

ETHYLENE PRODUCTION BY THE VALENCIA ORANGE TREE AS RELATED TO THE USE OF ABSCISSION CHEMICALS¹

W. C. COOPER, W. H. HENRY, AND C. J. HEARN
Horticultural Field Station, USDA

Orlando

ABSTRACT

Various tissues of the 'Valencia' (*Citrus sinensis* [L.] Osb.) orange tree produce ethylene. Small fruitlets, during a 6-week period following petal fall, produce ethylene in excess of that required to cause abscission. Application of 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutaramide [Cycloheximide] stimulates ethylene production in these fruitlets and increases fruitlet drop. After mid-May, ethylene production by the small green fruit occurs at very low rates, and cycloheximide does not greatly influence ethylene production at that stage. In mature leaves, cycloheximide greatly stimulates ethylene production only in the abscission zone and petiole; whereas, 2-chloroethylphosphonic acid stimulates ethylene production by the entire leaf.

INTRODUCTION

During investigations on the chemical control of citrus fruit abscission, it has become apparent that ethylene is necessary for abscission. We have measured the ethylene content of citrus fruit treated with various abscission chemicals and found that they contain enough ethylene to account for the fruit abscission (3).

It is the purpose of this paper to show that ethylene is a common product of citrus tissues; that physiologically potent concentrations of ethylene occur naturally in trees during the period of spring flush and that further stimulation by abscission chemicals at this time may contribute to leaf and small fruit drop. An understanding of this situation is needed in order to determine what concentration of the chemicals

to use, or whether to use the chemical at all during the period of spring flush.

METHODS AND MATERIALS

The work was confined to experiments with the 'Valencia' (*C. sinensis* [L.] Osb.) orange between March 17 and July 17, 1970. On March 17, April 21, May 21, and July 17, three 15-year-old bearing trees at the Chase and Company Groves, Windermere, Florida, were sprayed with 5, 10, and 20 ppm of 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl] glutaramide [cycloheximide]. On March 17, when the trees had a flush of new growth and were in bloom, additional 3-tree lots were sprayed with 500 ppm 2-chloroethylphosphonic acid (Ethrel)² and 2 percent erythorbic acid. Five gallons of test solution, to which 0.1% Upjohn surfactant 120² was added, was applied to each tree.

Ten flowers or small immature fruit were collected for ethylene production measurements from each tree at various intervals after treatment. The flowers or fruits were sealed in 250-ml flasks for 24 hours and held at laboratory temperature after which time the air in the flasks was analyzed for ethylene content by gas chromatography. When the fruit became too large for the flasks, 1.3 liter plastic containers were used, and the number of fruit per container was reduced to four.

On March 18 and 21 (1 and 4 days after application of abscission chemicals) ten 2-inch long immature new vegetative shoots and five 4-inch long mature leafy shoots were collected from each tree for ethylene production analyses. The leaves on the mature shoots were dissected into petioles and blades: the petioles included all of the abscission zone separating the blade and petiole. The immature shoots were tested only in the intact condition. Ethylene production measurements were made on all tissues in a manner similar to that described above for flowers and small fruit. The data were calculated on the millimicroliters of gas per gram

¹This report deals with the current status of research on the agricultural uses of growth regulators. It does not contain recommendations for use of these chemicals nor does it imply the uses discussed here have been registered. All uses of growth regulators must be registered by appropriate State and Federal agencies before they can be recommended. All chemical uses described in this report should be applied in accordance with directions on the manufacturer's label as registered under the Federal Insecticide, Fungicide, and Rodenticide Act.

²Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

fresh weight of tissue per hour ($m\mu l/g/hr$), based on a 24-hr-incubation period.

RESULTS AND DISCUSSION

The new developing shoots with immature leaves produced ethylene at rates of about $3.5 m\mu l/g/hr$, as compared to $0.5 m\mu l/g/hr$ for blades of mature leaves and $0.1 m\mu l/g/hr$ for mature stems (Table 1). Cycloheximide, at 20 ppm, tripled ethylene production in these new shoots. High rates of ethylene production in immature shoots of citrus have been associated with high auxin (a naturally occurring growth hormone) levels in the immature tissue (4), but there is no evidence to indicate that changes in auxin levels are associated with the cycloheximide treatment.

Leaves and stems on the mature shoots were producing ethylene, but at lower rates than that of the immature shoots. Cycloheximide, as with the new shoots, stimulated ethylene production in stems (Table 1). The bark slipped readily on these stems, indicating a nondormant condition.

In earlier work (5), we occasionally detected an enhancement of ethylene production in mature leaves treated with cycloheximide, but it was never very large or consistent. Recently, Jackson and Osborne (7) reported a localization of the major ethylene-production response of senescent leaves in the petioles for leaves of *Prunus serrulata senriko*. When we measured the ethylene production separately in blades and petioles of leaves treated with cycloheximide, we found that the petioles were producing more than five times as much ethylene as the blades. Thus, in the earlier work, when we only meas-

ured the ethylene production by the whole leaf, the large bulk of the low ethylene-producing blade tended to mask the relatively high ethylene-producing rates at the site of the abscission zone in the petiole.

Ethylene production patterns (data not shown), by new shoots and mature stems treated with erythorbic acid and 2-chloroethylphosphonic acid, were quite similar to those shown for cycloheximide-treated tissue. However, in contrast to erythorbic acid and cycloheximide, 2-chloroethylphosphonic acid induced "hyperethyleneism" of the leaf blades, as well as of the leaf petioles (Table 2). It is known that 2-chloroethylphosphonic acid breaks down chemically into ethylene, and the detection of a large-ethylene production by the entire leaf treated with this chemical was expected. On the other hand, we do not fully understand why ethylene production in the cycloheximide- and erythorbic-treated leaves is localized in the petiole. When these two chemicals are applied to the rind of mature fruit, they injure the rind, and the injured rind produces ethylene. There was no visible evidence of injury to either the leaf blades or petioles in these experiments.

