussion

Distinct compositional and physiological differences existed between YS and normal red tissue. The results of all compositional and physiological measurements between tissue types were similar among cultivars. Therefore, the data from all four cultivars were pooled.

Composition. Total dry matter content and AIS were significantly higher in YS tissue (Table 1). Percent soluble solids content was lower in the shoulder than blossom region, and was the lowest in the yellow tissue type. Fructose was the principal sugar found in both tissue types. Higher levels of fructose existed in the shoulder region, but generally no difference was found between the fructose content of YS and RS tissues. Similar levels of glucose were found between regions and tissue types. Sucrose concentration was higher in the shoulder region, especially in the yellow tissue type, than in the blossom-end.

Acidity parameters differed between the yellow and red tissues (Table 2). Yellow shoulder tissue was significantly higher in pH than RB tissue. The higher pH was accompanied by about three times less titratable acidity in the YS tissue. This reduction was attributed to lower amounts of the major organic acid, citric, in the yellow tissue. Similar amounts of malic acid were found in the YS and RS tissue, but the shoulder region contained higher amounts of malic acid than did the blossom-end.

Differences in mineral content existed between YS and red tissue (Table 3). Yellow shoulder tissue contained less than half the P and K levels of the red tissue. There was no difference in P or K content between the shoulder and bottom region of normal red fruit. Calcium content was substantially greater in the yellow than red tissue. Nitrogen content was reduced in the shoulder region of both YS and normal fruit. There was no difference in Mg content between YS and red tissue.

A cation–anion balance generally exists within cells, and the reduced K concentration presumably was responsible for the reduced citric acid concentration in the yellow tissue. Potassium was the major cation and citric acid the major organic acid anion. Other workers indicated K accounted for 86% to 90% of the total cations in blotch-free tomato fruit (3, 4) and highly significant positive correlations were found between fruit K concentration and titratable acidity (4, 6). The reduced K levels in YS tissue may have been due to a diminished capacity for K uptake and accumulation by cells in that tissue. Redistribution of pre-existing K from the shoulder region and translocation from the pericarp to locule tissue also may have occurred. It did not appear that K from the shoulder tissue was redistributed into the RB tissue, because fruit exhibiting YS did not have abnormally high levels of K in the pericarp of RB tissue. It is not known if a critical K level exists in the tissue, below which YS will be expressed; and if the abnormal levels of K (and citric acid) are the cause or the effect of YS. Yellow shoulder tissue was obviously deficient in lycopene and had a different pattern of pigment change during ripening compared to the red tissue. This pattern also may have been mediated by suboptimal levels of tissue K. Total carotenoids and lycopene content in particular, were reduced in tomato fruit from plants of low K fertility (14). The significance of a lower P level in tissue exhibiting YS is not known. Yellow shoulder expression was not associated with an imbalance in tissue total N or Mg.

The differences in titratable acidity, citric acid, soluble solids, AIS, and pH between yellow and red tissue were consistent with the previous results from England between blotchy and red tissue (5, 19). Dry matter content, however, was higher in YS than red tissue, but was lower in blotchy than red tissue (5, 19). Reducing sugar content was similar between yellow and red tissue but was lower in blotchy than red tissue (7, 19). No significant difference in K concentration was found between blotchy and normal tissue (5), but whole fruit with blotchy ripening symptoms contained less K than comparable blotch-free fruit (17). Yellow shoulder was apparently a more extreme form of blotchy ripening compared to the tissue used in the English work, which used greenhouse-grown tomatoes. Based on the

Table 1. Percent dry matter, alcohol-insoluble solids (AIS), soluble solids (SS), and sugar content in different tissue types and regions of tomato fruit.

Tissue			Percent	Percent	Sugar		
Type	Region	Percent dry matter	AIS	SS	Fructose (%)	Glucose (%)	Sucrose (%)
Yellow	Shoulder	6.94	2.4	4.2	1.60	1.18	0.077
Red	Blossom	6.28	1.4	4.9	1.39	1.19	0.003
		*[z]	*	*	*	NS	*
Red	Shoulder	---	1.4	4.4	1.51	1.20	0.018
Red	Blossom	---	1.3	4.9	1.37	1.13	0.001
			NS	*	*	NS	*

[z]Values between tissue regions were significantly different (*) or not significantly different (NS) at the 5% level by the Student's *t* test for paired comparisons. Each value represents the average of 10 replications × 4 different cultivars.

