Physiological Response to Chilling Temperatures of Intermittently Warmed Cucumber Fruit

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Abstract. Symptoms of chilling injury were reduced by intermittently warming cucumber fruit (*Cucumis sativus* L. cv. Poinsett 76) from 2.5 to 12.5C for 18 hr every 3 days. Fruit continuously held at 2.5C for 13 days developed severe pitting and decay after 6 days at 20C, while fruit continuously held at 12.5C or intermittently warmed showed no pitting or decay during subsequent holding at 20C. The increased rate of C_2H_4 production during the first warming period, from 12 $nl \cdot (kg \cdot hr)^{-1}$ at 2.5C to 201 $nl \cdot (kg \cdot hr)^{-1}$ at 12.5C, was significantly greater than that during the second or third warming periods, i.e., 53 to 98 and 53 to 55 $nl \cdot C_2H_4 / (kg \cdot hr)$, respectively. Respiration increased 3-fold during the initial warming period, but only 2-fold during subsequent warming periods. Leakage of cellular ions from excised disks of mesocarp tissue was around 6% and 10% of the total ion content of the tissue for control and intermittently warmed fruit, respectively, but increased to 17% for fruit that were continuously held at 2.5C for 10 days. After 320 hr (three cycles) of chilling and warming, chilled fruit showed significantly lower ethylene-forming enzyme activity than the control or intermittently warmed fruit. Fruit held at 12.5C contained 0.09 to 0.34 $nmol \cdot g^{-1}$ of ACC. ACC levels were 6.23 $nmol \cdot g^{-1}$ in fruit exposed to 2.5C for 320 hr. In contrast, intermittently warmed fruit only showed 30% and 27% increases in ACC content during the first and second warming periods, respectively. Periodic warming appears to allow chilled fruit to acclimate to subsequent periods of chilling. Chemical names used: 1-aminocyclopropane-1-carboxylic acid (ACC).

Although the storage life of freshly harvested fruits and vegetables is usually prolonged at temperatures near 0C, many horticultural crops of tropical and subtropical origin are chilling-sensitive and are injured if held at nonfreezing temperatures below 12C (Lyons, 1973; Saltveit and Morris, 1989). Cucumbers are chilling-sensitive and are injured if held at temperatures < 10C for more than 3 days (Eaks and Morris, 1956). Chilling-sensitive crops can develop symptoms of chilling injury either during storage at chilling temperatures, or subsequently during marketing at nonchilling temperatures. Injury symptoms include the formation of sunken, dark-colored watery areas (pits) and increased susceptibility to decay and fungal growth (Ryan and Lipton, 1979).

Eaks and Morris (1956) reported increased respiration and disease susceptibility and rapid senescence of cucumber fruit held at 0 or 5C. Earlier findings of Mack and Janer (1942) revealed a similar increase in CO_2 production of cucumber during storage at 2 to 3C. Wang and Adams (1980, 1981) observed an increase in C_2H_4 production when cucumbers that had been chilled for 4 days at 2.5C were transferred to 25C. However, they did not observe an increase in respiration or C_2H_4 production during the chilling period (Wang and Adams, 1980). Increased production of C_2H_4 by chilled cucumbers could reduce quality, since it has been shown that exposure to $\mu l \cdot C_2H_4 / liter$ (ppm) air mixtures accelerates the senescence of cucumber fruit (Saltveit and McFeeters, 1980).

Postharvest temperature treatments that reduce symptoms of chilling injury include conditioning at near-chilling temperatures and intermittent warming during chilling (Morris, 1982; Wang, 1982). Chilling injury has been reduced in fruits of bell pepper (Wang and Baker, 1979), grapefruit (Davis and Hoffmann, 1973), and tomato (Saltveit and Cabrera, 1987), and tomato seedlings

and ornamental (Morris, 1982; Wheaton and Morris, 1967) by conditioning them at cool, nonchilling temperatures before chilling. Intermittent warming has been reported to reduce chilling injury in bell peppers (Wang and Baker, 1979), citrus (Davis and Hofmann, 1973; Eaks, 1965), cucumbers (Wang and Baker, 1979; Hirose, 1985), okra (Ilker, 1976), potatoes (Hruschka et al., 1968), and peaches and nectarines (Anderson, 1982; Wang and Anderson, 1982).

