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Induction of Abscission at Hypobaric Pressures

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ABSTRACT

The use of hypobaric pressures has increased the precision of abscission research by enabling us to differentiate between abscission action of ethylene and abscisic acid. When cycloheximide is sprayed on fruit attached to trees, enhanced levels of ethylene occur in the fruit and, subsequently, the fruit abscises. When ethylene in the fruit is eliminated by hypobaric pressures, the fruit does not abscise. Thus, ethylene is the effector of fruit abscission that results from cycloheximide treatment. When abscisic acid is applied to the fruit through stem uptake and ethylene is removed by hypobaric pressures, rapid fruit abscission occurs, which is presumably caused by abscisic acid itself. Thus, either ethylene or abscisic acid will induce abscission of citrus. Likewise, the abscission of debladed petioles of *Coleus* plants appears to be effected either by ethylene or abscisic acid.

The dominant role of endogenous ethylene in both leaf and fruit abscission has gained considerable support in recent years. Jackson and Osborne (10) have shown that large quantities of ethylene are synthesized in the abscission zone tissues of leaves just before abscission. In mature, ripening apples (*Pyrus malus* L.), high levels of ethylene occurring in the abscission zone before fruit fall appear to result from diffusion of the gas from adjacent flesh containing high levels of ethylene (2). In fruit of oranges (*Citrus sinensis* [L.] Osbeck), which have no respiration climacteric, enhanced levels of ethylene are induced in the fruit by treatment with CHI[1], and this ethylene diffuses to the adjacent abscission zone and initiates abscission (5). Thus, whether the production of ethylene occurs naturally or is stimulated by chemical treatment, the gas is produced endogenously by the fruit and subsequently initiates the synthesis of enzymes essential for abscission.

A new abscission regulator was introduced in 1963, when Ohkuma *et al.* (13) demonstrated the presence of what is now known as abscisic acid in rapidly abscising fruit of cotton (*Gossypium hirsutum*). Correlations between increased levels of endogenous ABA and the incidence of abscission have been obtained with cotton fruit (1), leaves of *Betula* (7), and debladed petioles of *Coleus blumei* (4). Applied ABA promotes abscission of leaf abscission zone explants of apple (14) and cotton and *Coleus* (15). None of these workers showed that the abscising plant parts were producing ethylene.

There is now substantial evidence that part of the effect of ABA on abscission is mediated through ethylene production. Ripening pears (*Pyrus communis* L.) produce both ethylene and ABA before fruit fall (16, 17). The treatment of apple fruits with ABA promotes abscission (8); but, since such ABA-treated fruits produce ethylene, we cannot be sure whether ABA, ethylene, or both substances is responsible for the fruit abscission. ABA, when sprayed on oranges, does not promote either ethylene production or abscission; but when taken up into the fruit through a cut stem, it promotes both ethylene production and abscission (6). In experiments with *Phaseolus vulgaris* L. petiole explants, Jackson and Osborne (11) showed that, although ethylene and ABA applied separately promote abscission (ethylene being the most effective), no increase in abscission is detected when ABA and ethylene are applied together.

Since both ABA and ethylene have abscission activity and since both occur naturally in most plant tissue, it would aid in our understanding of the physiology of abscission if one or both of the abscission-promoting substances could be eliminated from the tissue. In the case of CHI-induced orange fruit abscission, it is important to know whether abscission would take place if ethylene were eliminated from CHI-injured fruit. Likewise, if ethylene were eliminated from debladed *Coleus* petioles, would abscission take place?

Since ethylene is a gas, it can be readily removed from tissue by low (hypobaric) pressures (3). The accelerated outward diffusion or escape of gases from tissues held under hypobaric pressures prevents the accumulation of endogenously produced gases such as ethylene. Burg and Burg (3) employed hypobaric pressures to remove endogenous ethylene from stored banana (*Musa* X *paradisiaca* L.), and thereby greatly prolonged the storage life of the fruit. Imaseki (9) used the same technique to remove endogenous ethylene from sliced sweet potatoes (*Ipomoea batatas* [L.] Lam.). In the present communication, the authors employed hypobaric pressures to determine the effect on fruit abscission of removing endogenous ethylene from orange trees and orange fruit explants treated with CHI and with ABA. Tests were also conducted on petiole abscission on debladed *Coleus* plants.

MATERIALS AND METHODS

We engineered a system of 12 bell jars (18 inches in diameter, 30 inches high, and with 3.75 cubic feet capacity) to maintain a leakproof vacuum of 150 mm Hg with a flow of 2 cubic feet of a gas mixture (99.7% O_2 and 0.3% CO_2) per hour for each chamber. When the gas mixture is reduced to ⅕ atm, it approximates the ratio of $O_2 + CO_2$ normally found in air at 1 atm. The gas mixture in some of the chambers was enriched by the addition of ethylene to provide 5 μl/l ethylene to the gas mixture.

