The mineral concentration variations among cultivars were similar to that seen earlier for one cultivar (Ruby Queen) grown at four widely separated locations (11). Carrot cultivars have greater variation in root mineral content (1) than seen for beets in the present study.

High mineral concentrations in the beet root outer peel were consistent with the high amounts of apoplastic tissue in this layer. The concentrations of N, K, Mg, Ca, and Mn were similar to those found in highly apoplastic beet leaf petioles (10). A similar combination of high mineral concentrations with apoplastic tissue was seen in potato tuber cortex (4). The outer peel concentration of Fe was closer to that in leaf blades than other plant parts. The outer peel had more P and less Na than beet petioles or blades.

The variations in mineral content in different root layers have nutritional importance because peeling removes proportionally more minerals on a volume basis. Beet cultivar had little effect on root mineral composition. As yet there is no information on the relation of root size to mineral distribution.

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Changes in Acidic and Basic Peroxidase Activities during Tomato Fruit Ripening

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Abstract. **Activities of the acidic and basic peroxidases from tomato fruit (*Lycopersicon esculentum* Mill. cv. Flora Dade) were determined at six ripening stages, from green to red-ripe fruits. Both the acidic and basic peroxidases reached a maximum during the climacteric, at the pink stage, but the relative increase in basic peroxidase activity was much more pronounced. Changes in the peroxidase, IAA oxidase, and ACC oxidase activities of the basic peroxidases paralleled the changes in ethylene production. However, in the presence of calcium, the degree of activation of peroxidase was constant throughout ripening, whereas the IAA and ACC oxidase activities of the basic peroxidases were only activated at the pink stage.**

Changes in peroxidase (EC 1.11.1.7) activity during fruit ripening have been the subject of many studies (2, 5, 6, 14), with total peroxidase (4) or the different peroxidase fractions, mainly soluble and ionically bound fractions being the areas investigated (2, 6, 14). Electrophoretic studies with tomato fruit indicate that fractions consist of different isozymes whose activities change according to the developmental stages of the fruits (14). Since isozymes have very different affinities toward substrates and are inhibited by an excess of substrate [either the hydrogen peroxide or the hydrogen donor (3)], the exact evaluation of the real peroxidase activity of a crude extract is almost impossible. Thus, little is known of the quantitative changes in the activities of peroxidase isozymes during fruit ripening. However, such information is important because the two main forms present in plants, the acidic and the basic peroxidases, exhibit different substrate specificities and physiological roles (4). Among these roles, the basic form may be involved in the degradation of IAA (4) and the conversion of ACC to ethylene (1, 4). In the present investigation, we report on the changes during tomato fruit ripening of the total, acidic and basic peroxidase activities and of the 1*H*-indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activities of the basic peroxidases. These determinations were performed on naturally ripened tomato fruits thoroughly characterized by their CO_2 and ethylene production rates, firmness, and pigment content.

Field-grown 'Floradade' tomato fruits were harvested and segregated into the following six ripening stages: green, G; mature green, MG; breaker, B; pink, P; orange red, OR; and red ripe, RR. Ethylene production and respiratory rate were determined on three fruits for each ripening stage 28 hr after the fruits were picked. Ethylene and CO_2 measurements were carried out as described by Nicolas et al. (10). Pulp firmness (residual force after 1-cm penetration of a 3-mm-diameter tip) was measured, as described by Nicolas et al. (9), on 40 fruit for each ripening stage. The same 40 fruits were then used to constitute eight sub-samples of five fruits for each ripening stage; the fruit were immediately prepared by dicing, blending, deep-freezing, and grinding in liquid N_2 and storing at −20C for lycopene and peroxidase analysis.

The lycopene content was determined spectrophotometrically according to Lime et al. (8).

Peroxidase was extracted as follows: 3.5 g NaCl was added to 60 g of tomato powder and the suspension was homogenized for 90 min at 4C in a rotary agitator. The resulting tomato homogenate was then centrifuged at 40,000× *g* for 15 min. The filtered supernatant is referred to as the crude extract. Each

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Table 1. Comparison of ACC oxidase activities of the acidic and basic forms of tomato peroxidase.