Ethylene synthesis progresses from the amino acid methionine to S-adenosylmethionine to ACC to C_2H_4 (Yang, 1980). ACC is converted to C_2H_4 by the ethylene-forming enzyme (EFE). The rate at which C_2H_4 is produced in most tissues is governed by the rate at which ACC is synthesized, although under some conditions the EFE activity may be the rate-controlling step.

The objective of our present study was to determine the effect of intermittent warming during chilling on the respiration and C_2H_4 metabolism of cucumber fruits, and to evaluate the effect of intermittent warming on alleviating symptoms of chilling injury.

Materials and Methods

Plant materials. 'Poinsett 76' cucumber fruits were hand-harvested from the Peto Seed Research Center in Woodland, Calif. Nine uniform fruit, blocked for size and shape among treatments and free from injury, were used in each treatment. Fruit were placed in shallow plastic trays that were covered loosely with a plastic film to minimize water loss.

Temperature management. The time to warm and/or cool individual cucumber fruit when transferred between the two experimental temperatures of 2.5 and 12.5C was determined by periodically recording the temperature shown by a thermometer inserted in the seed cavity of each of nine fruit used only for this purpose. The half-time for either warming or cooling was about an hour.

There were three temperature treatments: 1) control fruit were continuously held at 12.5C; 2) chilled fruit were continuously held at 2.5C; and 3) intermittently warmed fruit were held at 2.5C for 3 or 4 days and warmed at 12.5C for 18 hr before

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transfer back to the chilling temperature. Intermittently warmed fruit were subjected to from one to three temperature cycles. All fruit were subsequently evaluated at 20C. Each experiment was repeated with similar results.

Pitting and decay. The incidence of pitting and decay was determined subjectively by an 8-point Hedonic scale, where 0 = no pitting or decay (0% of the surface area was pitted or decayed), 2 = slight (1% to 5%), 4 = moderate (6% to 15%), 6 = severe (16% to 75%), and 8 = very severe (> 75%). Measurements were made 0, 2, 4, and 6 days after transfer to 20C.

Measurement of ethylene and CO₂ production. Production of C₂H₄ and CO₂ were calculated from an analysis of 1-ml samples of the head space gas accumulated in 4-liter glass jars (Saltveit and McFeeters, 1980). Three replicates of three fruit were used in each determination. Rates of C₂H₄ and CO₂ production were calculated from measurements taken at 2.5, 12.5, and 20C.

Measurement of ion leakage. Epidermal mesocarp disks were excised with a stainless steel cork borer from the central region of each fruit, trimmed of seed cavity tissue to 4 mm thickness with a stainless steel razor blade, and washed for 1 min in two changes of 20 ml deionized water. Three 4-mm-thick × 9-mm-diameter disks, weighing a total of ≈ 1 g, were incubated in a 100-ml beaker containing 30 ml of 0.3 M mannitol and shaken at 100 cycles per min. Conductivity measurements were taken with an Exttech Conductivity Meter Model 480 (Waltham, Mass.) 0.5 and 1 hr after adding the mannitol. Preliminary experiments had shown that, after a nonlinear increase for the first 20 min, the conductivity of the mannitol solution increased linearly for up to 3 hr from both chilled and nonchilled disks (data not shown). The beakers containing the tissue and mannitol were weighed and the contents boiled for 5 min. After cooling to room temperature, weights were adjusted to the original weights with deionized water and total conductivity was measured after 30 min of shaking. To compensate for differences among the samples, results are expressed in terms of relative leakage; i.e., the change in conductivity of the solution during the 1-hr sampling period as a percent of the conductivity of the solution after boiling.

Determining EFE activity and ACC content. The EFE activity and ACC content were also analyzed in washed 4 × 9-mm-diameter epidermal mesocarp disks. For the determination of EFE activity, disks were placed epidermis down in 15 × 60-mm plastic petri dishes and 10 µl of deionized water or 10 µM ACC was applied to each disk. After 6 hr, four disks from each treatment were blotted dry and transferred to 16 × 100-mm test tubes that were capped with rubber serum stoppers. After 1 hr of incubation, a 1-ml sample of the headspace gas was withdrawn and injected in a Carle (Loveland, Colo.) gas chromatograph with a flame ionization detector to quantitate the C₂H₄ produced. EFE activity was calculated as the difference between C₂H₄ production with and without ACC.

ACC was extracted from three disks in 10 ml of 90% ethanol in a tared 16 × 100-mm test tube for 7 hr at 70C, and assayed as reported by Lizada and Yang (1979).

