DIFFERENTIAL ABSCISSION OF CITRUS LEAVES, MATURE AND IMMATURE FRUITS BY ETHYLENE, ETHREL, AND CYCLOHEXIMIDE

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ABSTRACT

Various levels of ethylene, 2-chloroethylphosphonic acid (Ethrel), and cycloheximide stimulated abscission in explants of mature 'Valencia' orange fruit but did not affect abscission of immature (9 to 11 weeks old) fruit explants. The pull force to remove mature fruit dropped substantially within 4 days after treatment, while that of immature fruit was not affected. Cycloheximide was effective in accelerating abscission only when fruit were dipped in a solution of the chemical. Stem feeding of fruit with cycloheximide inhibited abscission. 'Pineapple' orange leaf explants were found more resistant to ethylene than explants from mature fruit.

INTRODUCTION

The use of abscission-accelerating chemicals as an aid to mechanical harvesting of citrus is receiving considerable attention from several research groups in Florida. A wide variety of chemicals have been screened for possible abscission activity (10, 11, 15, 22, 23). From such studies, iodoacetic acid first emerged (15) as an effective abscission accelerator on citrus but was later abandoned due to excessive tree defoliation. Ascorbic acid was later reported (9, 10, 24) to promote citrus fruit abscission when used at relatively high concentrations. Cycloheximide and Ethrel were also reported (11) to accelerate abscission of 'Valencia' oranges. A combination of Ethrel (200 ppm) and ascorbic acid (1 lb/tree) was reported (25) to promote fruit loosening, but caused severe tree defoliation in 'Valencia' orange trees.

Currently (2), ethylene is regarded as a hormone that exerts numerous physiological effects on plants (6), among which regulation of abscission is most prominent. Field applications of ethylene on citrus trees under a tent produced abscission in mature 'Valencia' fruit, but 5 to 6 hours exposure to the gas was necessary (22). On the other hand, soil injection with ethylene resulted in severe tree defoliation and dieback (22).

Because citrus trees are evergreen, an abscission chemical for early or midseason citrus crops should preferentially promote fruit, but not leaf, abscission. For late-maturing cultivars, e.g., 'Valencia' orange, the selection of an abscission chemical becomes more critical due to overlapping of crops. Evidence is presented on the differential abscission response of 'Pineapple' orange leaf and fruit explants to ethylene, and also of mature vs immature 'Valencia' oranges to 3 abscission accelerators.

MATERIALS AND METHODS

Effect of ethylene and 2,4-dichlorophenoxyacetic acid (2,4-D) on abscission of 'Pineapple' orange leaf and fruit explants.—Leaf explants were excised from fully expanded, dark green 'Pineapple' orange leaves. Each explant consisted of 1 cm petiolar and 0.3 cm leaf midrib portions (17). Following removal of the petiolar wings and leaf blade tissues, the explants were supported, distal end upward, on a layer of 1.6% agar in petri dishes and held at 25 ± 1C. Thirty explants were randomly assigned to each treatment.

Fruit explants from mature 'Pineapple' oranges were harvested from the same trees with 4 to 5 inch stems. The force needed for detachment of fruit from their stems was initially measured on a 20-fruit sample with the Chatillion tensiometer (15).

Ethylene treatments.—Shortly after excision, leaf and fruit explants were exposed to 10 ppm ethylene for 8 hours in a sealed system. Controls were held in air for 8 hours. At the end of the exposure period, the stems were reclipped and held under distilled water.

2,4-D treatments.—Leaf explants were administered the synthetic auxin 2,4-D (2×10^{-4}M)

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at the rate of 2 μl/ explant applied distally with a microsyringe. Fruit explants were treated with 2 x 10^{-4}M 2,4-D through their stems for 8 hours and then transferred to distilled water. The explants were checked for abscission at 24-hour intervals.

In order to study the antagonism between 2,4-D and ethylene, 'Pineapple' orange leaf explants were treated with 20 ppm 2,4-D shortly after excision. Forty-eight hours later, they were exposed to 4 ppm ethylene in closed containers for 24 hours. Control explants were initially treated with distilled water and held for 24 hours in airtight containers without ethylene.

Abscission response of mature and immature 'Valencia 'oranges to ethylene, Ethrel, and cycloheximide.—Mature and immature (1.25 to 1.5 inches equatorial diameter, approximately 9 to 11 weeks from full bloom) 'Valencia' oranges were clipped with 4 to 5 inch stems. Following washing and drying, the fruit were divided into 20-fruit samples and placed in glass jars. Ethylene was injected to create atmospheres containing 10, 100 and 1,000 ppm ethyene in air. The jars were kept sealed for 8 hours following which the stems were reclipped and placed in distilled water.