The vacuum system of 12 bell jars was set up on a greenhouse bench. The bell jars were large enough to accommodate small, 2-feet high, potted *Citrus* trees (bearing fruit) or potted *Coleus* plants. During the summer, we enclosed the system in

[1] Abbreviations: CHI: cycloheximide; FRF: fruit removal force.

a Plexiglas box and provided refrigeration to keep air temperatures within the vacuum jars near those of the greenhouse air.

The experiments reported herein were conducted on calamondin (*Citrus reticulata* var. austera X *Fortunella* sp) trees, bearing 10 fruit per tree; "Persian" lime (*C. aurantifolia* [Christm.] Swingle) trees, bearing 5 fruit per tree; "Valencia" orange (*C. sinensis* [L.] Osbeck) fruit plus stem explants; and *Coleus blumei,* Princeton Clone plants.

Three CHI-treated (20 μl/l) citrus trees were placed in the hypobaric chambers within 15 min after the experimental spray was applied. Three untreated (control) trees were also placed in the hypobaric chambers. Likewise, treated and untreated trees were placed on an adjacent greenhouse bench at atmospheric pressures.

The fruit-removal force after 1 week at hypobaric pressure was determined with a Chatillon[2] pull force gauge by applying a straight pull at a zero angle parallel to the axis of the fruit, in order to transmit the force equally to all sides of the juncture of the woody peduncle and the exocarp of the fruit (5). The average value of the FRF on the 10 fruit on each of the three replicate trees provides a good abscission index (5).

In the experiment with Valencia orange fruit-plus-stem explants, the cut ends of the stems were placed in solutions of 100 μg/ml ABA. Others were placed with the stem in water, and a third lot was placed with stems in water after dipping the fruit momentarily in a 20-μg/ml solution of CHI. Ten explants were used for each treatment.

The terminal buds of the *Coleus* plants were examined carefully, and only those with a vegetative terminal bud and nine pairs of leaves were selected (12). The lower seven pairs of leaves were debladed. Three were placed in the hypobaric chambers, and three others (controls) were placed in an adjacent chamber held at atmospheric pressure. The same experiment was repeated on three additional plants per treatment.

Observations were made when each debladed petiole abscised without having been touched. The abscised petioles were incubated in sealed 125-ml flasks for 24 hr, and a 1-ml sample of air was removed from the flasks with a hypodermic syringe for ethylene analysis by gas-liquid chromatography (5).

RESULTS

Fruit of Valencia orange, calamondin, and Persian lime, held at hypobaric pressure, showed the same CHI-induced surface lesions of flavedo tissue as that of fruit exposed to normal atmospheric pressure. However, the fruit, after 1 week at hypobaric pressure, showed only a small reduction in FRF as compared with fruit at atmospheric pressure (Tables I and II). We were unable to remove air from under the flavedo (outer portion of the exocarp) immediately after removing the fruit from the chambers; but after 30 min we extracted air that contained 3.254 μl/l ethylene, as compared to only 0.990 μl/l ethylene from the CHI-treated fruit held at 1.0 atm. These readings represent the average of determinations from six fruits per treatment. The data indicate that the endogenous system for CHI-induced ethylene (or wound ethylene) in citrus fruit is not only intact, but actually produces more ethylene after 1 week at hypobaric pressure than CHI-treated fruit exposed to atmospheric pressure. We did not determine how long the increased rate of ethylene production persisted after the 30-min determination.

[2] 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.

Table I. *Effect of Hypobaric Pressures on Fruit and Leaf Abscission of Calamondin Trees*

Trees were sprayed with 20 μg/ml CHI, CHI + C_2H_4 (5 μg/ml), and untreated. Results are after 1 wk in 3.75 cubic feet bell jars engineered to maintain a leakproof vacuum of 150 mm Hg with a flow of a mixture of 99.7% O_2 + 0.3% CO_2 at the rate of 2 cu ft/hr·jar. When this mixture is reduced to 0.2 atm, ratio of O_2 to CO_2-approximates that in air at 1 atm.

Atmospheric Pressure	Chemical Treatment	FRF[1]	Fruit[2] Drop	Leaf[2] Drop
atm		*kg*	%	
0.2	CHI	2.0	0	0
	CHI + C_2H_4[1]	0.5	25	4
	Control	2.3	0	0
1.0	CHI	0.6	25	5
	Control	2.3	0	0

[1] Ethylene was added to the O_2 + CO_2 mixture flowing through this jar to provide 5 μl/l ethylene.

[2] The sample size was six plants per treatment with 10 fruit per plant. The FRF data are average for 60 fruit per treatment. The leaf-drop data are average for six plants per treatment.

Table II. *Effect of Hypobaric Pressure on Fruit and Leaf Abscission of Persian Lime Trees*

Trees were sprayed with 20 μg/ml CHI. Results are after 1 wk in 2-cubic feet bell jars engineered to maintain a leakproof vacuum of 159 mm Hg with a flow of a mixture of 99.7% O_2 + 0.3% CO_2 at the rate of 2 cu ft/hr·jar. When this mixture is reduced to 0.2 atm, the ratio of O_2 to CO_2 approximates that in air at 1 atm.