Peroxidase forms	Activities		Ratio of ACC oxidase to peroxidase activity
	Peroxidase[z]	ACC oxidase[y]	
Acidic	0.21	2.5	11.9
Basic	0.17	16	94

[z]Results are expressed in $A_{470\ nm} \cdot s^{-1} \cdot ml^{-1}$ of purified peroxidase fraction.
[y]Results are expressed in $nmol \cdot hr^{-1} \cdot ml^{-1}$ of purified peroxidase fraction.

of the eight sub-samples of the six ripening stages were extracted on the same day. All subsequent steps were carried out at 4C. Crude extracts were dialyzed twice against sodium phosphate buffer pH 6 (5 mM) for 24 hr. The acidic and basic peroxidases of the dialysed extracts were separated and concentrated by ion-exchange gel chromatography (2).

All activities were assayed at 30C. The peroxidase activity was determined spectrophotometrically at 470 nm using guaiacol (20 mM) and H_2O_2 (8 mM) in phosphate buffer pH 6 (100 mM) in a total volume of 3 ml. One unit of peroxidase activity caused a change of one absorbance unit per second under the assay conditions. IAA oxidase activity was determined according to Thomas et al. (15). Cell-free ethylene formation by the basic peroxidases was determined in 17-ml vials fitted with serum caps using ACC (10 mM), $MnCl_2$ (0.01 mM), and 0.1 unit of peroxidase activity (0.05 to 0.2 ml, depending on the fraction being assayed) in Tris·HCl buffer pH 7.9 (100 mM) in a total volume of 2.3 ml. The amount of ethylene produced was measured each hour between 3 and 7 hr after the beginning of the reaction. For the determination of the effect of calcium (5 mM), Mes buffer, pH 6 (100 mM), was used instead of phosphate buffer.

Firmness changes (Fig. 1) were very rapid during the early stages of ripening (MG–B–P stages), which corresponded to the climacteric rise in CO_2 and C_2H_4 production (Fig. 2). Lycopene did not appear until ethylene synthesis began (B stage), and increased sharply from then on (Fig. 1). In agreement with Jeffery et al. (7), these changes were clearly related to tomato fruit ripening and ethylene synthesis, and they confirmed that the six maturity stages studied are representative of fruit ripening as determined visually. Moreover, the six maturity stages were significantly different (at the 0.1% level) from one another whatever the ripening characteristic considered, with the exception of pigments for the B and P stages.

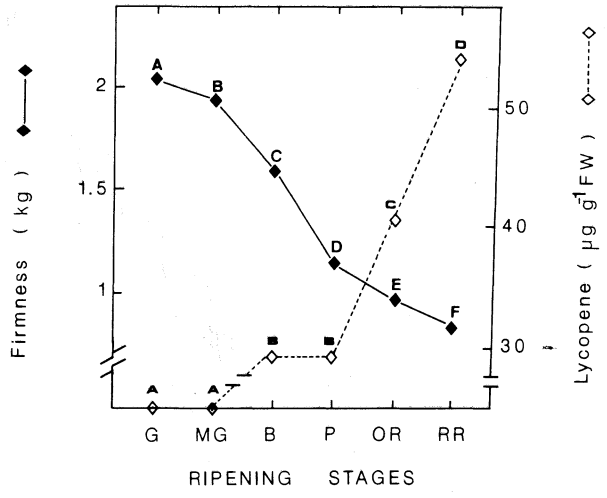

Fig. 1. Changes in firmness (♦) and lycopene (◇) during tomato fruit ripening. Mean separation between ripening stages according to Duncan's multiple range test, at the 1% level of significance.

