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Chilling Injury and Electrolyte Leakage in Fruit of Different Tomato Cultivars[1]

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Additional index words. *Lycopersicon esculentum*, postharvest

Abstract. **Mature-green tomato fruit from 2 chilling-sensitive cultivars and 2 chilling-tolerant breeding lines, all derived from *Lycopersicon esculentum* Mill., were harvested and chilled at 5°C for 0, 2, 7, and 15 days. Mature-green fruit analyzed immediately after chilling showed higher electrolyte leakage in chilling-sensitive than chilling-tolerant lines. However, electrolyte leakage from chilled fruit that were ripened before analysis was higher in normal-sized than in cherry cultivars and seemed to be a function of fruit type rather than of chilling sensitivity. Unchilled field samples of these cultivars harvested at the mature-green, turning, and full-ripe stages and analyzed immediately showed an electrolyte leakage pattern similar to that found for comparable chilled mature-green fruit. No significant differences were found in calcium content (total, bound, or soluble) or in pectinase activity between chilling-sensitive or -tolerant cultivars.**

Many plants of tropical and subtropical origin are susceptible to chilling injury when exposed to nonfreezing temperatures in the range of 0 to 15°C. In terms of first observed response, time studies on cellular ultrastructure of tomato seedling cotyledons with chilling at 5° showed that membrane alterations preceded other changes and that different types of organelles varied in susceptibility (10). Such membrane changes alter physiological functions, including cellular permeability.

Increased leakage of solutes was first observed as one aspect of chilling injury in sweet potato (12). Chilling has since been shown to increase permeability of the plasmalemma membrane in various species, as measured by a decreased rate of ion uptake at chilling temperatures (17) or by increased solute or electrolyte leakage (6, 9, 19). Although electrical conductivity measurements of electrolyte leakage have been used to demonstrate differences after chilling in membrane permeability between chilling-sensitive and -tolerant tissues (11, 13), few have used it as a means of quantifying chilling injury within closely related species. Leaves from *Passiflora* sp. originating from lowland tropics showed higher electrolyte leakage rates on chilling and were less resistant to chilling temperatures than species from high altitudes (15). Related ^{3}H-leucine and ^{85}Rb leakage studies with leaves of *Lycopersicon* sp. indicated similar results (16). Our studies deal with tomato fruit rather than leaves, and with more closely related species than altitudinal variants.

Since chilling retards normal tomato ripening, pectinase activity may also be affected as reported for peach (2) and cucumber (7). Polygalacturonase activity, a component of pectinase, was absent in the nonripening tomato mutant *rin* (3). Calcium content is another factor that has been implicated in chilling effects. A strong correlation was found between endogenous calcium levels and the incidence of chilling injury in avocado (5).

Tomato fruit are subject to chilling injury particularly at the mature-green stage when they are normally shipped, and differences in tolerance to chilling injury have been found between different breeding lines of tomato (4). However, the effects of chilling are usually apparent only after exposure to ripening temperatures. Thus, one of the objectives of these experiments was to find an indication of chilling injury before the development of visual symptoms. We studied fruit of several tomato cultivars, also found to differ in chilling sensitivity, to determine if electrolyte leakage rates could be used as an index of chilling injury susceptibility in tomato fruit and to determine if either pectinase activity or calcium content could also be correlated with susceptibility.