Atmospheric Pressure	Chemical Treatment	FRF[1]	Fruit Drop	Leaf[1] Drop
atm		*kg*	%	
0.2	CHI	4.5	0	0
1.0	CHI	0	100	0
1.0	Control	5.9	0	0

[1] The sample size was six plants per treatment with five fruit per plant. The FRF data are the average for 30 fruit per treatment. The leaf-drop data are the average for six plants.

Table III. *Effect of Hypobaric Pressure on FRF of Fruit Explants of Valencia Orange Treated with ABA[1] and CHI*

Stems of explants were soaked in 100 μg/ml ABA for 5 days. Fruits were sprayed with 20 μg/ml CHI and stems placed in water for 5 days. Experiment was conducted in July 1972.

Atmospheric Pressure	Chemical Treatment	FRF[1]
atm		
0.2	100 μg/ml ABA to stem	1.2
	20 μg/ml CHI to fruit	2.6
	Control	3.0
1.0	100 μg/ml ABA to stem	1.0
	20 μg/ml CHI to fruit	0.6
	Control	2.7

[1] The sample size was 10 explants per treatment, with one fruit per explant. The FRF data are the average for 10 explants.

When 5 μl/l ethylene were added to the trees under hypobaric pressure, fruit loosening occurred at a rate similar to that observed on CHI-treated trees held at atmospheric pressures

Table IV. *Effect of Hypobaric Pressures on Abscission of Petioles of Debladed Coleus Plants*

Atmospheric Pressure	Ethylene Treatment	Petiole Abscission Time		C_2H_4 Production by Petioles at Time of Abscission[1]
		Lower petioles	Upper petioles	
atm		*days*		*μml/g·hr*
0.2	None	5	8	—[2]
0.2	5 μl/l	1	2	4.366
1.0	None	2	3	0.500

[1] At the time of deblading the petioles, they were producing 0.125 μml/g·hr ethylene.

[2] It was not possible to determine ethylene production by the hypobaric petioles at the time of abscission. The important consideration here is that it escapes from the tissue as fast as it is produced and does not accumulate in the tissue.

(Table I). This is evidence that the inhibition of fruit loosening occurring at hypobaric pressure is due to the reduction or near elimination of ethylene from the tissue and not to any physical damage by the low pressure. Thus, we conclude that CHI accelerates citrus fruit abscission only under conditions in which ethylene is allowed to accumulate in the fruit.

When ABA is applied through stem uptake to Valencia oranges, it results in a rapid acceleration of fruit abscission at both hypobaric and atmospheric pressures (Table III). Since endogenous ethylene does not accumulate in fruit held at hypobaric pressure, it appears that ABA alone was the abscission-accelerating factor in the ABA-treated fruit. On the other hand, ethylene was the obvious abscission factor in the CHI-treated fruit in the same test. In interpreting the data in Table III, however, it should be pointed out that these Valencia orange fruit were senescent (fruit held on the trees for 2–3 months past normal harvest time) and were more sensitive to both ABA and ethylene action than fruit harvested several months earlier.

The aging petioles of *Coleus* plants abscised 2 to 3 days after deblading on plants held at atmospheric pressure, as compared to 5 to 8 days when held at hypobaric pressure (Table IV). Ethylene was being produced at the rate of 0.5 μml/g·hr by the debladed petioles of the control plants at atmospheric pressure. If ethylene is produced by the petioles of hypobaric plants, the ethylene undoubtedly escapes rapidly and does not accumulate in the petioles. When ethylene was added to the hypobaric plants, petiole abscission occurred quite rapidly. Thus, ethylene obviously promotes petiole abscission in *Coleus* and appears to be a factor in abscission of debladed petioles of plants held at atmospheric pressure. Yet, petiole abscission does occur in hypobaric plants, but it occurs much more slowly than when ethylene is present.

DISCUSSION

Normally, the abscission process in citrus fruit commences coincident with the accumulation of ethylene and with the appearance of visible injury of the flavedo (5). We know that ethylene production results from CHI-induced stress and injury to the tissue (5, 6). Since the results given in this paper show that when ethylene is prevented from accumulating in the fruit abscission is inhibited, we conclude that the fruit-abscission response from CHI treatment is indeed mediated by an intervening step in which ethylene is produced. Ethylene is, therefore, the effector rather than some other abscission factor produced by the CHI treatment. However, ABA also accelerates citrus fruit abscission in the absence of ethylene. Thus, both ABA and ethylene may be considered as abscission regulators in citrus.

In the experiments with *Coleus* plants, the abscission of debladed petioles appears to be mediated by both ethylene and some other endogenous factor. Endogenous ABA is known to increase in debladed petioles of *Coleus* during aging and abscission (4). Our results indicate that ethylene is also generated in the aging petioles, and this ethylene hastens abscission. Petiole abscission, however, does occur in aging petioles at hypobaric pressures where ethylene does not accumulate, and this abscission may possibly be caused by endogenous ABA.

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