Cycloheximide at 0, 5, 10, and 20 ppm and Ethrel at 0, 10, 100, 500, and 1,000 ppm were applied to samples of mature and immature 'Valencia' fruits by dipping in solutions of the chemicals for 30 seconds. Triton X-100 was incorporated in each solution at 0.05% as a wetting agent. Dipping and stem feeding applications of 20 ppm cycloheximide to mature 'Valencia' fruit were also compared.

Effect of Ethrel and cycloheximide tree sprays on abscission of mature and immature 'Valencia' oranges.—Ethrel was sprayed at 100 and 200 ppm and cycloheximide at 10 ppm on branches of fully grown 'Valencia' trees bearing both mature and immature fruits, the latter ranged from 1.5 to 1.75 inches in equatorial diameter. Triton X-100 was used at 0.05% to improve coverage. Control branches were sprayed with distilled water containing only the wetting agent. The pull force on 20-fruit samples of both mature and immature fruits was determined initially and at 4, 5, 7, and 8 days after treatment.

RESULTS

Response of 'Pineapple' orange leaf and fruit explants to ethylene and 2,4-D.—Ethylene initially accelerated abscission in fruit explants, but not in leaf explants (Fig. 1). 2,4-D retarded abscission in both leaf and fruit explants (Fig. 2). At 96 hours after excision, 2,4-D-treated leaf explants exhibited no abscission while 10% of fruit explants treated with 2,4-D had abscised. Leaf explants exposed to 4 ppm ethylene 48 hours after an initial treatment with 2,4-D showed a lower abscission rate than did the controls (Table 1). Day and Erickson (12) reported similar findings although extremely high concentrations of ethylene (15,620 ppm) and 2,4-D (222 ppm) were used.

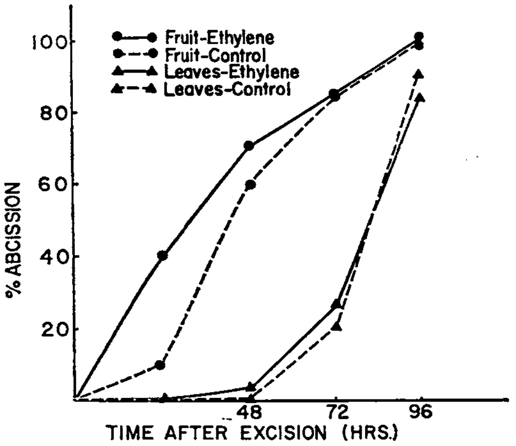

Figure 1.—Effect of ethylene (10 ppm for 8 hours) on abscission of explants of mature 'Pineapple' orange fruit and leaves.

Response of mature vs immature 'Valencia' oranges to 3 abscission-promoting chemicals.—Various concentrations of ethylene applied to mature and immature 'Valencia' fruits for 8 hours hastened abscission only of mature fruit (Table 2). Immature fruit exhibited no decline in pull force even when exposed to 1,000 ppm ethylene.

Abscission in mature 'Valencia' oranges was

Table 1.--Effect of 2,4-D and ethylene treatments on abscission of 'Pineapple' orange leaf explants.

Treatment		% abscission		
		Time after initial treatment (hrs)		
Initial	After 48 hrs	24	48	72
2,4-D	Ethylene	0.0	0.0	33.3
2,4-D	Air	0.0	0.0	0.0
H_2O	Ethylene	0.0	6.7	90.0
H_2O	Air	0.0	3.3	46.7

Table 2.--Abscission response of mature and immature 'Valencia' orange fruit explants to various concentrations of ethylene.

Ethylene concentration (ppm)	Pull force (lbs)*			
	Mature fruit		Immature fruit	
	Initial	At 4 days	Initial	At 4 days
0	19.1 ± 2.04	12.8 ± 2.77	12.3 ± 1.72	14.9 ± 2.53
10		7.8 ± 3.40		14.3 ± 2.4
100		7.0 ± 2.50		14.1 ± 3.16
1000		5.1 ± 3.16		12.7 ± 2.88

*Data represent means ± standard deviation.

accelerated only when fruit were dipped in cycloheximide solution, but was inhibited by the chemical when administered through the stems (Table 3). Inhibition of abscission by cycloheximide has been shown to result from blocking *de novo* synthesis of protein necessary for cell separation (3). When cycloheximide was applied to mature and immature fruits by dipping, abscission was accelerated only in mature fruit (Table 4).