Materials and Methods

Five tomato entries were used in this study: 'New Yorker' (normal size, chilling-sensitive); Line 79-546 (normal size, chilling-tolerant); 'Early Cherry' (cherry size, chilling-sensitive); 'Small

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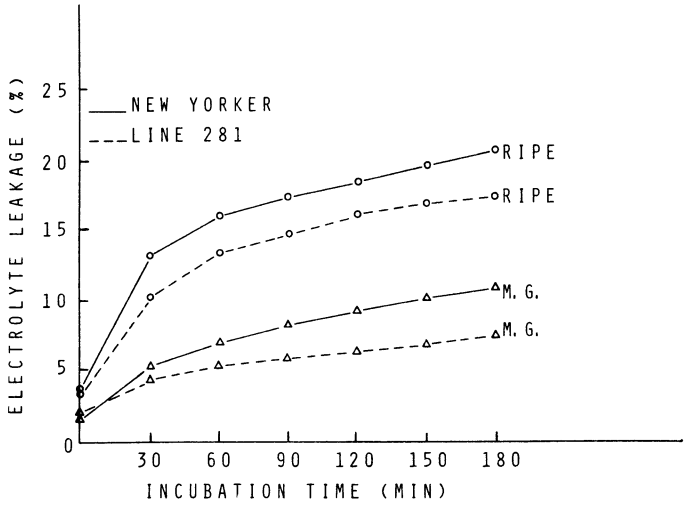

Fig. 1. Time course of electrolyte leakage from tomato pericarp discs of 2 cultivars, 1979: a) Mature-green chilled 7 days at 5°C (MG); b) Chilled and subsequently ripened (Ripe).

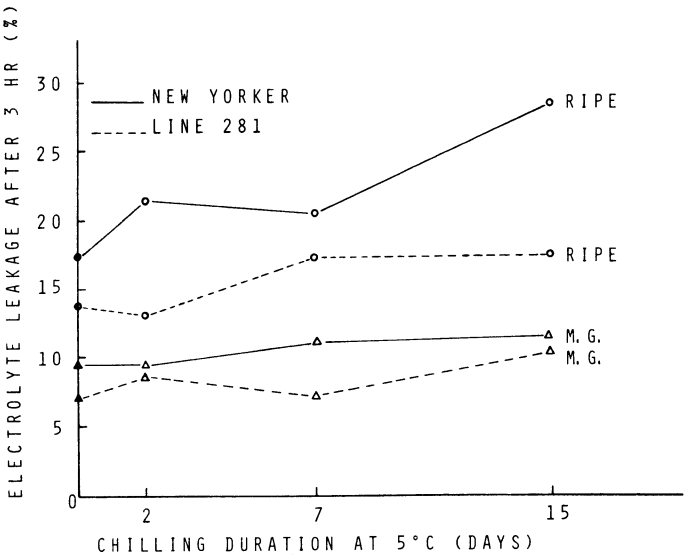

Fig. 2. Effect of chilling duration on electrolyte leakage from fruit of 2 tomato cultivars, 1979: a) Mature-green (MG); b) Chilled and subsequently ripened (Ripe).

Cherry' (cherry size, chilling-tolerant); and Line 281 (cherry size, chilling-tolerant).

All tomatoes were seeded in the greenhouse (April). Six-week-old seedlings were transplanted to the field in a completely randomized block design. Each cultivar or line contained 3 replications of 40 transplants spaced 45 cm apart and grown on black plastic. Fertilization, cultivation, irrigation, and fungicide practices were carried out as recommended. Fruit were harvested at the mature-green stage and dipped for 3 min in sodium hypochlorite solution containing 200 ppm active chlorine. After towel drying they were separated into the following chilling treatments:

1. a) control-no chilling b) plus 20°C ripening
2. a) 2 days at 5°C b) plus 20°C ripening
3. a) 7 days at 5°C b) plus 20°C ripening
4. a) 15 days at 5°C b) plus 20°C ripening

A single harvest was made in 1979, which was a preliminary experiment using only 2 entries ('New Yorker' and Line 281). The 5 entries were harvested twice in 1980.

Electrolyte leakage. From each of 3 randomly selected fruit in each treatment, 3 pericarp discs (12 mm diameter) were taken from the equatorial region of each fruit. The discs were weighed, rinsed 3 times in millipore water, and placed in 100 ml of 0.4M mannitol solution at room temperature. Electrical conductivity readings of the solution were taken at 30-min intervals over a 3-hr period as a measure of electrolyte leakage from the discs, using a Markson Electromark conductivity meter. After 3 hr the flasks were autoclaved for 30 min and cooled, and a final conductivity reading was taken for total electrolytes. All leakage data were expressed as a percentage of the total electrolyte readings (6).

