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 12.5°C for 18 hr every 3 days. Fruit continuously held at 2.5°C for 13 days developed severe pitting and decay after 6 days at 20°C, while fruit continuously held at 12.5°C or intermittently warmed showed no pitting or decay during subsequent holding at 20°C. The increased rate of C$_2$H$_4$ production during the first warming period, from 12 nl·(kg·hr)$^{-1}$ at 2.5°C to 201 nl·(kg·hr)$^{-1}$ at 12.5°C, was significantly greater than that during the second or third warming periods, i.e., 53 to 98 and 53 to 55 nl C$_2$H$_4$/kg·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 2.5°C 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 12.5°C contained 0.09 to 0.34 nmol·g$^{-1}$ of ACC. ACC levels were 6.23 nmol·g$^{-1}$ in fruit exposed to 2.5°C 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 0°C, many horticultural crops of tropical and subtropical origin are chilling-sensitive and are injured if held at nonfreezing temperatures below 12°C (Lyon, 1973; Saltveit and Morris, 1989). Cucumbers are chilling-sensitive and are injured if held at temperatures < 10°C 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 5°C. Earlier findings of MacK and Janer (1942) revealed a similar increase in CO$_2$ production of cucumber during storage at 2 to 3°C. Wang and Adams (1980, 1981) observed an increase in C$_2$H$_4$ production when cucumbers that had been chilled for 4 days at 2.5°C were transferred to 25°C. However, they did not observe an increase in respiration or C$_2$H$_4$ production during the chilling period (Wang and Adams, 1980). Increased production of C$_2$H$_4$ by chilled cucumbers could reduce quality, since it has been shown that exposure to µl C$_2$H$_4$/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 peppers (Wang and Baker, 1979), grapefruit (Davis and Hoffman, 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$_2$H$_4$ (Yang, 1980). ACC is converted to C$_2$H$_4$ by the ethylene-forming enzyme (EFE). The rate at which C$_2$H$_4$ 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$_2$H$_4$ 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 coveredtorsing step.

The time to warm and/or cool intindividual cucumber fruit when transferred between the two experimental temperatures of 2.5 and 12.5°C 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 12.5°C; 2) chilled fruit were continuously held at 2.5°C; and 3) intermittently warmed fruit were held at 2.5°C for 3 or 4 days and warmed at 12.5°C 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 CO production. Production of CH$_4$ and CO 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 CH$_4$ and CO 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 Extech 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 C$_2$H$_4$ produced. EFE activity was calculated as the difference between C$_2$H$_4$ 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 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 the fruit warmed for 12 or 18 hr; i.e., activities of 21 and 11 µl C$_2$H$_4$/(kg·hr), respectively. In subsequent intermittent warming experiments, warming periods of 18 hr were used.

Ethylene production. The rate of C$_2$H$_4$ 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 C$_2$H$_4$ production from 5.4 to 95.5 nl·(kg·hr)$^{-1}$ within 18 hr of warming. Cooling these

| Additional days at 20C | Pitting | Decay |
|------------------------|---------|-------|
| 0                      | 1.0     | 0.0   |
| 2                      | 4.2*    | 0.0   |
| 4                      | 7.3*    | 2.6*  |
| 6                      | 7.9*    | 6.0*  |

*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% 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.

*Means were separated within columns by using Dunnet’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 CH$_4$ production with and without added ACC. Comparison by Dunnet’s test, $P = 0.05,*$. Plus ACC, EFE—all differ from 0 hr; minus ACC, only 18 hr differs from 0 hr.
fruit to 2.5°C was quickly followed by a reduction in the rate of C\textsubscript{2}H\textsubscript{4} production from 95.5 to 8.5 nl·(kg·hr\textsuperscript{-1}) within 6 hr of cooling. After 6 days (i.e., 144 hr), the rate of C\textsubscript{2}H\textsubscript{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\textsubscript{2}H\textsubscript{4} production from 5.2 to 61 nl·(kg·hr\textsuperscript{-1}) and from 6.7 to 35 nl·(kg·hr\textsuperscript{-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\textsubscript{2}H\textsubscript{4} production when the fruit continuously held at 12.5°C were transferred to 20°C. The rate of C\textsubscript{2}H\textsubscript{4} production from all the fruit continued to increase, reaching 371, 156, and 35 nl·(kg·hr\textsuperscript{-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\textsubscript{2}H\textsubscript{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·(kg·hr\textsuperscript{-1}) during the 320-hr storage period at 12.5°C. The average rate of production during this period was 30 ± 12 nl·(kg·hr\textsuperscript{-1}). As in the previous experiments with one warming cycle, C\textsubscript{2}H\textsubscript{4} production remained around 34 ± 3 nl·(kg·hr\textsuperscript{-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\textsubscript{2}H\textsubscript{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\textsubscript{2}H\textsubscript{4} production had increased around 2-fold to 36 nl·(kg·hr\textsuperscript{-1}), and then increased an additional 20% to around 44 nl·(kg·hr\textsuperscript{-1}) during the 320-hr (≈13 days) sampling period. The increased production of C\textsubscript{2}H\textsubscript{4} after 9 days (230 hr) of continuous chilling is similar to the increase in CO\textsubscript{2} production previously reported (Mack and Janer, 1942; Eaks and Morris, 1956). A dramatic 12.5-fold increase in C\textsubscript{2}H\textsubscript{4} production to 554 nl·(kg·hr\textsuperscript{-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·(kg·hr\textsuperscript{-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\textsubscript{2}H\textsubscript{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\textsubscript{2}H\textsubscript{4} production. The increased rate of C\textsubscript{2}H\textsubscript{4} production during the first warming period from 12 nl·(kg·hr\textsuperscript{-1}) at 2.5°C to 201 nl·(kg·hr\textsuperscript{-1}) at 12.5°C was significantly greater than during the second or third warming periods; 53 to 98, and 53 to 55 nl·(kg·hr\textsuperscript{-1}), respectively. The Q\textsubscript{10} of the increase in C\textsubscript{2}H\textsubscript{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\textsubscript{2}H\textsubscript{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\textsubscript{2}/(kg·hr), were measured from fruit continuously held at 12.5°C, while rates of 7 to 9 mg·(kg·hr\textsuperscript{-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\textsubscript{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\textsubscript{2} production at 2.5°C (i.e., at the end of each chilling period) steadily increased from 7.9 to 11.7 mg·(kg·hr\textsuperscript{-1}) during the first 230 hr of the experiment, before declining to 7.3 mg·(kg·hr\textsuperscript{-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\textsubscript{10} of 2, about double for the control fruit at 12.5°C.