Ethylene production by flowers and small fruitlets is shown in Table 3 and fruitlet drop is shown in Table 4. For fruitlet drop, we tagged three branches on each tree and counted

Table 1. Ethylene production by new shoots and leaves and stems of mature shoots of Valencia orange one day after treatment with 20 ppm cycloheximide (CHI) on March 19, 1970

Treatment	C ₂ H ₄ production ($m\mu l/g/hr$) of various plant parts			
	New shoots	Mature stems	Mature leaf blade	Mature leaf petiole
Control	3.547 a*	.155 a**	.521 a	1.414 a
CHI	10.813 b	2.857 a	1.540 b	8.589 b

*Data are means of 3 trees used for each treatment. Values followed by the same letter are not significantly different at the .01 level, according to Duncan's multiple range test.

**The values for control vs. CHI were statistically significant at the .05 level.

Table 2. Effect of various abscission chemicals on ethylene production by blades of mature leaves of Valencia orange*

Treatment	C ₂ H ₄ production ($m\mu l/g/hr$)
Control	.521 a**
20 ppm CHI***	1.540 a
2% erythorbic acid	.605 a
500 ppm CEPHA	18.607 b

*Trees sprayed on March 19 and leaves collected on March 20.

**Data are means of 3 trees used for each treatment. Values followed by the same letter are not significantly different at the .05 level, according to Duncan's multiple range test.

***Cycloheximide.

Table 3. Effect of cycloheximide (CHI) on ethylene production by flowers and immature fruits

of Valencia orange*							
C ₂ H ₄ production (m μ l/g/hr) at indicated stage of fruit development and date of collection							
	Open	Pistles at	Yellow	Green	Green	Green	Green
	flowers	petal fall	fruitlets	fruitlets	fruitlets	fruit	fruit
			(0.3g.fr.wt)	(0.5g.fr.wt)	(2.0g.fr.wt)	(8g.fr.wt)	(40g.fr.wt)
(ppm CHI)	3/20	3/25	4/21	4/21	4/21	5/21	7/16
Control	1.050 a**	7.615 a	3.008 a	.133 a	.050 a	.091 a	.043 a
5	1.520 bc	14.650 b	11.603 ab	2.488 b	.137 a	1.041 bc	.053 a
10	1.590 bc	21.905 c	20.647 b	1.473 ab	.498 b	.866 b	.062 a
20	1.900 c	13.855 b	17.163 b	2.529 b	.648 c	1.737 c	.025 a

*All flowers and fruits collected 2 days after treatment.

**Data are means of 3 trees used for each treatment. Values followed by the same letter are not significantly different at the .05 level, according to Duncan's multiple range test.

flowers or small fruitlets before treatment, and again after 2 and 8 weeks.

On March 21, the open flowers produced less ethylene than the pistles or small fruitlets after petal fall. Cycloheximide, applied to trees in full bloom on March 21, caused a slight increase in ethylene production by the flowers, but this had no significant effect on fruit drop after 2 and 8 weeks (Table 4). However, when the trees were sprayed with cycloheximide on April 21 (when the fruits weighed from 0.3 to 2.0 g fr wt), there was a marked increase in ethylene production by the fruitlets (Table 3) and in fruitlet drop (Table 4). Generally, the fruitlets turned yellow before abscising. Abscising, yellow

fruitlets on control trees were producing 3 m μ l/g/hr of ethylene, and larger green fruitlets on the same tree, which did not abscise, were producing only 0.50 m μ l/g/hr of ethylene.

Ethylene production by cycloheximide-treated green fruit on May 21 was below 3 m μ l/g/hr, and there was no significant effect of treatment on green fruit drop (Table 4). Ethylene production by green fruit on trees treated with cycloheximide in July was almost nil, and there was no fruit drop on these trees at that time.

Bain (2) has shown that the growth of the immature Valencia orange fruit, during the first 6 wk after petal fall, takes place largely by cell division in the rind. It is during this period of fruit development that we have observed the highest rates of naturally occurring ethylene production. These data confirm the earlier findings of Aharoni (1) and Eaks (6) that rates of ethylene production in the ontogeny of the fruit are highest during the 6-wk period after petal fall.

GENERAL DISCUSSION

These data show that various tissues of the Valencia orange tree are capable of producing ethylene. The highest rates of production were found in the new vegetative shoots and in the young fruitlets after petal fall. Cycloheximide

Table 4. Effect of cycloheximide (CHI) at three dates of application on immature fruit drop of Valencia oranges

Treatment (ppm CHI)	Trees sprayed 3/18/70			Trees sprayed 4/21/70			Trees sprayed 5/21/70	
	% fruit drop after 2 wk	4 wk	9 wk	% fruit drop after 2 wk	10 wk	% fruit drop after 2 wk	8 wk	
Control	27 a*	82 a	98 a	58 a	55 a	18 a	20 a	
5	21 a	95 b	98 a	63 a	74 b	10 a	12 a	
10	14 a	96 b	98 a	67 a	72 ab	8 a	11 a	
20	16 a	95 b	98 a	73 a	85 b	7 a	10 a	

*Data are means of 3 trees used for each treatment. Values followed by the same letter are not significantly different at the .05 level, according to Duncan's multiple range test.