Pectinase analysis. Pectinase activity in the tomato fruit was determined by use of the cucumber bioassay of Mussel and Morré (14). Tomato enzyme extracts were prepared by blending 5 g freeze-dried tomato pericarp (100 g fresh weight) in 100 ml of 5% (w/v) NaCl containing 1% (w/v) PVPP in a Waring blender for 1 min. After incubation for 1 hr at 4°C and pH 8 (using 1N NaOH), the homogenate was filtered through Miracloth and the pH of the filtrate was readjusted to pH 5 (3).

The volume of the reaction mixture was 5 ml. For mature-green tomato fruit this contained 1 ml tomato enzyme extract and 4 ml of citrate-phosphate buffer (0.01M), with controls of 1 m NaCl (pH 5) and 4 ml buffer. For ripened fruit, tubes contained 0.5 ml tomato enzyme extract and 4.5 ml buffer, with controls of 0.5 ml NaCl (pH 5) and 4.5 ml buffer. To each tube, 5 rinsed, blotted, and weighed cucumber fruit mesocarp sections (5 mm thick by 5 mm in diameter) were added and incubated at room temperature for 4 hr, after which they were strained and washed for 3 min under a strong spray of water. After standing in millipore water for 1 hr, the sections were blotted dry and reweighed. Each treatment was replicated 3 times, each enzyme extract 3 times, and each control 6 times.

Standard curves were prepared using commercial pectinase (Sigma Biochem. Corp. E.C.3.2.1.15). The weight loss of cuc-

Table 1. Electrolyte leakage from chilled mature-green fruit of 5 tomato cultivars (1980, Harvest I).

Chilling duration at 5°C (days)	Electrolyte leakage (%)				
	Normal-sized cultivars		Cherry cultivars		
	New Yorker	Line 79-546	Early Cherry	Small Cherry	Line 281
0	11.24 a[z]	9.02 bc	10.78 ab	8.70 c	8.36 c
2	9.73 b	10.99 ab	12.16 a	11.45 ab	7.72 c
7	13.47 a	10.91 b	13.44 a	8.81 c	8.80 c
15	17.89 b	14.00 c	20.57 a	13.82 c	10.40 d

[z]Mean separation in rows by Duncan's multiple range test, 5% level.

umber sections in the enzyme solutions (both standards and tomato extracts) was corrected by subtracting the weight loss of the controls, giving the net weight loss due to pectinase activity. Pectinase activity of tomato enzyme extract was then determined from standard curves.

Calcium analysis. Freeze-dried tomato pericarp (5 g) from 15 day-chilled and subsequently ripened fruit was extracted with stirring in 10 ml millipore water for 1 hr, centrifuged (15,000 g for 15 min), and filtered (18). The filtrates were used for soluble calcium analysis; residues were washed 3 times and dried for determination of bound calcium. Untreated dried pericarp was used for determination of total calcium. Bound calcium and total calcium samples were ashed at 500°C and dissolved in nitric acid. Calcium was analysed by plasma emission spectrometry.

Results and Discussion

The time course of electrolyte leakage from tomato discs over the 3-hr period showed increased leakage with time (Fig. 1). Therefore, only the data at 3 hr will be presented. In 1979, both cultivars showed a slight tendency for enhanced electrolyte leakage with increasing chilling time (Fig. 2). After ripening, electrolyte leakage was much greater, and was higher in 'New Yorker' than in Line 281. However, these cultivars differed not only in chilling sensitivity but also in fruit type—normal versus cherry. Hence, in 1980 other entries were included so that chilling-sensitive 'New Yorker' could be compared with tolerant Line 79-546 (both normal type), and also direct comparisons could be made within cherry types.

