Tomato Fruit Chilling Tolerance in Relation to Internal Atmosphere after Return to Ambient Temperature

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Additional index words. *Lycopersicon esculentum*, CO₂

Abstract. Mature-green 'Vedette' tomato (*Lycopersicon esculentum* Mill.) fruit were stored with (+P) or without (-P) peduncles at 1°C. During storage and after return to ambient temperature, pigment content and electrolyte leakage of pericarp tissue and fruit internal atmosphere composition were monitored. The +P fruit showed severe chilling injury (CI) symptoms—shriveling and brown discoloration of the surface—on transfer to 20°C after at least 8 days of exposure to low temperature. The chilling-injured fruit did not ripen normally; i.e., pigmentation did not change at 20°C. The -P fruit were largely unaffected and ripened normally at 20°C. Severely injured tomatoes showed an apparent decrease in electrolyte leakage after transfer to ambient temperature. The CO₂ content of the -P fruit internal atmosphere was significantly lower than in +P tomatoes after return to 20°C. The peduncle scar has a greater permeability to gases than the skin and facilitates gas exchange with the external atmosphere. The accumulation of CO₂ in the internal atmosphere of the chilled +P fruit after transfer to 20°C apparently promoted CI symptom development.

Mature-green tomato fruit are sensitive to chilling when stored at temperatures below 13°C (Autio and Bramlage, 1986; Wang, 1989). The main chilling-injury (CI) symptoms are abnormal ripening and increased postharvest decay (Autio and Bramlage, 1986; Cheng and Shewfelt, 1988).

Controlled or modified atmospheres have been used to increase tomato storage life at nonchilling temperatures (Buescher, 1979; Geeson et al., 1985; Nakhasi et al., 1991; Parsons et al., 1970; Salunkhe and Wu, 1973). These storage techniques also have been used in conjunction with low temperature. In some studies, modifying the storage atmosphere failed to alleviate tomato CI (Forney and Lipton, 1990; Kader and Morris, 1975; Tomkins, 1963), while in other studies, chilling symptoms were delayed or reduced (Brown et al., 1989; Hobson, 1987).

The present report stems from our observation that mature-green tomato fruit without peduncles (-P) stored at low temperature showed less severe CI symptoms than fruit with the peduncle still attached (+P) after return to ambient temperature. As a preliminary study of using modified atmospheres to alleviate CI, the influence of fruit internal atmosphere composition on CI symptom develop-

ment was examined in mature-green +P or -P tomatoes stored at 1°C and subsequently returned to 20°C.

Mature-green (U.S. Dept. of Agriculture Marketing Service, 1976), greenhouse-grown 'Vedette' tomatoes were harvested from a local farm. The fruit were selected for uniformity in size and absence of lesions, and their surfaces were sanitized in a 1% (v/v) hypochlorite solution. The tomatoes were divided into two lots: the peduncles and calyxes were removed from one lot (-P), while the other lot was left intact (+P). The fruit were stored in darkness at 1°C under a continuous flow of humidified air at 85% relative humidity. Three fruit from each lot were analyzed on day 0, 6, 8, and 21 for pericarp pigment content, electrolyte leakage, and internal atmosphere composition. After 6, 8, and 10 days of chilling, three fruit were returned to 20°C and analyzed after 7 days. Some unchilled fruit were kept at 20°C to measure the internal atmosphere during normal ripening at the mature-green, turning, and red-ripe stages.

The fruit were visually evaluated for CI symptoms, including surface pitting, shriveling, skin discoloration, and uneven ripening.

Ripening was assessed based on pigment content. Seven-millimeter-diameter disks were sampled from the outer pericarp tissue with a cork borer. Two grams of freshly excised disks was homogenized in 10 ml of 60 hexane : 40 acetone (v/v) for 2 min at 9500 rpm using an Ultra-turrax homogenizer (model T 25; Janke and Kunkel, Hohenstaufen, Germany). The homogenate was vacuum-filtered. The residue was rinsed twice with 10 ml of extraction solvent, and the combined extracts were centrifuged for 5 min at 500× g. The upper phase was collected and evaporated to dryness under N₂. The residue was dissolved in 5 ml of

chloroform, and the optical density was measured (model 8451A Diode Array; Hewlett Packard, Mississauga, Ont., Canada) at 480 nm for carotenoids, mainly lycopene, and at 664 nm for chlorophyll. Pure pigment solutions (Sigma, St. Louis) were used to prepare standard curves.

