

# Effect of Exposure to Subfreezing Temperatures on Ethylene Evolution and Leaf Abscission in Citrus

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## ABSTRACT

Citrus leaves exposed to subfreezing temperatures evolved ethylene at rates between 0.1 and 38.3 microliters per kilogram fresh weight per hour whereas untreated leaves evolved between 0.01 and 0.50 microliter per kilogram fresh weight per hour. Leaves not injured by freezing temperatures did not abscise, and ethylene evolution was near normal after 2 days. Freeze-injured leaves continued evolving high ethylene levels 4 or 5 days subsequent to freeze injury, and many of the freeze-killed leaves abscised. Supportive evidence suggested freeze-induced ethylene was involved in freeze-induced leaf abscission; whereas freeze-inhibited abscission was not due to a lack of ethylene but injury to other metabolic systems necessary for abscission.

Above normal ethylene evolution by leaves is one of the initial events preceding leaf abscission (17). Ethylene, applied at hormonal levels to leaves or leaf explants, increases RNA and protein synthesis (4, 5, 15); stimulates cell-wall dissolution (18); increases cellulase activity in the abscission zone (1, 2); and induces leaf abscission (2-5, 10-12, 18). Anatomical evidence also has associated protein synthesis in the abscission zone with cell-wall dissolution and abscission of ethylene-treated leaves (22). Several have suggested that ethylene is the mediating hormone in leaf and fruit abscission and plant senescence (1, 11, 13, 17).

Environmental factors may either induce or inhibit leaf abscission (6, 7). This is particularly true with subfreezing conditions where mild freezes may cause leaf abscission and severe freezes may inhibit abscission (6, 8). Recently, Cooper *et al.* (14) reported higher rates of ethylene evolution from freeze-injured citrus tissues than from noninjured tissues.

This report relates changes in ethylene evolution and respiration with abscission of leaves exposed to different subfreezing temperatures.

## MATERIALS AND METHODS

**Plant Materials.** Test plants were uniform 12- to 18-month-old seedlings of sour orange, *Citrus aurantium* Linn. and "Pine-

apple orange," *C. sinensis* (L.) Osbeck. Prior to freezing, the plants were grown in 6-inch pots in a screenhouse.

In addition, 6- to 15-year-old field-grown trees of the following varieties were sampled prior to and following exposure to subfreezing temperatures February 4, 1970: "Valencia" and "Pineapple" oranges, *C. sinensis* (L.) Osbeck; "Marsh" grapefruit, *C. paradisi* Macf.; and "Orlando" tangelo, *C. paradisi* Macf. × *C. reticulata* Blanco. "Dancy" tangerines, *C. reticulata* Blanco, and "Pineapple" oranges were also sampled after exposure to subfreezing temperatures on January 21, 1971.

**Freeze Tests.** Seedling plants were exposed to freeze temperatures between -3.3 and -6.7 C for 4 hr. At the beginning of each freeze test, 15 plants of each variety were placed in a freeze chamber at 1.7 C, and the temperature was lowered to and raised from the test temperature at 1 C per hour. A corresponding number of plants were kept in a screenhouse as controls. Field trees in central Florida were exposed to natural freezes, with minimum temperatures between -3.3 C and -4.7 C on February 4, 1970, and to -4.4 C to -5.6 C on January 21, 1971 (for less than 1 hr). Three single tree replicates of each variety were sampled.

**Freeze Injury and Leaf Abscission.** Freeze injury ratings on seedlings were made 2 weeks after each freeze test, and consisted of calculating the percentage of leaves or twigs killed. The term "killed" refers to tissues which exhibit a rapid desiccation, loss of chlorophyll, and no recovery after freezing, although some metabolic activity, *i.e.*, respiration, ethylene evolution, *etc.*, can be measured several days after freezing. Leaf abscission counts were made daily after each freeze test by gently shaking each plant and counting the abscised leaves. Abscission of the leaf blade from petiole of detached leaves was determined by visual observation of separation or applying slight pressure on the leaf blade in a horizontal position. Observations on leaf injury and abscission on field-grown trees were made 1 week after the freeze.

