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Short Communication

Ethylene Evolution Stimulated by Chilling in Citrus and Persea Sp.

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Chilling temperatures (below 10° but above the freezing point of the tissue) injure fruits of cold-sensitive crops native to tropical and subtropical areas (1, 4, 5). Sweet orange (*Citrus sinensis* Osb.) fruits, even though mature internally, may not lose their chlorophyll on the trees when grown in tropical climates. However, oranges grown on the northern fringes of the subtropical regions of Florida, Texas, Arizona, and California lose their green color at the onset of cool autumn weather (4).

Yellowing and shedding of leaves occur in the trifoliate orange [*Poncirus trifoliata* (L.) Raf.] in subtropical and temperate regions (6). Most citrus varieties, however, are evergreen, in the sense that the leaves normally remain green all fall and winter.

The physiological reasons for these chilling-induced phenomena are not known. Cooper *et al.* (3) reported that chilling temperatures of 5° increased the evolution of ethylene in calamondins (*C. reticulata* var. *austera*? X *Fortunella* sp?); and thereafter, the fruit lost its chlorophyll content rapidly. We now have gas chromatographic evidence that chilling causes ethylene production in fruit of grapefruit (*C. paradisa* Macf.), sweet oranges, and avocados (*Persea americana* Miller), and in leaves of the deciduous trifoliate orange before leaf abscission.

Results and Discussion

Experiments on Fruit Ripening. Five Redblush grapefruit trees, growing in 5-gal cans and bearing 10 mature but green fruit and 10 green, immature fruit, were placed in a plant growth chamber programmed to maintain a 20° temperature for a 12-hr photoperiod and 5° for a 12-hr dark period (20/5°). A similar number of trees were held in an air-conditioned greenhouse at approximately 25/20° for a 12-hr natural daylight photoperiod. The trees remained under these conditions for 14 days. Air samples of the internal atmosphere of fruits were taken, as described by Burg and Burg (2), by submerging the fruit in water and inserting a hypodermic syringe underneath the peel or inside the center of the fruit and withdrawing 2 ml of air. The air was analyzed by flame ionization gas chromatography to determine the ethylene content. After 4 days, the free air space under the peel of the fruit in the programmed climate of 20/5° contained 100 ppb C_2H_4, compared to 4 ppb for fruit on trees held at 25/20° (table I). After 14 days of treatment, the peel of the 10 mature fruit on each of the 5 trees held at 20/5° lost their chlorophyll content and developed a yellow color. The peel on all fruit on all trees exposed to a 25/20° climate remained green for 2 months.

In experiment No. 2 (table I) ethylene was measured in fruit of a Pineapple orange tree exposed to a natural climate in early October, before the onset of cool night temperatures, and again in late October following a period of 7 cool nights in which air temperatures dropped to 5 to 10°. The ethylene content of the free air space under the peel of fruit harvested in early October before the onset of cool nights was only 15 ppb, whereas 100 ppb ethylene was detected in fruit harvested in late October after a period of cool weather (table I). The peel of fruits on the trees remained green in early October but, following the cool weather in late October, the peel color turned rapidly from green to orange.

The Robinson tangerine (*C. reticulata* Blanco), an early ripening variety, responded even more dramatically to a chilling treatment. In experiment No. 3 (table I), Robinson tangerine fruits with a trace of yellow peel color harvested on October 10, 1968, and stored at 5°, produced 200 ppb ethylene in the free air space under the peel as compared to only 10 ppb for fruit held at 20°. The 5° fruit remained green during a 10-day storage period but, when held for an additional 4-day period at 20°, it lost its chlorophyll and developed an orange-red color. In contrast, fruit held continuously for a full 14-day period at 20° showed only a slight loss of chlorophyll.

Twenty fruits of 3 varieties of avocados were harvested on November 18, and the fruit of each variety was divided into 2 lots and placed in storage at 5° and 20°. The fruit was not enclosed in jars. After 3 days at 20°, large quantities of ethylene (expressed in ppm) occurred in the free air space under the rind and in the central cavity of the fruit of the Lula variety and considerably smaller quanti-

Table I. *Effect of Chilling Treatment on Accumulation of Ethylene in Free Air Space Under Peel of Citrus Fruits*

Expt. No.	Initial treat-ment date	Plant material	Temp conditions	C_2H_4 under peel after 4 days (10-fruit avg)	Peel color Before treat-ment	Peel color After 14 days
			deg	*ppb*		
1	9/1/68	Grapefruit on small tree	Programmed climate 25/20° for 14 days	4	Green	Green
	9/1/68	Grapefruit on small tree	Programmed climate 20/5° for 14 days	100	Green	Yellow
2	10/1/68	Pineapple orange on large tree in orchard	Natural climate 22/15° for 14 days	15	Green	Green
	10/28/68	Pineapple orange on large tree in orchard	Natural climate 20/5° for 7 days followed by 20/15° for 7 days	100	Green	25 % Orange
3	10/10/68	Detached fruit Robinson tangerine	Programmed climate 20/20° for 14 days	10	10 % Yellow	25 % Yellow
	10/10/68	Detached fruit Robinson tangerine	Programmed climate 20/5° for 10 days followed by 20/20° for 4 days	200	10 % Yellow	100 % Orange-red

Table II. *Effect of Chilling Treatment on Accumulation of Ethylene in the Atmosphere of Fruit of 3 Avocado Varieties*

3-day temp treatment	Part of fruit sampled	Concn. C_2H_4 after treatment Lula	Simpson	Booth 7
deg		*ppm*	*ppm*	*ppm*
5	Under peel	1	45	24
5	Center	6	45	42
20	Under peel	134	17	9
20	Center	110	28	15

ties in the Simpson and Booth 7 varieties (table II). The fruit of all 3 varieties softened during the 3-day period, but the softening was excessive for the Lula variety, which produced the most ethylene. as compared to the Simpson and Booth 7 varieties.