Electrolyte leakage was determined as an indirect measure of membrane damage and CI. About 2 g of freshly excised disks from three fruit per treatment was placed in 100 ml 0.4 M mannitol and incubated at 25°C for 4 h. Electrical conductivity of the bathing solution was determined at 25°C using a conductance meter (model 35; YSI, Yellow Springs, Ohio) after incubation and again after autoclaving at 121°C for 30 min to liberate all electrolytes. Electrolyte leakage was expressed as percentage of total electrolytes.

To determine the internal atmosphere, fruit were immersed in water, and 1 ml of internal atmosphere was removed with a syringe. The peduncles of the intact fruit were removed under water—just before sampling. Carbon dioxide, O₂, and N₂ were determined by a gas chromatograph (GC) (model 8500; Perkin Elmer, Mississauga, Ont., Canada) fitted with a thermal conductivity detector and two columns: Porapak Q (90 × 0.3 cm, 80/100 mesh) and molecular sieve (180 × 0.3 cm, 80/100 mesh; Supelco, Oakville, Ont., Canada). The oven was isothermal at 60°C.

Two experiments were carried out during this study and they yielded similar results. The data from the first experiment are reported. Data are means ± SD from triplicate measurements for each treatment. Each datum was the average of three determinations per fruit for electrolyte leakage and internal atmosphere and of two pigment determinations.

Tomatoes developed only slight surface pitting after 21 days of low-temperature storage. Fruit did not ripen during chilling at 1°C, as assessed by the absence of red pigment. No CI symptoms developed during 7 days at 20°C after 6 days of chilling, and the fruit began to ripen normally. After 8 and 10 days of chilling, however, severe CI symptoms developed on +P fruit, while -P fruit were largely unaffected. After 7 days at 20°C following 8 and 10 days of chilling, +P tomatoes showed no sign of ripening and displayed severe shriveling and brown skin discoloration. One-third of the -P fruit was slightly affected by chilling and the rest ripened normally. A preliminary screening of tomato cultivars indicated that the difference in response to chilling between +P and -P 'Vedette' fruit may not apply to all cultivars.

Although chlorophyll content was reduced by ≈50% after 21 days of chilling at 1°C, no lycopene accumulated during this period (Fig. 1). This result indicates that no significant ripening occurred at low temperature. After 6 days of chilling, transferring the fruit to ambient temperature led to lycopene accumulation and chlorophyll depletion in +P and -P fruit. Only -P tomatoes accumulated lycopene after transfer to 20°C following 8 and 10 days of chilling.

Received for publication 13 Sept. 1992. Accepted for publication 23 Oct. 1992. This research was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) strategic grant no. STR0114645. M.B. was a recipient of an NSERC postgraduate fellowship. Contribution no. 49 of the Centre de Recherche en Horticulture, Université Laval; contribution no. 266 of the Food Research and Development Center (FRDC), Agriculture Canada, Saint-Hyacinthe.

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Fig. 1. Pigment contents of pericarp tissue of fruit with (+) or without (-) peduncles during storage at 1°C and after transfer to 20°C for 7 days following 6 (6+7), 8, or 10 days of chilling. (a) Chlorophyll, (b) lycopene. Vertical bars indicate sd.

Electrolyte leakage from the pericarp tissue progressively increased during storage at 1°C (Fig. 2). The increase was similar for +P or -P fruit. Only after 21 days of chilling did electrolyte leakage reach a higher value for +P tomatoes. After transfer to 20°C, electrolyte leakage decreased for both fruit lots. The +P fruit electrical conductivity during storage at ambient temperature consistently returned to the values measured at day 0 before chilling. The -P fruit conductivity never reached this low value after storage for 7 days at 20°C following 6, 8, or 10 days of chilling.

The internal atmosphere CO_2 content decreased during low-temperature storage, from 2.4% on day 0 to $\approx 1.0\%$ at day 21 (Fig. 3). At each sampling date, CO_2 content was lower in -P than in +P fruit. After transfer to 20°C, CO_2 content increased in all fruit but to a greater extent in +P fruit, which developed severe CI symptoms. The increase in CO_2 content during storage at 20°C in -P fruit became greater with length of exposure to low temperature. After

10 days of chilling, CO_2 content reached almost the same level as that in +P fruit. Fruit 0₂ content was affected little by exposure to low temperature and subsequent storage at 20°C, and remained between 16% and 21%. Normal fruit ripening at ambient temperature had little influence on internal atmosphere composition (Table 1). Fruit contained a slightly lower CO_2 content at the red-ripe than at the mature-green stage. The +P fruit accumulated some CO_2 at the turning stage.