**Ethylene and CO<sub>2</sub> Measurements.** Three leaf samples, each consisting of five leaves, were harvested from plants of each treatment and sealed in 125-ml flasks with vicine caps. After 24-hr incubation at room temperature, gas samples were removed by syringes and analyzed for ethylene and CO<sub>2</sub> by gas-solid chromatography. Ethylene was chromatographed on a 1/8-inch × 6-ft activated alumina column and CO<sub>2</sub> on a silica gel column, and helium was used as a carrier (40 ml/min). The thermal conductivity detector was maintained at 150 C and 225 ma (20) during both analyses.

Ethylene is expressed as microliters per kilogram fresh weight

per hour, except where indicated, and CO<sub>2</sub> as millimicroliters per kilogram fresh weight per hour. The minimum detectable ethylene level was  $5 \times 10^{-3}$  microliters per liter, and zero readings indicated that ethylene was below this limit.

**Exogenous Ethylene Application.** Two sour orange seedlings from each lot of plants exposed to -5.6 C, -6.1 C, and -6.7 C and controls were treated with 10  $\mu$ l/liter ethylene for 24 hr after removal from the freeze chamber.

Ethylene levels which induce abscission of blades from petioles of detached sour orange leaves were determined by exposing leaves in closed 250-ml flasks to different levels of ethylene up to 20 days. Leaves were removed from the plant and aged 24 hr prior to ethylene treatment; all leaves were evaluated daily for abscission, resealed in a clean flask, and retreated.

**Ethylene Removal Tests.** In one test, detached frozen (-5.0 C) and unfrozen sour orange leaves were incubated in closed containers in the presence or absence of an ethylene absorber (Purafil<sup>1</sup> pellets—KMnO<sub>4</sub>). Ethylene and abscission were measured daily, and all containers were vented daily.

## RESULTS

**Leaf Abscission and Ethylene Evolution versus Freeze Temperature.** Sour orange seedlings exposed to -5.0 C to -6.7 C had progressively greater freeze injury with lower freeze temperatures (Table I). There was no wood injury at -5.6 C, but 50% was killed at -6.1 C and 100% at -6.7 C. Leaf abscission, however, was highest following exposure to -5.6 C and was absent after exposure to -6.7 C. Initial leaf abscission occurred after 3 days from freezing, and total leaf abscission was complete after 7 days. Abscission occurred at the leaf-petiole and petiole-stem abscission zones. Leaf curl, a sign of stress, occurred on all leaves exposed to subfreezing temperatures and disappeared only from uninjured leaves after 2 to 3 days. Above normal ethylene evolution occurred in leaves 1, 2, and 3 days from freezing with the highest rates occurring after 2 and 3 days. Ethylene rates from leaves killed by -6.1 C and -6.7 C, where little or no abscission occurred, were greater than from leaves killed by -5.0 C where abscission did occur. Exposure of freeze-injured plants to 10  $\mu$ l/liter ethylene did not induce further leaf abscission, although the ethylene level was adequate to cause leaf abscission on unfrozen plants (Table I).

Pineapple orange seedlings, which are more cold sensitive than sour orange, were exposed to the same range of freeze temperatures, and progressively greater injury occurred at the lower temperatures (Table II). More injury occurred to Pineapple orange seedlings at -5.0 C than to sour orange seedlings, and there was less leaf abscission. Leaf abscission was greatest on plants exposed to -3.3 C and -5.0 C and did not occur on those exposed to -6.7 C. Ethylene evolution, although above normal after exposure to -3.3 C to -6.7 C, was low after exposure to -5.0 C and -6.7 C. Leaf CO<sub>2</sub> evolution after exposure to -3.3 C was above normal, but was greatly retarded by -5.0 C and -6.7 C.