Results

Pitting and decay. Pitting and decay were observed neither on the control fruit held at 12.5C nor on the intermittently warmed fruit, either during the 13-day temperature treatment or during 6 additional days of storage at 20C (Table 1). In contrast, continuously chilled fruit developed slight pitting (i.e., a score of 1) after 13 days of chilling. The severity of pitting increased to

Table 1. Pitting and decay of cucumber fruit stored for 13 days at 2.5C.^z

Additional days at 20C	Defect ^y	
	Pitting	Decay
0	1.0	0.0
2	4.2 *	0.0
4	7.3 *	2.6 *
6	7.9 *	6.0 *

^zThe incidence of pitting and decay was determined subjectively by an 8-point Hedonic scale, where 0 = no pitting or decay (0% of the surface covered), 2 = slight (1% to 5%), 4 = moderate (6% to 15%), 6 = severe (16% to 75%), and 8 = very severe (> 75% of the surface covered). Pitting and decay were absent in fruit held continuously at 12.5C or intermittently warmed from 2.5 to 12.5C for 18 hr every 2 or 3 days.

^yMeans were separated within columns by using Dunnett's test ($P = 0.05$,*) to compare each longer period with zero time.

Fig. 1. Ethylene-forming enzyme activity (EFE) of chilled cucumber fruit with intermittent warming. The fruit were chilled at 2.5C for 11 days with 0, 6, 12, or 18 hr of warming to 12.5C after 5 days of chilling. EFE activity is expressed as the difference between C₂H₄ production with and without added ACC. Comparison by Dunnett's test, $P = 0.05$. Plus ACC, EFE—all differ from 0 hr; minus ACC, only 18 hr differs from 0 hr.

moderate (4.2) and very severe (7.9) after the chilled fruit had been warmed at 20C for 2 and 6 days, respectively. Fungus, which was identified as *Aspergillus* sp. (black mold), was observed in pitted areas after 2 days at 20C only on fruit that had been continuously chilled at 2.5C for 13 days.

EFE activity of intermittently warmed fruit. The level of EFE activity measured after 11 days of chilling at 2.5C was significantly lower for fruit that had the chilling period interrupted after 5 days by warming to 12.5C for 6, 12, or 18 hr than for fruit continuously chilled (Fig. 1). Fruit that were not warmed had almost twice as much EFE activity as did the fruit warmed for 12 or 18 hr; i.e., activities of 21 and 11 µl C₂H₄/(kg·hr), respectively. In subsequent intermittent warming experiments, warming periods of 18 hr were used.

Ethylene production. The rate of C₂H₄ production remained between 5 and 10 nl·(kg·hr)⁻¹ and between 10 and 20 nl·(kg·hr)⁻¹ for fruit held continuously at 2.5 or 12.5C, respectively (Fig. 2). Warming fruit to 12.5C after 72 hr at 2.5C resulted in a rapid 18-fold increase in the rate of C₂H₄ production from 5.4 to 95.5 nl·(kg·hr)⁻¹ within 18 hr of warming. Cooling these

Fig. 2. Effect of intermittent warming on C_2H_4 production of cucumber fruit. The fruit were either held continuously at 2.5 or 12.5°C for 144 hr, or held at 2.5°C for 72 hr, 12.5°C for an additional 18 hr, and then at 2.5°C for the remainder of the 144-hr treatment period. All fruit were warmed to 20°C after 144 hr. Vertical error bars representing the SE of the mean are contained within the symbols.

fruit to 2.5°C was quickly followed by a reduction in the rate of C_2H_4 production from 95.5 to 8.5 $nl \cdot (kg \cdot hr)^{-1}$ within 6 hr of cooling. After 6 days (i. e., 144 hr), the rate of C_2H_4 production had started to increase in the fruit continuously held at 12.5°C. This increase coincided with yellowing and appearance of fungi on some fruit.

All fruit were transferred to 20°C after 144 hr. Substantial increases in the rate of C_2H_4 production from 5.2 to 61 $nl \cdot (kg \cdot hr)^{-1}$ and from 6.7 to 35 $nl \cdot (kg \cdot hr)^{-1}$ were observed 9 hr after the continuously chilled and intermittently warmed fruit, respectively, were transferred to 20°C (Fig. 2). There was only a slight rise in the rate of C_2H_4 production when the fruit continuously held at 12.5°C were transferred to 20°C. The rate of C_2H_4 production from all the fruit continued to increase, reaching 371, 156, and 35 $nl \cdot (kg \cdot hr)^{-1}$ after 24 hr at 20°C for the 2.5°C, intermittently warmed, and 12.5°C fruit, respectively.