In 1980, chilling-sensitive 'New Yorker' at the mature-green stage had higher electrolyte leakage than did Line 79-546, while chilling-sensitive 'Early Cherry' showed higher leakage than did either 'Small Cherry' or Line 281 (Table 1). Although only data from the first harvest are presented, similar results were determined for the second harvest. As in 1979, the 1980 data for fruit ripened after chilling showed that 'New Yorker' had higher leakage than did Line 281 (Fig. 3). However, since both large fruited cultivars had higher electrolyte leakage than did the cherry types, this appeared to be more related to fruit size or type than to chilling sensitivity. This was the case even though visual symptoms of chilling injury expressed after ripening were more severe in chilling-sensitive than in chilling-tolerant cultivars.

There was no difference in soluble, bound, or total calcium content of the cultivars tested (15 days, chilled and ripened), with a range of 0.295 to 0.384% total calcium. Also no differences were found in pectinase activity between chilling-sensitive or -tolerant cultivars, either at the mature-green stage (value near zero) or after ripening (up to 0.025 units pectinase activity/0.5 ml tomato extract, equivalent to 56% weight loss of cucumber mesocarp).

Thus, in conclusion, enhanced electrolyte leakage was observed in tomato fruit after chilling at the mature-green stage, as previously reported for tomato (11) and cucumber (13). Not all chilling-sensitive fruit appear to show such differences, as electrolyte leakage was reported to be unaffected by chilling in both peach (8) and bell-pepper (13), while for eggplant it was said to be either affected (1) or not affected (13). In the present study there were also differences in electrolyte leakage from fruit of chilling-sensitive and chilling-tolerant cultivars, as there were from leaves of *Passiflora* sp. showing differing chilling sensitivities (15).

Interestingly enough, when leakage from unchilled field samples of the 5 cultivars at different maturity stages were compared,

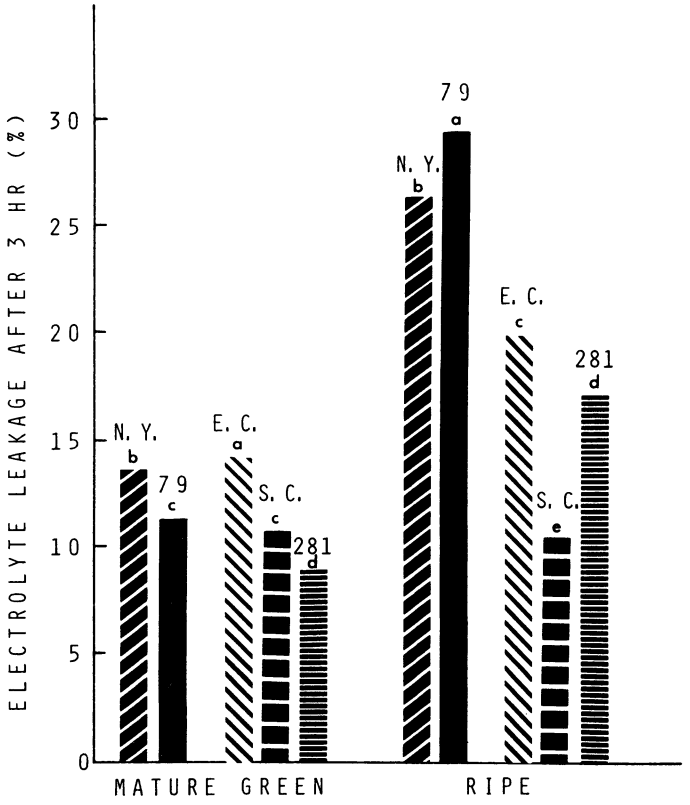

Fig. 3. Effect of chilling (mean of 2, 7, 15 days at 5°C) on electrolyte leakage from fruit of 5 tomato cultivars (1980, Harvest 1): a) Mature-green; b) Chilled and subsequently ripened. (Mean separation within a maturity stage by Duncan's multiple range test, 5% level.)

leakage patterns similar to those of chilled mature-green fruit were seen (Fig. 4). Again, the chilling-sensitive 'New Yorker' had higher electrolyte leakage than did chilling-tolerant Line 79-546, and 'Early Cherry' showed higher leakage than did either 'Small Cherry' or Line 281.