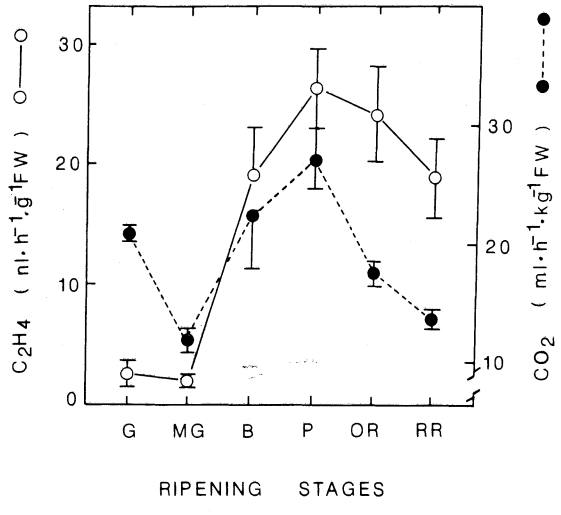

Fig. 2. Changes in ethylene production (○) and respiratory (●) rate during tomato fruit ripening. Vertical bars represent SD on average of three fruits.

Total peroxidase activity in crude extracts showed a minimum before the onset of ripening (MG stage), followed by high levels of activity during ripening (Fig. 3A). The same phenomenon was observed in apple by Gorin and Heidema (5). However, the establishment of a relationship between total peroxidase changes and ripening is difficult, since tomato peroxidase consists of a number of isozymes whose activities vary greatly during fruit ripening (2, 14). Separation of acidic and basic forms present in the crude extracts shows that, although the activities of both the acidic and basic forms peaked at the P stage (Fig. 3A), the relative increase in activity of the basic peroxidases was more important than that of the acidic peroxidases. The activity of the basic peroxidases, which varied between 9% and 17% of that of the total peroxidase (Fig. 3B), increased markedly at the onset of ripening (a 240% increase between MG and P stages), while the rise in acidic peroxidases activity was far less pronounced (only a 55% increase during the same period). Thus, it appears that, among all the changes of peroxidase activity followed during tomato ripening, those of the basic form are the most closely related to the changes in ethylene production (Fig. 2).

Moreover, in agreement with (1, 4), we also observed that in vitro ACC oxidase activity of peroxidase was mainly associated with the basic form (Table 1). It is shown in Fig. 4 that the changes in IAA and ACC oxidase activities of basic peroxidase during tomato ripening closely paralleled the changes in its peroxidase activity. By contrast, Ca, a known activator of the basic peroxidases (11), had different effects according to the substrate and stage of ripening. The increase in peroxidase activity induced by Ca (Fig. 4A) was fairly constant throughout ripening (from G to RR stages). On the other hand, Ca caused an increase in IAA and ACC oxidase activities only at the P stage, i.e., the period of maximum ethylene production, whereas it inhibited these activities at the G and MG stages (Fig. 4 B and C). While the meaning of the inhibition by Ca of the IAA and ACC oxidase activities during the preclimacteric stages remains obscur, these results suggest the appearance during the climacteric of at least one basic peroxidase isozyme whose associated activities are Ca-sensitive. The appearance of two new basic peroxidase isozymes at the MG stage has been shown by Thomas et al. (14). Since IAA and ACC oxidase activities exhibited the same pattern of changes, even in the presence of Ca, it is