All levels of Ethrel over 10 ppm hastened abscission of mature 'Valencia' fruit explants (Table 4). Abscission in immature fruit was not accelerated by any of the Ethrel levels except the relatively high concentration of 1,000 ppm.

When 'Valencia' tree branches were sprayed with 100 ppm Ethrel, the pull force was not decreased for either mature or immature fruits at 4, 5, 7, or 8 days after treatment (Table 5). Spraying with 200 ppm Ethrel caused slight loosening of mature fruit, while it did not affect immature fruit. The pull force of mature fruit dropped by 50%, 4 days after spraying, with

Table 3.--Effect of stem feeding vs dipping in cycloheximide on abscission of mature 'Valencia' orange fruit explants.

Treatment	Pull force (lbs)*	
	Initial	After 4 days
Stem feeding		
10 ppm cycloheximide	17.2 ± 2.43	18.7 ± 3.79
Control	17.2 ± 2.43	9.8 ± 3.31
Dipping		
10 ppm cycloheximide	17.3 ± 2.44	5.0 ± 3.36
Control	17.3 ± 2.44	10.2 ± 2.24

*Data represent means ± standard deviation.

Table 4.--Abscission of mature and immature 'Valencia' orange fruit explants in response to various levels of Ethrel and cycloheximide.

Treatment	Pull force (lbs)*			
	Mature fruit		Immature fruit	
	Initial	At 4 days	Initial	At 4 days
Ethrel (ppm)				
0	22.2 ± 2.96	6.0 ± 2.43	13.7 ± 1.69	15.8 ± 2.71
10		8.3 ± 2.67		15.1 ± 2.37
100		5.8 ± 2.27		15.4 ± 2.31
500		2.7 ± 1.92		14.9 ± 3.88
1000		1.9 ± 1.44		6.6 ± 3.61
Cycloheximide (ppm)				
0	18.1 ± 2.17	7.5 ± 3.64	11.3 ± 1.52	14.0 ± 2.22
5		5.4 ± 2.56		12.0 ± 3.35
10		4.9 ± 1.51		12.6 ± 3.01
20		4.6 ± 2.38		13.3 ± 2.67

*Data represent means ± standard deviation.

10 ppm cycloheximide while immature fruit showed no response.

Discussion

Although ethylene is noted for its acceleration of abscission in many types of leaf explants (1, 5, 7, 13), the development of a sufficient degree of senescence seems to be an essential prerequisite for initiation of the abscission response (1, 4, 8, 13). For 48 hours after excision, 'Pineapple' orange leaf explants exhibited little abscission response to ethylene applied shortly after excision, while explants from mature fruit abscised faster in response to the abscission-accelearting gas (Fig. 1). These findings indicate that citrus fruit explants were more senescent and, thus, more responsive to ethylene at the time of excision than were leaf explants. Senescence changes which precede abscission include loss of chlorophylls, protein, and RNA (4, 18). Earlier, it was reported (16) that 'Pineapple' orange leaf explants remained unresponsive to ethylene for 20 hours after excision, but responded after 28 hours. Similar findings have been reported on bean (13) and cotton leaf explants (1, 13).

Retardation of abscission in 'Pineapple' orange leaf and fruit explants by 2,4-D (Fig. 2) was probably caused by halting or reversing of processes associated with senescence. Field application of 2,4-D was reported to reduce preharvest drop, a manifestation of senescence, in 'Pineapple' (14) as well as in 'Valencia' oranges (20). Wilson and Hendershott (24) observed less methylation and breakdown of pectins in

Table 5.--Effect of Ethrel and cycloheximide field sprays on abscission of mature and immature 'Valencia' orange fruits.