Citrus trees exposed to a natural freeze of -3.3 C to -4.7 C exhibited low temperature stress conditions (leaf curl) but no permanent injury or leaf abscission (Table III). Leaf ethylene evolution rates for the 24-hr period after the freeze were in the range previously associated with leaf abscission (Tables I

Table I. Effect of Freezing Temperature and Ethylene Treatment on Abscission of Unhardened Leaves of Sour Orange Seedlings

Treatment	Ethylene Evolution <sup>1</sup>			Visible Injury		Abscission
	0-24 hr	24-48 hr	48-72 hr	Leaves killed	Twigs killed	
	$\mu$ l/kg fr wt/hr			%		
Unfrozen	0.03	0.04	0.03	...	...	0
Unfrozen + C <sub>2</sub> H <sub>4</sub> <sup>2</sup>	...	...	...	...	...	74
Frozen -5.0	0.14	1.50	4.64	59	0	59
Frozen -5.0 + C <sub>2</sub> H <sub>4</sub> <sup>2</sup>	...	...	...	59	0	80
Frozen -5.6	7.47	17.72	25.19	100	0	87
Frozen -5.6 + C <sub>2</sub> H <sub>4</sub> <sup>2</sup>	...	...	...	100	0	82
Frozen -6.1	4.67	38.25	18.65	100	50	21
Frozen -6.1 + C <sub>2</sub> H <sub>4</sub> <sup>2</sup>	...	...	...	100	50	27
Frozen -6.7	0.00	9.35	13.08	100	100	0
Frozen -6.7 + C <sub>2</sub> H <sub>4</sub> <sup>2</sup>	...	...	...	100	100	0

<sup>1</sup> A new set of leaves was detached from each plant daily for ethylene measurements.

<sup>2</sup> Plants exposed to 10  $\mu$ l/liter ethylene for 24 hr. Frozen plants exposed to ethylene 24 hr after removal from freeze chamber.

Table II. Ethylene and CO<sub>2</sub> Evolution, Freeze Injury, and Abscission of Unhardened Leaves of "Pineapple" Orange Seedlings Exposed to Three Freeze Temperatures

Temperature Treatment	CO <sub>2</sub> Evolution <sup>1</sup>			Ethylene Evolution			Visible Injury		Leaf Abscission
	0-24 hr	24-48 hr	48-72 hr	0-24 hr	24-48 hr	48-72 hr	Leaves killed	Twigs killed	
	$m\mu$ l/kg fr wt/hr			$\mu$ l/kg fr wt/hr			%		
C									
26.6	100.2			0.04			0	0	0
-3.3	115.2	331.0		9.58	33.26		24	4	22
-5.0	63.4	52.1		0.55	0.64		100	94	31
-6.7	31.6	7.4	9.3	0.11	0.21	0.16	100	100	0

<sup>1</sup> See Table I.

Table III. Effect of Natural Subfreezing Temperatures on Ethylene Evolution and Subsequent Abscission of Hardened Leaves of Four Citrus Varieties<sup>1</sup>

Variety	Leaf Curl <sup>2</sup>			Ethylene Evolution			Leaf Abscission
	2/3	2/4	2/6	2/3	2/4	2/6	
				$\mu$ l/kg fr wt/hr <sup>3</sup>			%
Valencia orange	0	1	0	0.05	0.62	0.10	0
Pineapple orange	0	1	0	0.05	1.31	0.11	0
Orlando tangelo	0	3	0	0.18	2.24	0.12	0
Marsh grapefruit	0	2	0	0.20	2.96	0.11	0

<sup>1</sup> Temperatures of -3.3 C to -4.7 C experienced on the morning of February 4, 1970 for less than 1-hr duration.

<sup>2</sup> 0: none; 1: slight; 2: moderate; 3: severe.

<sup>3</sup> 24-hour period after harvest.

<sup>1</sup> Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

and II). However, these rates were shortlived and near normal 2 days after the freeze.

**Ethylene Evolution versus Injury Site in Leaf.** Ethylene evolution from Dancy tangerine and Pineapple orange leaf blades and petioles which were separated at the abscission zone was measured 3 days after exposure to a natural freeze (Table IV). High ethylene evolution rates were measured in freeze-killed leaf blades and petioles. Leaf blade abscission from the petiole was near complete 3 days from the freeze when either the leaf blade or both blade and petiole were killed.