Experiments with multiple cycles of warming were performed next. The rate of C_2H_4 production from control fruit that were continuously held at 12.5°C doubled during the 13-day experiment (Table 2). It slowly increased from 17.5 to between 31 and 36 $nl \cdot (kg \cdot hr)^{-1}$ during the 320-hr storage period at 12.5°C. The average rate of production during this period was 30 ± 12 $nl \cdot (kg \cdot hr)^{-1}$. As in the previous experiments with one warming cycle, C_2H_4 production remained around 34 ± 3 $nl \cdot (kg \cdot hr)^{-1}$ during 2 subsequent days of storage at 20°C for the fruit that had been continuously held at 12.5°C.

Low rates of C_2H_4 production were also observed during the first 170 hr (≈ 7 days) of continuous storage at 2.5°C (Table 2). When sampled after ≈ 10 days at the end of the third chilling cycle (i. e., 230 hr) or at the end of the third warming cycle (i. e., 248 hr) however, the rate of C_2H_4 production had increased around 2-fold to 36 $nl \cdot (kg \cdot hr)^{-1}$, and then increased an additional 20% to around 44 $nl \cdot (kg \cdot hr)^{-1}$ during the 320-hr (≈ 13 days) sampling period. The increased production of C_2H_4 after 9 days (230 hr) of continuous chilling is similar to the increase in CO_2 production previously reported (Mack and Janer, 1942; Eaks and Morris, 1956). A dramatic 12.5-fold increase in C_2H_4

production to 554 $nl \cdot (kg \cdot hr)^{-1}$ occurred when the continuously chilled fruits were transferred from 2.5°C to 20°C after 13 days. Ethylene production declined to 52 $nl \cdot (kg \cdot hr)^{-1}$ after an additional day at 20°C; a level of production significantly higher than that during the last measurement at 2.5°C.

Fruit in all three temperature treatments had similar rates of C_2H_4 production after 55 hr; these rates had not changed significantly after 73 hr for the chilled and control fruit. In contrast, warming fruit to 12.5°C at 73 hr resulted in an almost 20-fold increase in C_2H_4 production. The increased rate of C_2H_4 production during the first warming period from 12 $nl \cdot (kg \cdot hr)^{-1}$ at 2.5°C to 201 $nl \cdot (kg \cdot hr)^{-1}$ at 12.5°C was significantly greater than during the second or third warming periods; 53 to 98, and 53 to 55 $nl \cdot (kg \cdot hr)^{-1}$, respectively. The Q_{10} of the increase in C_2H_4 production upon warming 10 degrees from 2.5 to 12.5 was around 17 for the first warming period and 1.8 and 1.1 during the two subsequent warming periods. Rates of C_2H_4 production were similar for both chilled and intermittently warmed fruit at 320 hr and again after 2 days at 20°C.

Respiration rate. Low respiration rates, ≈ 10 to 20 $mg CO_2 \cdot (kg \cdot hr)^{-1}$, were measured from fruit continuously held at 12.5°C, while rates of 7 to 9 $mg \cdot (kg \cdot hr)^{-1}$ were measured from fruit continuously chilled at 2.5°C (Table 2). Since the measurements were made at 12.5 and 2.5°C, respectively, a 2-fold difference in respiration rates was expected. The Q_{10} during the 13-day storage period ranged from 1.2 to 2.2, with an average of 1.8 ± 0.4 between the fruit continuously held at 2.5 and 12.5°C.

Carbon dioxide production by intermittently warmed fruit increased as the fruit were warmed from 2.5 to 12.5°C. The rate of CO_2 production at 2.5°C (i. e., at the end of each chilling period) steadily increased from 7.9 to 11.7 $mg \cdot (kg \cdot hr)^{-1}$ during the first 230 hr of the experiment, before declining to 7.3 $mg \cdot (kg \cdot hr)^{-1}$ at the end of the last chilling period. The respiration rate after 320 hr was similar for the chilled and intermittently warmed fruit and, as expected with a Q_{10} of 2, about double for the control fruit at 12.5°C.