The rate of CO\textsubscript{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 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 µl C\textsubscript{2}H\textsubscript{4}/(kg·hr) 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 µl C\textsubscript{2}H\textsubscript{4}/(kg·hr), before starting a steady decline in activity to 6.4 µl C\textsubscript{2}H\textsubscript{4}/(kg·hr), 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
At 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 12.5°C (Table 3). Warming fruit to 12.5°C from 148 to 298 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 2.5°C. 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 12.5°C by the end of the fourth chilling cycle. Upon warming to 20°C, 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 12.5°C from 28.2 µl C₂H₄/(kg·hr) after 1 day at 20°C.

ACC content. The level of ACC in fruit continuously held at 12.5°C 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 2.5°C had levels of ACC similar to the fruit held at 12.5°C. 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 2.5°C with 18-hr periods of intermittent warming at 12.5°C every 2.5 to 3 days for 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 (≈ 7 days) of continuous chilling, while increased pitting and decay after 4 days of chilling 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 2. Ethylene production and respiration rates of cucumber fruit stored at 2.5°C, 12.5°C, and intermittently warmed (IW). IW fruit were held 2.5°C and warmed to 12.5°C for 18 hr every 2 to 3 days. Chilling and warming cycles refer to the IW fruit.]

| Cycle | Time of measurement | Total hr | 2.5°C C₂H₄/(kg·hr) | 12.5°C C₂H₄/(kg·hr) | IW C₂H₄/(kg·hr) | mg CO₂/(kg·hr) |
|-------|---------------------|----------|-------------------|-------------------|----------------|----------------|
| 0     | No chilling         |          | 0                 | 18 j              | 19 de          |                |
| 1     | End of chilling     | 55       | 12 j              | 20 ij             | 12 j           | 9.1 g          |
|       | End of warming     | 73       | 16 j              | 15 j              | 201 b          | 8.2 g          |
| 2     | End of chilling     | 148      | 19 ij             | 27 hi             | 53 e           | 8.5 g          |
|       | End of warming     | 169      | 16 j              | 26 i              | 98 e           | 8.9 g          |
| 3     | End of chilling     | 230      | 35 g              | 34 gh             | 53 e           | 9.2 g          |
|       | End of warming     | 248      | 37 fg             | 35 g              | 55 e           | 8.3 g          |
| 4     | End of chilling     | 320      | 44 f              | 31 h              | 45 f           | 7.3 g          |
| 1 day | 20°C                | 344      | 554 a             | 36 g              | 78 d           | 54 a           |
| 2 days | 20°C               | 368      | 52 e              | 31 h              | 41 fg          |                |

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

The C₂H₄ and CO₂ production of IW fruit were measured at 2.5°C (*), 12.5°C (*), or 20°C (*).
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 \( \text{C}_4 \text{H}_4 \) production during the first warming period, and the decrease in the intensity of this burst at the subsequent warming periods. The burst in \( \text{C}_4 \text{H}_4 \) production could result from an increase in the activity of the EFE, an increase in the substrate for \( \text{C}_4 \text{H}_4 \) production, or a decrease in the compartmentalization of the reactants.

Chill-induced \( \text{C}_4 \text{H}_4 \) 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 EFE activity times ACC content divided by the rate of ion leakage.

The first burst in \( \text{C}_4 \text{H}_4 \) 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 \( \text{C}_4 \text{H}_4 \) 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 \( \text{C}_4 \text{H}_4 \) 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 \( \text{C}_4 \text{H}_4 \) is not the only limiting factor in the production of chill-induced \( \text{C}_4 \text{H}_4 \) production—some other restriction must apply because chill-induced \( \text{C}_4 \text{H}_4 \) 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 \( \text{C}_4 \text{H}_4 \) production, except for the intermittent warming treatment. Fruit continuously held at 2.5C and 12.5C had correlation coefficients between \( \text{C}_4 \text{H}_4 \) 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 \( \text{C}_4 \text{H}_4 \) 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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