The results of this study suggest a link between tomato internal atmosphere composition after fruit were returned to ambient temperature and fruit response to chilling, as assessed by ripening and electrolyte leakage. After exposure to low temperature, differences in internal atmosphere composition of +P and -P fruit became apparent mainly during storage at 20°C, when differences in ripening, electrolyte leakage, and CI symptoms developed between +P and -P fruit. The +P tomatoes that accumulated CO_2 during postchilling

Table 1. Content (percent) of CO_2 in the internal atmosphere of nonchilled tomato fruit, with (+P) or without (-P) peduncles, during normal ripening at 20°C.

Ripening stage	CO_2 content (%)	
	+P	-P
Mature-green	1.82 ± 0.56	1.82 ± 0.56
Turning	2.39 ± 0.52	1.06 ± 0.14
Red-ripe	1.33 ± 0.53	1.39 ± 0.30

storage at 20°C lost their ability to ripen after 8 days of chilling. Chilled-P fruit accumulated less CO_2 at 20°C and maintained their ability to ripen, even after 10 days of chilling.

The reduction in CO_2 during low-temperature storage (Fig. 3) probably reflects a reduced fruit respiration rate at 1°C. The increase in CO_2 in +P fruit internal atmosphere from 2.4% to $>4\%$ during storage at 20°C following chilling may result from an enhanced respiration rate, as reported for many chilling-sensitive crops, including tomatoes (Lyons and Breidenbach, 1990), following low-temperature treatment. The peduncle scar, which is much more permeable to gases than the skin (Bertola et al., 1990), allowed more CO_2 to escape from the -P tomatoes, and the CO_2 content in these fruit remained $<3\%$ after 6 and 8 days of chilling. The increasing accumulation of CO_2 in -P fruit at 20°C with longer exposure to chilling temperatures (Fig. 3) may reflect increasing CI.

The accumulation of CO_2 in the internal atmosphere of +P fruit after they were returned to ambient temperature may result from a stress-induced stimulation of respiration and from an increased resistance of the tissues to CO_2 diffusion out of the fruit. The increased tissue resistance to gas diffusion would follow intercellular space flooding due to the damaged membranes' enhanced permeability to water.

Although electrolyte leakage—an indirect measure of membrane damage—increased slightly with chilling time, it correlated neither with internal CO_2 content nor with CI symptoms. No difference in electrical conductivity was noticed between +P or -P fruit during chilling, except after 21 days, although +P fruit consistently contained higher CO_2 concentrations. The fruit chilled for 21 days showed the greatest electrolyte leakage but contained the least CO_2 .

After 10 days of chilling and 7 days at 20°C, -P fruit had accumulated $>4\%$ CO_2 in their internal atmosphere—almost as much as +P fruit—but showed relatively little electrolyte leakage and no CI symptoms. The absence of severe symptoms may reflect slower CO_2 accumulation in -P fruit and, therefore, shorter exposure to high CO_2 .

Chilled fruit electrolyte leakage values decreased during subsequent storage at 20°C, while CO_2 accumulated and CI symptoms developed. This apparent decrease in electrolyte leakage after return to ambient temperature—when CI symptoms appear and when membrane damage is expected to be maximal—is an indication of extensive membrane damage (Bergevin et al., 1993; Côté and Willemot, 1992; Furmanski and Buescher,

Fig. 2. Electrolyte leakage from pericarp tissue of tomato fruit with (+) or without (-) peduncles. Other details as described for Fig. 1.

Fig. 3. Internal atmosphere CO₂ content of tomato fruit with (+) or without (-) peduncles. Other details as described for Fig. 1.

1979). The return of the conductivity values to prechilling levels in +P fruit and CO₂ accumulation >4% were further indications of greater damage to fruit membranes.

The relatively small change in internal atmosphere composition during normal ripening (Table 1) shows that CO₂ accumulated in the chilled fruit after they were returned to 20°C because of the previous low-temperature treatment, not simply because of the consequence of the climacteric rise in respiration during postchilling ripening.

The results of the present study indicate that the internal atmosphere of chilled tomato

fruit during subsequent storage at 20°C influenced CI symptom development. The +P fruit accumulated >4% CO₂ and eventually developed CI symptoms. A 5% CO₂ content may cause physiological disorders similar to CI (Kader and Morris, 1975; Parsons et al., 1970). Carbon dioxide should not be considered a direct cause of CI symptoms, however, as +P fruit chilled for 6 days and returned to ambient temperature did not develop symptoms, although they accumulated >4% CO₂. High CO₂ apparently enhanced only CI or CI symptom development, possibly by aggravating cell membrane destabilization initiated by low-

temperature stress. CI may then result from prolonged exposure to low-temperature and postchilling high-CO₂ stress.

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