The rate of CO_2 production increased 3-fold during the first warming period, but only increased around 2-fold during the two subsequent warming periods. All fruit showed increased respiration when warmed at 20°C.

Ion leakage. The rate of ion leakage from mesocarp disks excised from fruit continuously held at 12.5°C remained at $\approx 6\%$ of the total ions per hour during the entire 12.5°C storage period, while it fluctuated between 6% and 9% for fruit kept at 2.5°C for 169 hr (Fig. 3). From 230 hr on, the rate of ion leakage remained substantially different for each temperature treatment. It had increased to between 8.5% and 11% for intermittently warmed fruit and to between 16% and 18% for continuously chilled fruit. Ion leakage increased only minimally when either fruit continuously held at 12.5°C or intermittently warmed fruit were transferred to 20°C. In contrast, ion leakage from chilled tissue rose substantially, from 17% to 23%, upon warming.

EFE activity. The EFE activity of fruit continuously held at 12.5°C showed a variable, but slowly increasing, rate of activity from 23 to around 40 $\mu l C_2H_4 \cdot (kg \cdot hr)^{-1}$ during the 320 hr (13 days) of the experiment (Table 3). In contrast, fruit continuously chilled at 2.5°C showed a brief increase in activity at 55 hr from 23 to 43 $\mu l C_2H_4 \cdot (kg \cdot hr)^{-1}$, before starting a steady decline in activity to 6.4 $\mu l C_2H_4 \cdot (kg \cdot hr)^{-1}$, which continued even after transfer to 20°C.

EFE activity of intermittently warmed fruit during their first chilling cycle was similar to fruit held continuously at 2.5°C. At

Table 2. Ethylene production and respiration rates of cucumber fruit stored at 2.5C, 12.5C, and intermittently warmed (IW). IW fruit were held 2.5C and warmed to 12.5C for 18 hr every 2 to 3 days. Chilling and warming cycles refer to the IW fruit.^z

Cycle	Time of measurement	Total hr	nl C ₂ H ₄ /(kg·hr)			mg CO ₂ /(kg·hr)		
			2.5C	12.5C	IW ^y	2.5C	12.5C	IW ^y
0	No chilling	0		18 j			19 de	
1	End of chilling ^x	55	12 j	20 ij	12 j	9.1 g	17 e	9 g
	End of warming ^w	73	16 j	15 j	201 b	8.2 g	13 f	25 c
2	End of chilling ^x	148	19 ij	27 hi	53 e	8.5 g	13 f	10 g
	End of warming ^w	169	16 j	26 i	98 c	8.9 g	10 fg	18 e
3	End of chilling ^x	230	35 g	34 gh	53 e	9.2 g	17 e	12 f
	End of warming ^w	248	37 fg	35 g	55 e	8.3 g	18 e	22 cd
4	End of chilling ^x	320	44 f	31 h	45 f	7.3 g	16 e	7 g
	1 day at 20C ^v	344	554 a	36 g	78 d	54 a	32 b	53 a
	2 days at 20C ^v	368	52 e	31 h	41 fg			

^zMeans separation within C₂H₄ and CO₂ production are by Duncan's multiple range test, *P* = 0.05.

^yThe C₂H₄ and CO₂ production of IW fruit were measured at 2.5C (*), 12.5C (*), or 20C (v).

Fig. 3. Effect of chilling and intermittent warming on ion leakage from 4 × 9-mm-thick disks excised from cucumber fruit held at 2.5 (chilled) or 12.5C (control) for 320 hr. Fruit held at 2.5C were warmed to 12.5C for around 18 hr every 2 to 3 days (warmed).

the end of chilling in the second cycle, however, EFE activity of intermittently warmed fruit was significantly lower than continuously chilled fruit, and both values were significantly lower than fruit continuously held at 12.5C (Table 3). Warming fruit to 12.5C from 148 to 169 hr caused no significant change in EFE activity, while there was a marked, unaccountable rapid decline in the EFE activity in fruit continuously chilled at 2.5C. Rates of EFE activity remained elevated in intermittently warmed fruit during the third cycle, finally reaching a level of activity similar to fruit continuously held at 12.5C by the end of the fourth chilling cycle. Upon warming to 20C, however, the EFE activity of intermittently warmed fruit rapidly declined to levels of activity similar to the continuously chilled fruit. In contrast, the rate increased in fruit continuously held at 12.5C from 28.2 μ l C₂H₄/(kg·hr) at the end of the fourth chilling cycle to 39 μ l C₂H₄/(kg·hr) after 1 day at 20C.