The differences in electrolyte leakage from chilled tomato fruit were observed before the development of visual chilling injury symptoms, and could be used as an indication of chilling injury susceptibility using mature-green fruit. However, since electro-

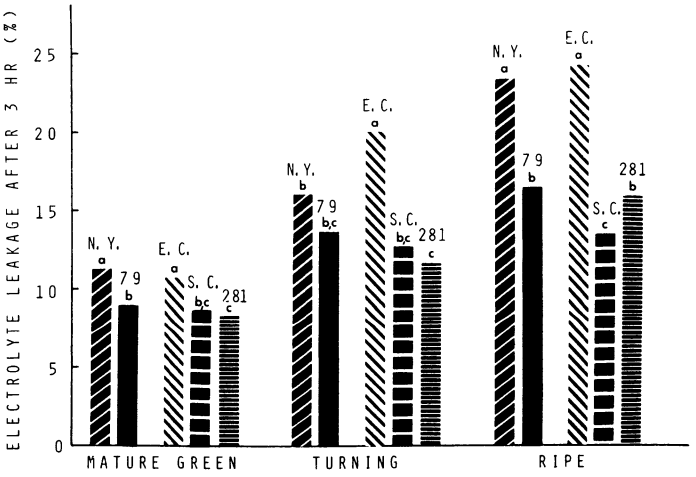

Fig. 4. Electrolyte leakage from unchilled field tomato fruit: effects of cultivar and maturity stage, 1980. (Mean separation within a maturity stage by Duncan's multiple range test, 5% level.)

lyte leakage studies of unchilled field samples followed that same pattern for all maturities tested, this may eventually be more useful in predicting chilling injury susceptibility.

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Effects of Ethephon-gibberellin Combinations on Yield, Size, and Quality of Muskmelon[1]

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Additional index words. *Cucumis melo*

Abstract. **Mixtures of (2-chloroethyl)phosphonic acid (ethephon) plus gibberellic acid (GA_3) were applied to 'Edisto-47' muskmelon (*Cucumis melo* L.) plants at the 3–4 true leaf stage for 3 growing seasons. Ethephon at 240 mg/liter + GA_3 at 100 mg/liter consistently increased the marketable yield of melons over the control. However, at 480 mg/liter ethephon, increased GA_3 concentration from 50 to 150 mg/liter decreased yields. Average fruit weight and length–diameter ratios were increased by all ethephon + GA_3 combinations, compared to the untreated control. Increased soluble solids and sweetness by 240 mg/liter ethephon + 100 or 150 mg/liter GA_3 combinations were associated with increased fruit weight.**

Ethephon affects the number of fruit produced by cucumber (9), squash (4, 11, 12), and muskmelon (7, 12) primarily by affecting sex expression. Although ethephon treatment can increase yield and enhance ripening, it can also result in unfavorable quality attributes to muskmelon including reduced yields (8, 15), deformed fruit (7), reduction of soluble solids (8, 15), undesirable flavor, and softened fruit (15). GA_3 treatment of cucurbits has been shown to affect sex expression (3, 5, 14) and to enhance fruit size (3). This study was conducted to examine the possible interactive effects of field applications of ethephon and gibberellin mixtures on yield, size, and quality of muskmelon.

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Materials and Methods

'Edisto-47' muskmelon plants were grown in plastic trays containing 63 × 58 mm cells filled with Jiffy mix. Four-week