After 3 days' storage at 5°, the internal atmosphere of Simpson and Booth 7 fruit contained substantially more ethylene than when held at 20°; whereas, that in the Lula variety contained relatively little. After 10 days the flesh of the fruit of the Simpson and Booth 7 varieties showed a brown discoloration, typical of chilling injury; whereas that of the Lula was free of chilling injury. Thus, there is an association of a higher ethylene production in fruit of the chilling-sensitive Simpson and Booth 7 varieties when held at 5° than when held at 20°. On the other hand, the fruit of the chilling-tolerant Lula variety showed low ethylene accumulation when held at 5°, as compared to extremely high ethylene accumulation in fruit held at 20°.

Biale *et al.* (1), measuring ethylene evolution from whole fruit held in sealed jars, failed to find any significant amount of ethylene evolution from Fuerte (a chilling-sensitive variety) fruit held at 5°, whereas large amounts were found at 20°. Burg and Burg (2) have shown that the peel of avocados offers a great deal of resistance to the movement of gas out of the fruit. Thus, there are likely to be large differences between ethylene concentrations in the atmosphere inside and outside of the fruit.

Table III. *Evolution of Ethylene by Leaves of Rich Trifoliate Orange and Redblush Grapefruit During October and November 1968 at Leesburg, Florida*

Species	Date of	Color of leaves At harvest	Color of leaves After 3 days in flask	C_2H_4 in 50 ml flask per 10 g fr wt 1 day	C_2H_4 in 50 ml flask per 10 g fr wt 3 days
				ppb	
Trifoliate or.	10/3	Green	Green	10	...
Trifoliate or.	10/23	Green	Green	51	...
Trifoliate or.	11/27	Trace yellow	1/3 yellow	260	500
Grapefruit	11/17	Green	Green	10	30

Experiments on Leaf Abscission. The involvement of ethylene in the loss of chlorophyll of citrus fruit, in response to chilling temperatures, suggested that ethylene may participate in the coloration and abscission of leaves of deciduous trees. Ethylene evolution from leaves was measured by incubating the leaves at 20° in 50 ml flasks sealed with vicine caps, and sampling the air from the flasks at intervals of 1 to 4 days. Data represent averages of ethylene content of 4 replicate samples, except as noted otherwise.

The leaves of trifoliate orange trees at Leesburg, Florida, remained green all fall until November, at which time they developed a yellow color concomitantly with an enhancement of ethylene evolution (table III). The ethylene apparently triggered the decomposition of chlorophyll in the plastids, and unmasked the autumn yellow color in the trifoliate orange leaves prior to leaf fall. In contrast to the ethylene evolution by autumn-colored leaves of trifoliate orange, only a trace of ethylene was detected from mature leaves in the outer canopy trees of the evergreen grapefruit following the onset of cool weather in November at Leesburg, Florida. The leaves, which were approximately 6 months old in November, retained their deep green color all fall and winter.

Conclusion

Our results indicate that chilling enhances ethylene production in citrus fruit and in the leaves of the trifoliate orange, a deciduous species and near relative of citrus. Coincidentally with the high ethylene production in leaves of trifoliate orange and fruit of oranges and grapefruit by chilling, the leaves and fruit lose green color, probably by decomposition of chlorophyll. The carotenoid pigments are unmasked, giving the autumnal yellow coloration to leaves and fruit.

The only visible evidence of chilling injury to citrus fruit was the decomposition of chlorophyll, but the chilling probably caused changes in the normal metabolism of the peel that resulted in the ethylene production. With the avocado, ethylene production probably resulted from chilling injury, even though the injury was not visible for some time after the ethylene accumulation.

Literature Cited

1. Biale, J. B. 1954. Fruit respiration and ethylene production. Plant Physiol. 29: 168–73.
2. Burg, S. P. and E. A. Burg. 1965. Gas exchange in fruits. Physiol. Plantarum 18: 870–84.
3. Cooper, W. C., G. K. Rasmussen, and J. J. Smoot. 1968. Induction of degreening of tangerines by preharvest applications of ascorbic acid, and other ethylene-releasing agents. Citrus Industry 49(10): 25–27.
4. Eaks, I. L. 1960. Physiological studies of chilling injury in citrus fruits. Plant Physiol. 35: 632–36.
5. Erickson, L. C. 1959. Color development in Valencia orange. Proc. Am. Soc. Hort. Sci. 75: 257–61.
6. Stathakopoulos, N. P. and L. C. Erickson. 1966. Induction of dormancy in *Poincirus trifoliata* Raf. by controlled environmental conditions. Proc. Am. Soc. Hort. Sci. 89: 216–21.