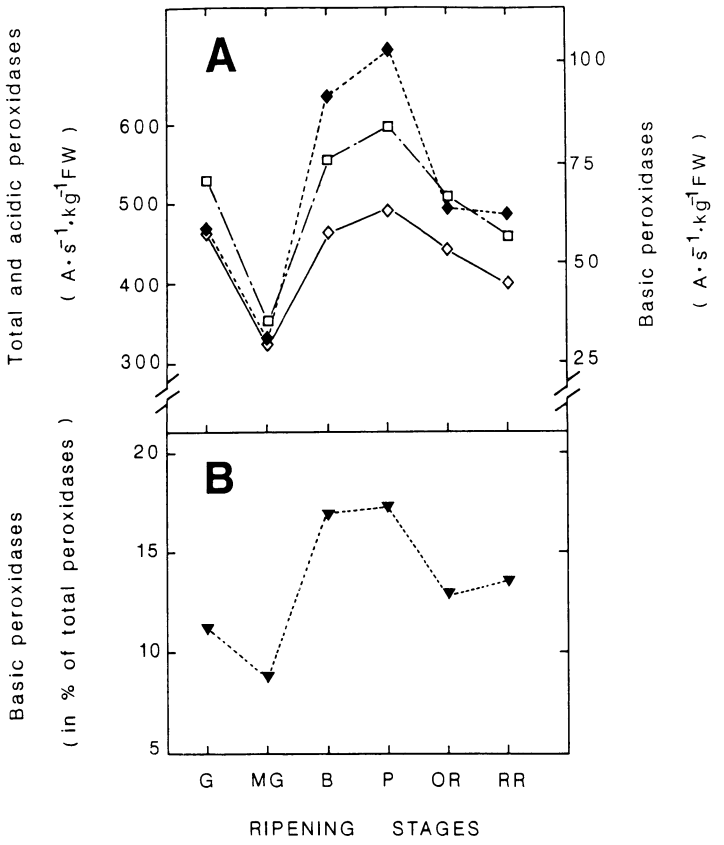

Fig. 3. Changes in peroxidase activities during tomato fruit ripening. (**A**) total (□), acidic (◇), and basic (◆) peroxidases and (**B**) basic peroxidases in percent of total peroxidases (▼). Error bars do not exceed the dimension of symbols.

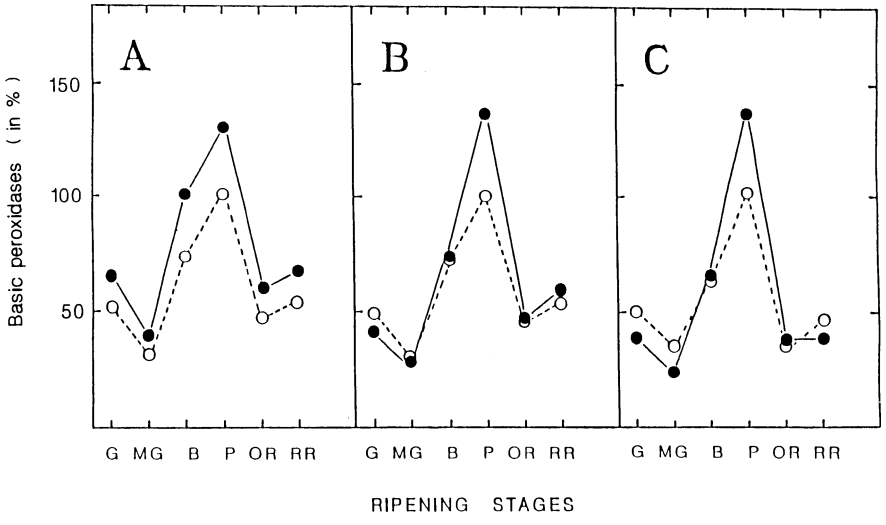

Fig. 4. Changes in peroxidase (**A**), IAA oxidase (**B**), and ACC oxidase (**C**) activities of the basic peroxidases during tomato fruit ripening. (○) Without added Ca. (●) With added Ca. Results are expressed in percent of the highest activities without added Ca (at the P stage).

likely that the same mechanism might be involved in IAA and ACC degradations. Such a view is supported by previous observations on one peanut peroxidase isozyme, where polyphenoloxidase and IAA oxidase activities are associated with the peroxidase activity (12), and on one tomato peroxidase isozyme, where polyphenoloxidase activity is associated with the peroxidase activity (13).

As the changes in all the measured activities of the basic peroxidases parallel those in ethylene production rates, it would be worthwhile to determine if a relationship exists between ethylene formation and basic peroxidases. Although the basic peroxidase-mediated degradation of ACC does not fit with the requirements for the conversion of ACC to ethylene in vivo (16), the involvement of the basic peroxidase in wound-ethylene formation has nevertheless been suggested (1). As the physiological roles of basic peroxidases are still unclear, further work is needed to assess the meaning of the concomitant changes in basic peroxidase and in ethylene production during tomato ripening.

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