Leaf petioles from freeze-killed sour orange leaves, which were cut to include the abscission zone and 2 mm of the leaf blade, evolved ethylene at a high rate compared to unfrozen petioles; leaf blades from freeze-killed leaves abscised from their petioles (Table V).

**Ethylene Removal versus Abscission.** Freeze-killed sour orange leaves were placed in airtight containers in the presence or absence of an ethylene absorber (Purafil pellets). Ethylene was measured daily, after which the containers were vented and the leaves evaluated for abscission. The containers were then resealed to allow ethylene to accumulate for the next 24-hr period. Freeze-killed leaves, in the absence of an ethylene

Table IV. Effect of Natural Subfreezing Temperatures on Ethylene Evolution from and Abscission of Partially Hardened Leaf Blades and Petioles in Relation to Injury<sup>1</sup>

Freeze-Injury Condition and Tissue	Dancy Tangerine		Pineapple Orange	
	Ethylene evolution <sup>2</sup>	Abscission <sup>3</sup>	Ethylene evolution <sup>2</sup>	Abscission <sup>3</sup>
	$\mu\text{l/kg fr wt/hr}$	%	$\mu\text{l/kg fr wt/hr}$	%
Uninjured leaf blade	0.39	0	0.02	0
Uninjured leaf petiole	0.60		0.13	
Killed leaf blade	15.15	100	14.35	100
Uninjured leaf petiole	0.00		0.10	
Killed leaf blade	18.25	100	5.29	100
Killed leaf petiole	12.72		6.93	

<sup>1</sup> Temperatures of  $-4.4$  C to  $-5.6$  C experienced on the morning of January 21, 1971 for less than 1-hr duration.

<sup>2</sup> Ethylene measured for 24-hr period, 3 days after freeze.

<sup>3</sup> Abscission of leaf blade from petiole 3 days after freeze. Two replicates of five leaf parts were used for each treatment.

Table V. Ethylene Evolution from Various Parts of Detached Freeze-Injured, Unhardened Sour Orange Leaves

Treatment and Tissue	Ethylene Evolution <sup>1</sup>	Abscission <sup>2</sup>
	$\mu\text{l/kg fr wt/hr}$	%
Untreated		
Whole leaf	0.50	0
Leaf blade	0.58	
Leaf petiole <sup>3</sup>	1.94	
Freeze-killed <sup>4</sup>		
Whole leaf	7.77	100
Leaf blade	13.00	
Leaf petiole <sup>3</sup>	14.19	

<sup>1</sup> Ethylene measured 24-hr period after removing plants from freeze chamber.

<sup>2</sup> Abscission of leaf blade from petiole after 6 days.

<sup>3</sup> Petioles included abscission zone and 2 mm of leaf blade. Two replicates of five leaves or leaf parts were used for each treatment.

<sup>4</sup> Plants exposed to  $-5.0$  C for 4 hr.

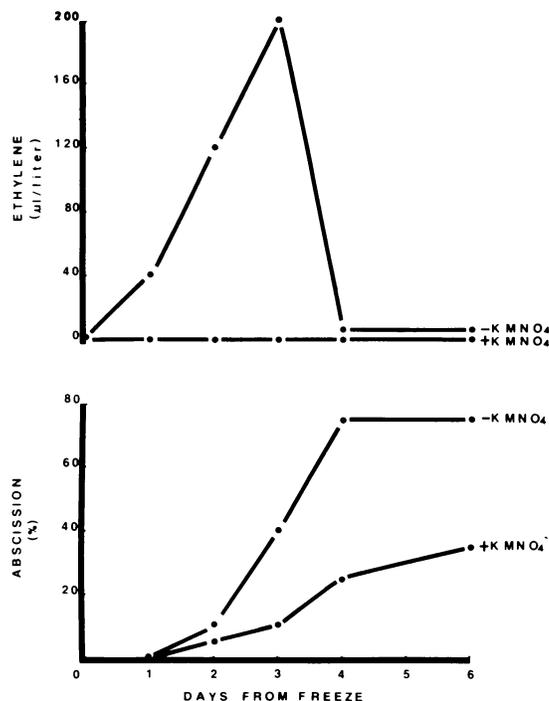


FIG. 1. Abscission (leaf blade from petiole) of and ethylene evolution from detached unhardened sour orange leaves in the presence or absence of an ethylene absorber after exposure to  $-5.0$  C for 4 hr. Ten leaves per container and two containers per treatment were used.