Treatment	Pull force (lbs)*				
	Initial	Days after spray			
		4	5	7	8
Mature fruit					
100 ppm Ethrel	18.2 ± 2.3	17.9 ± 4.6	17.0 ± 3.4	18.2 ± 2.6	18.3 ± 2.2
200 ppm Ethrel	17.6 ± 2.6	14.9 ± 3.9	15.6 ± 2.9	16.5 ± 2.3	16.1 ± 2.6
10 ppm cycloheximide	17.6 ± 1.9	8.8 ± 6.4	9.3 ± 4.7	9.8 ± 3.4	10.7 ± 3.8
Control	16.7 ± 2.5	17.7 ± 2.3	16.8 ± 1.7	18.1 ± 2.4	16.9 ± 2.4
Immature fruit					
100 ppm Ethrel	14.2 ± 1.8	15.3 ± 3.2	14.4 ± 2.3	15.1 ± 1.7	14.7 ± 1.9
200 ppm Ethrel	15.5 ± 2.2	16.9 ± 2.6	16.9 ± 3.2	16.5 ± 3.1	16.0 ± 2.7
10 ppm cycloheximide	13.9 ± 1.4	15.1 ± 2.3	15.6 ± 2.2	15.5 ± 1.7	14.3 ± 1.9
Control	15.6 ± 1.9	17.1 ± 2.3	15.3 ± 1.7	16.5 ± 2.1	16.1 ± 2.9

*Data represent means ± standard deviation.

cells of the separation layer of 2,4-D-treated 'Pineapple' oranges than in nontreated fruit. In banana fruit, Vendrell (21) reported 2,4-D to delay the onset of climacteric rise in respiration and suggested that the auxin causes a reversion of tissues to a more juvenile state. Although the 'Pineapple' orange fruit used in these studies were senescent, as indicated by their fully colored rind and by their immediate response to ethylene, they were still responsive to 2,4-D. Such simultaneous responses of fruit explants suggest that the two-stage abscission phenomenon (19) reported in many types of leaf explants (1, 16, 17, 19) in response to auxins and ethylene does not fully apply to mature citrus fruit.

The differential responses of mature vs immature fruit explants and of mature fruit vs leaf explants to the various abscission chemicals point out the importance of physiological age in determining the abscission response of a given organ. A senescent organ, e.g. mature, fully degreened fruit, is more likely to abscise under the influence of an abscission-accelerating chemical than a younger organ, e.g. healthy, green leaves, and expanding fruit. It has been shown (11) that low concentrations (5 to 25 ppm) of cycloheximide sprays promoted loosening of mature 'Valencia' orange fruit with very slight leaf drop, while higher levels (50 to 500 ppm) resulted in fruit removal with very severe defoliation. It would, therefore, appear that mature fruit are more susceptible to low levels of cycloheximide than are most of the leaves on the tree. Burg and Burg (7) reported that the sensitivity of banana fruit to the ripening effect of ethylene increased with age. In beans, Chatterjee and Leopold (8) reported that older leaves required less time to abscise under the influence of β-alanine, which stimulates ethylene production (1), than younger leaves.

Failure of Ethrel sprays (100 and 200 ppm) on 'Valencia' tree branches to accelerate abscission of either mature or immature fruits seem to indicate that the concentrations applied were not adequate. However, a combination of 200 ppm Ethrel and ascorbic acid (1 lb/tree) resulted in fruit loosening and in heavy defoliation of 'Valencia' orange trees (24) indicating possible synergism. Loosening of mature fruit induced

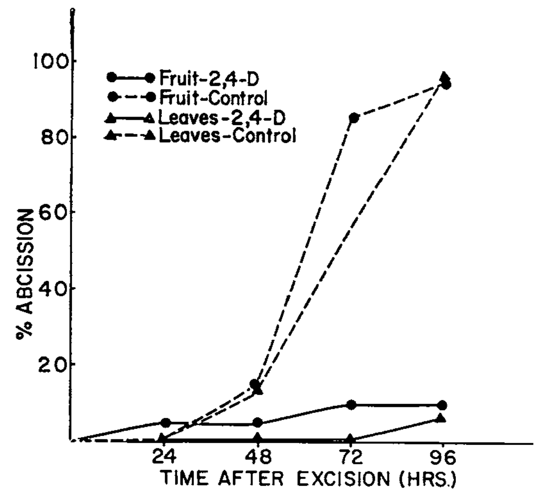

Figure 2.—Effect of 2,4-D (2 x 10-4 M) on abscission of explants of mature 'Pineapple' orange fruit and leaves.

by 10 ppm cycloheximide sprays on 'Valencia' trees (Table 5) does not seem to be practically adequate for mechanical harvesting purposes. Slightly higher concentrations could bring about a more desirable effect.

Age-related differences in the abscission response of various citrus organs might be beneficially exploited for selective removal of mature, ready-to-harvest fruit without causing extensive losses of leaves or immature fruit. One approach might be the use of abscission chemicals at marginal levels, levels that can initiate abscission only in mature fruit. Further field testing, however, must decide whether these differential responses can be applied practically.

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