ACC content. The level of ACC in fruit continuously held at 12.5C was variable, but averaged 0.2 ± 0.1 nmol·g⁻¹ (fresh weight) during the experiments (Table 3). During the first 169 hr, fruit continuously held at 2.5C had levels of ACC similar to the fruit held at 12.5C. ACC content of chilled fruit dramatically increased > 10-fold to 1.8 nmol·g⁻¹ (fresh weight) by

248 hr, and > 40-fold to 6.2 nmol·g⁻¹ (fresh weight) by 320 hr. In contrast, ACC levels in intermittently warmed fruit increased slightly more than 3-fold during the first and second warming periods. The levels of ACC in intermittently warmed fruit appeared to stabilize at around 0.50 nmol·g⁻¹ (fresh weight) for the duration of the experiment.

Discussion

Pitting and increased decay are two visible symptoms of chilling injury in cucumber fruit that were alleviated by interrupting the period of exposure to chilling at 2.5C with 18-hr periods of intermittent warming at 12.5C every 2.5 to 3 days for $\approx$ 13 days (Table 1). The physiological responses of increased C₂H₄ production and ion leakage that are also associated with chilling injury were reduced by this intermittent warming treatment (Table 2, Fig. 3). Another indicator of chilling injury, increased CO₂ production, however, was not reduced by intermittent warming (Table 2). The lack of a significant correlation between CO₂ and C₂H₄ production implies that the increase in CO₂ production was not induced by the increase in C₂H₄ production.

The two visual symptoms of chilling injury are probably interrelated, in that the breakdown of tissue that results in formation of pits would also provide a suitable environment for the growth of the weak saprophytic pathogens that colonize chilled cucumber fruit. Physiological responses to attack by pathogens may also be curtailed by chilling, but this aspect was not investigated.

The increase in ion leakage following chilling is probably an early manifestation of the collapse of tissue that produced the visual symptoms. However, a significant increase in the rate of ion leakage measured within a few hours of removal from chilling was not observed until after 170 hr ($\approx$ 7 days) of continuous chilling, while increased pitting and decay after 4 days at 20C were observed after only 5 days of chilling (Cabrera and Saltveit, 1989). Obviously, sufficient damage had occurred after 4 days of chilling to result in the subsequent development of visual symptoms, while almost twice that length of exposure was necessary to produce effects on membrane permeability that could be measured as increased ion leakage immediately after chilling. Alteration in this gross measurement of membrane permeability, therefore, appears to be one of the results, rather than the immediate cause, of chilling injury in cucumber fruit.

Table 3. Ethylene-forming enzyme (EFE) activity and ACC content of cucumber fruit stored at 2.5C, 12.5C, and intermittently warmed (IW). IW fruit were held at 2.5C and warmed to 12.5C for 18 hr every 2 to 3 days. The EFE activity was measured at 20C.^z

Cycle	Time of measurement	Total hr	EFE			ACC		
			$\mu\text{l C}_2\text{H}_4/(\text{kg}\cdot\text{hr})$			$\text{nmol}\cdot\text{g}^{-1}$ (fresh wt)		
			2.5C	12.5C	IW ^y	2.5C	12.5C	IW ^y
0	No chilling	0		23 c			0.25 f	
1	End of chilling ^x	55	43 a	23 c	39 a	0.23 f	0.23 f	0.15 fg
	End of warming ^w	73	---	---	---	0.24 f	0.37 e	0.50 d
2	End of chilling ^x	148	25 c	30 b	15 e	0.16 fg	0.16 fg	0.20 fg
	End of warming ^w	169	13 f	24 c	15 e	0.14 fg	0.11 g	0.74 c
3	End of chilling ^x	230	---	---	---	---	---	---
	End of warming ^w	248	6.4 g	40 a	18 cd	1.8 b	0.09 g	0.54 d
4	End of chilling ^x	320	6.4 g	28 b	21 b	6.2 a	0.14 fg	0.49 d
	1 day at 20C ^v	344	1.0 h	39 a	3.6 gh	---	---	---

^zMeans separation within EFE and ACC are by Duncan's multiple range test, $P = 0.05$.