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Table 2. pH, titratable acidity (TA), and organic acid content in different tissue types and regions of tomato fruit.

Tissue			TA	Organic acid	
Type	Region	pH	(ml 0.1 N NaOH)	Citric (%)	Malic (%)
Yellow	Shoulder	4.94	1.7	0.13	0.06
Red	Blossom	4.25	5.4	0.30	0.04
		*[z]	*	*	*
Red	Shoulder	4.34	4.6	0.32	0.07
Red	Blossom	4.30	5.2	0.29	0.05
		NS	*	NS	*

[z]Values between tissue regions were significantly different (*) or not significantly different (NS) at the 5% level by the Student's *t* test for paired comparisons. Each value represents the average of 10 replications × 4 different cultivars.

Table 3. Mineral content of different tissue types and regions of tomato fruit.

Tissue		Mineral content (percent dry wt basis)				
Type	Region	K	N	P	Ca	Mg
Yellow	Shoulder	1.45	1.22	0.16	0.26	0.08
Red	Blossom	3.09	1.79	0.36	0.09	0.09
		*[z]	*	*	*	NS
Red	Shoulder	3.42	1.54	0.39	0.13	0.10
Red	Blossom	3.25	1.95	0.42	0.08	0.12
		NS	*	NS	*	NS

[z]Values between tissue regions were significantly different (*) or not significantly different (NS) at the 5% level by the Student's *t* test for paired comparisons. Each value represents the average of 10 replications × 4 different cultivars.

Table 4. Oxygen uptake, CO_2 evolution, and C_2H_4 evolution of different tissue types and regions of tomato fruit.

Tissue		O_2 uptake	CO_2 evolution	C_2H_4 evolution
Type	Region	(μl/g per hr)	(μl/g per hr)	(μl/kg per hr)
Yellow	Shoulder	55.3	95.1	8.9
Red	Blossom	28.7	85.2	4.7
		*[z]	*	*
Red	Shoulder	---	50.4	6.3
Red	Blossom	---	61.2	4.3
		---	*	*

[z]Values between tissue regions were significantly different (*) or not significantly different (NS) at the 5% level by the Student's *t* test for paired comparisons. Each value represents the average of 10 fruit × 4 different cultivars.

compositional differences between tissue types, YS tissue should be considered abnormal and not merely delayed in ripening.

Respiration and C_2H_4. Wounding increased respiration rate and C_2H_4 evolution over that of whole fruit, but the wound response was assumed to be similar between yellow and red tissue. Respiration rate, expressed as O_2 uptake or CO_2 evolution, was significantly higher in YS tissue than normal RB tissue (Table 4). The difference was due to tissue type and not tissue location, since RB tissue had a higher respiration rate than RS tissue in fruit not exhibiting YS. Calculation of the respiratory quotient (μM CO_2 evolved/μM O_2 absorbed) may indicate the class of compounds being respired, or the oxidation state of the substrate. The respiratory quotient, calculated from Table 4, was higher in red tissue (2.97) than in yellow tissue (1.72). The high respiratory quotient of the red tissue may have been related to the use of citric acid as the primary substrate for respiration. The lower respiratory quotient of the yellow tissue was consistent with the abnormally low citric acid concentration.

Ethylene evolution in YS tissue was almost twice the amount in red tissue (Table 4). The magnitude of difference was more than location could account for, since RS tissue had only 1.5 times the rate of C_2H_4 evolution than RB tissue. Abnormal ripening of the yellow tissue could not be attributed to a low rate of C_2H_4 evolution. The two distinct tissue types (yellow vs. red) found in the same fruit provide a unique system for studying the role of C_2H_4 and other metabolic changes in the regulation of tomato fruit ripening.