absorber, evolved large quantities of ethylene 1 to 3 days after injury and 75% of the leaves had abscised after 4 days (Fig. 1). Ethylene was not detected in containers with an absorber, and abscission was delayed and reached only 35% after 6 days. Untreated sour orange leaves evolved ethylene at rates similar to those already shown (Tables I and V), and, in the presence or absence of an ethylene absorber, had not abscised after 6 days when the experiment was terminated.

**Ethylene Levels versus Leaf Abscission.** Unhardened sour orange leaves were detached, aged for 24 hr, and then exposed to different ethylene levels. The time required for 50% abscission of blades from petioles was as follows: 0.04  $\mu\text{l/liter}$  or untreated—greater than 20 days; 0.6–7.0; 1.1–6.5; 2.3–5.5; and 3.3–3.5. Leaves exposed to 2.1  $\mu\text{l/liter}$  for 48 hr and then removed required 16 days for 50% abscission, while those exposed to 3.0  $\mu\text{l/liter}$  for only 24 hr were similar to untreated leaves and required more than 20 days for 50% abscission.

## DISCUSSION

Citrus leaves exposed to subfreezing temperatures evolved above normal ethylene between 0.1 and 38.3  $\mu\text{l/kg fr wt/hr}$ , confirming the findings of Cooper *et al.* (14). Leaves which appeared normal and did not abscise after exposure to subfreezing temperatures evolved ethylene at rates between 0.6 and 3.0  $\mu\text{l/kg fr wt/hr}$ , but this occurred only during a 2-day period after freezing temperatures. Abscission of petioles from blades of detached sour orange leaves was not materially affected by exposure to 2.1 to 3.0  $\mu\text{l/liter}$  ethylene for 24 to 48 hr. This suggested the failure of the uninjured leaves to abscise after exposure to subfreezing temperatures may have been due to a lack of a sufficient duration of ethylene production. Ethylene evolution rates of untreated leaves varied between 0.02 and 0.50  $\mu\text{l/kg fr wt/hr}$ .

Leaves killed by freezing evolved ethylene at rates between

0.1 and 38.3  $\mu\text{l}/\text{kg fr wt}/\text{hr}$ , but not all leaves which were killed abscised. The higher ethylene evolution rates were in the range which induced abscission of petioles from blades of detached sour orange leaves in our tests and sweet orange leaves in others (10, 16). Freeze-killed leaf blades abscised from petioles regardless if the petioles were killed and evolving high ethylene levels or not, and abscission was associated with high ethylene evolution rates from injured tissues. High ethylene evolution rates were present from freeze-injured tissues surrounding and including the abscission zone between leaf blades and petioles. Abscission of the leaf blades from petioles occurred with freeze-killed leaves attached to or detached from the same freeze-injured plant. Removal of ethylene from the atmosphere surrounding freeze-killed leaves delayed the abscission of leaf blades from petioles. This was supportive evidence that ethylene was involved in freeze-induced leaf abscission.

Abscission of leaves killed by exposure to  $-6.7\text{ C}$  was inhibited, although ethylene evolution rates were comparable to those of abscising freeze-killed leaves. This low temperature inhibition of leaf abscission is comparable to that reported for citrus exposed to severe natural freezes (19, 21, 23). Exogenously applied ethylene (10  $\mu\text{l}/\text{liter}$ ) to these plants did not overcome this inhibition. Ethylene did not appear to be a limiting factor, but other metabolic processes associated with abscission probably were. Carbon dioxide evolution, used as an index of respiration (9, 10, 24), was greatly reduced in freeze-killed leaves which failed to abscise, confirming a previous report (24). It seems likely that freeze temperatures low enough to kill the tissue and inhibit leaf abscission probably cause extensive injury to many metabolic systems (24).

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