^yThe C_2H_4 and CO_2 production of intermittently warmed fruit were measured at 2.5C (*), 12.5C (w), or 20C (v).

Our regime of intermittent warming did not prevent the chill-induced increase in ion leakage, but did reduce the rate of increase to about one-half that of continuously chilled fruit (Fig. 3). While this lower rate was still almost twice that of fruit held at 12.5C, the treatment was sufficient to completely prevent the development of either pitting or decay in fruit held for an additional 6 days at 20C after chilling (Table 1). Intermittently warmed fruit were apparently able to overcome the moderate level of chilling injury that produced a doubling of leakage.

The most pronounced effects of intermittent warming were the large bursts of C_2H_4 production during the first warming period, and the decrease in the intensity of this burst at the subsequent warming periods. The burst in C_2H_4 production could result from an increase in the activity of the EFE, an increase in the substrate for C_2H_4 production, or a decrease in the compartmentalization of the reactants.

Chill-induced C_2H_4 production was not significantly correlated with increased EFE activity. While 3 days of chilling actually stimulated EFE activity, longer exposures to 2.5C resulted in the loss of EFE activity (Table 3). These results support those of Wang and Adams (1980), who reported that prolonged chilling damages the system that converts ACC to C_2H_4 . The decline in EFE activity in intermittently warmed fruit was generally less than that measured in continuously chilled fruit, while fruit held at 12.5C had slightly increasing EFE activities during the experiment. Since the EFE is thought to be membrane-bound, and increased ion leakage indicated that membrane damage was occurring during prolonged exposure to chilling temperatures, it is not surprising that EFE activity of continuously chilled fruit started to decline below the control level (Table 3) at the same time (≈ 170 hr) that the rate of ion leakage started to increase (Fig. 3).

ACC levels were also not significantly correlated with chill-induced C_2H_4 production in continuously chilled or intermittently warmed fruit. While ACC levels of intermittently warmed cucumbers increased 3-fold during the first two warming cycles, the increase in C_2H_4 production was around 20-fold and 2-fold during the first and second cycles, respectively (Tables 2 and 3). The correlation between ACC levels and C_2H_4 production by intermittently warmed fruit was not significant ($r = 0.53$). It is clear that synthesis of the substrate for C_2H_4 production,

i.e., ACC, is stimulated by prolonged chilling and by transfer to warming temperatures (Table 3). Increased ACC levels were found in intermittently warmed fruit after each warming and in continuously chilled fruit after 230 hr. Our results agree with those of Wang and Adams (1982) that the conversion of ACC to C_2H_4 , rather than the availability of the substrate, appears to be the limiting factor in the production of chill-induced C_2H_4 production.

The first burst in C_2H_4 production following warming could have resulted from a combination of the 70% increase in EFE activity and the 3-fold increase in ACC concentration (Table 3). However, the subsequent much smaller 2-fold increase in C_2H_4 production during the second warming cycle when EFE activity remained constant and ACC level increased 3.7-fold, and the lack of any increase in C_2H_4 production during the third warming cycle when both EFE activity and ACC content were elevated over that of the second cycle, are inconsistent with this explanation.

The conversion of ACC to C_2H_4 is not the only limiting factor in chill-induced C_2H_4 production—some other restriction must apply because chill-induced C_2H_4 production is not significantly correlated with either EFE activity or ACC content in any of the temperature treatments. If the three factors of ACC content, EFE activity, and membrane permeability are considered together, no significant correlation exists between them and C_2H_4 production, except for the intermittent warming treatment. Fruit continuously held at 2.5C and 12.5C had correlation coefficients between C_2H_4 production and the three factors of 0.27 and 0.51, respectively. The regression line calculated for the 12.5C treatment had a negative slope, indicating that C_2H_4 production was negatively correlated with the three factors. In comparison, intermittently warmed fruit had a significant correlation coefficient of 0.86 between C_2H_4 production and the product of EFE activity times ACC content divided by the rate of ion leakage.

Interrupting a period of chilling with a period of warming at nonchilling temperatures appears to allow the tissue to acclimate to chilling temperatures, as is shown by the reduced production of C_2H_4 at each progressive warming period. The chill-induced increases in ACC content, EFE activity, and membrane permeability were diminished by successive chilling and warming periods. Periodic warming appears to allow chilled fruit to acclimate

to subsequent periods of chilling. The method by which intermittent warming accomplishes these physiological changes requires further study.

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