Harvest stage. A substantial amount of table-ripe stage harvested fruit contained various degrees of YS expression (Table 5). 'Flora-Dade' fruit were slightly more YS-susceptible than those of 'Walter'. Exposure of the fruit surface to direct sunlight was not essential for YS development, but a greater proportion of fruit exposed to direct sunlight had YS compared to those located inside the canopy. The incidence of YS expression was practically eliminated by harvesting fruit at the mature-green stage and room-ripening at 23°C. This discovery indicated YS was an abnormality mediated by environmental conditions during the physiological period of ripening. Predisposition of the fruit to express YS may be initiated during the development and maturation stages, but expression apparently will be realized only under certain unfavorable environmental conditions during ripening of fruit still attached to the vine. The unfavorable conditions may involve the quantity and quality of incident radiation (9), high fruit temperature near the calyx (15), high relative humidity (9, 12), and low K fertility (12).

Vine-ripened tomatoes generally have a higher quality and better composition than mature-green harvested room-ripened fruit (2, 8, 11, 16). However, allowing fruit from nonuniform ripening gene cultivars to vine-ripen under certain environmental conditions may result in undesirable external quality, expressed as YS. External appearance is one of the first criteria consumers use in deciding which tomatoes to purchase, and fruit with YS symptoms would be inferior to uniformly red-colored fruit.

Maintaining high soil K fertility, planting uniform ripening

Table 5. Number of fruit with yellow shoulder expression in tomato fruit harvested table-ripe or mature-green. Mature-green fruit were room-ripened.

		Degree of severity of yellow shoulder expression[z]			
Cultivar	Harvest stage	None	Slight	Moderate	Severe
Flora-Dade	Table-ripe	60[y]	74	62	4
	Mature-green (room-ripened)	190	10	0	0
Walter	Table-ripe	116	64	20	0
	Mature-green (room-ripened)	196	4	0	0

[z]None = no yellow shoulder symptoms; slight = 0–5% of fruit surface area with yellow shoulder; moderate = 5–25% of fruit surface area with yellow shoulder; sever = 25–50% of fruit surface area with yellow shoulder.

[y]Based on a total of 200 fruit from each harvest stage.

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gene cultivars, or harvesting nonuniform ripening gene cultivars in the mature-green stage are several approaches that may be followed to minimize the incidence of YS.

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Seasonal Trends of Calcium and Iron Fractions in Sweet Cherry Leaves and Their Relationships

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Abstract. **Calcium in cherry (*Prunus avium* L.) tree leaves was separated into four fractions at 2- to 4-week intervals during the season. Concentrations of all fractions increased, according to curvilinear equations. The organic insoluble fraction was the most important during the first half of the cycle, whereas the soluble fraction was the most important during the second half. Bound and inorganic insoluble Ca contribute little to the total content. Iron extracted by 1 N HCl is highly correlated with the total concentration and increased as a quadratic equation. Accumulation of Fe seems to be related to the accumulation of Ca in the leaf tissue.**

The total concentration of a nutrient element in plant material does not indicate the physiological activity of that element. Thus, an apparent optimum concentration concommitant with an inadequate metabolic function is possible. Such inadequate relationships have been described for Ca (1, 9–11) and Fe (7, 14, 16, 22).

Fractionation of Ca was first reported more than 55 years ago (13). The basic idea is to extract plant tissue in a sequence of solvents (2, 3, 12, 15): Demineralized water (to remove Ca bound in soluble organic and inorganic salts), an inorganic sodium salt—chloride or nitrate—(calcium bound in pectate or protein), acetic acid (in phosphate and carbonate) and hydrochloric acid (in oxalate).

Iron fractionation procedures have been designed to elucidate the role of Fe in chlorophyll formation. This portion has been called "active iron" (16) and is a part of the Fe extracted in 1 N HCl. Iron extracted by this method is also a good parameter to diagnose iron deficiency chlorosis (4) and should be called "soluble iron."

The synergism between Fe and Ca has not been studied sufficiently. However, under certain circumstances, Ca concentration has been affected by iron (3, 8, 18).

As no work has, to the best of our knowledge, been reported on cherry trees, the intent of the present study was to establish a) the distribution of Fe and Ca total contents, b) the dynamics of the above-mentioned fractions, and c) the relationship between the analyzed parameters.

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