Abscission Chemicals in Relation to Citrus Fruit Harvest

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The use of abscission chemicals to loosen fruit as an aid to either mechanical or manual harvest is now on the verge of reality for citrus fruit destined for the juice processing industry. The abscission process is now understood, and the probability of obtaining registration for cycloheximide, an effective abscission chemical, is good. All satisfactory chemical abscission agents cause physical injury, which is undesirable for fresh fruit marketing but inconsequential for the juice processing industry. This places the fruit-loosening process in the category of a wound reaction. Wound ethylene generation by the rind, whether induced by cycloheximide or by mechanical wounding, acts as a triggering agent of an essential enzymatic abscission process in the abscission zone which is inhibited by both cycloheximide and auxin.

The U.S. 200-million-box annual orange corp is the United States' largest fruit corp. Because of a shortage of labor, there is an urgent need to mechanize the harvesting of citrus. Mature citrus fruits remain firmly attached to the tree for a considerable period of time. This strong attachment allows harvesting the fruit of a single variety over a period of several months, but interferes with mechanical harvesting. Our objective is to develop chemical treatments that will lower the attachment force of the fruit to the stem and permit the rapid and clean separation of all the fruit at the normal fruit abscission zone when the trees are mechanically harvested.

The presence of a diffusible substance in cotton that accelerated the abscission of cotton explants ultimately led to the discovery of what is now known as abscisic acid, a plant hormone capable of regulating a number of processes (Addicott *et al.*, 1964; El-Antably *et al.*, 1966). Although we find that abscisic acid has abscission activity on citrus when taken up through the stem to the abscission zone, it has relatively little fruit abscission effect when sprayed on citrus trees (Cooper *et al.*, 1968).

Ethylene is produced in measurable amounts in various tissues of many higher plants (Burg, 1968). It is produced in citrus leaves, fruit, and shoots under normal conditions, and in larger amounts after treatment with certain chemicals (Cooper *et al.*, 1968; Palmer *et al.*, 1969). Abscission of fruits and leaves often occurs in plants following chemical applications, and it is now apparent from the work of Abeles (1967) that endogenous ethylene controls or regulates abscission in plants. Jackson and Osborne (1970) have further shown that it is the endogenous ethylene in the petiole of the leaf that regulates the process.

Plant fumigation with ethylene has long been known to produce abscission responses (Neljubow, 1901). When ethylene is applied to a tented citrus tree, it is extremely effective in speeding fruit abscission (Cooper and Henry, 1968; Wilson, 1966). However, since ethylene is a gas, its use as an abscission chemical in the field is impractical. Chemicals that release ethylene in water solution, or chemicals that cause fruit to produce ethylene, may be practical.

2-Chloroethylphosphonic acid (CEPA) in water solution is a true ethylene-producing chemical (Yang, 1969). This chemical is reported to be effective in speeding the fruit abscission of apples (Edgerton and Greenhalgh, 1969) and cherries (Bukovac *et al.*, 1969). When water solutions of CEPA are sprayed on citrus trees, leaves and fruit of citrus trees evolve large quantities of ethylene (Cooper *et al.*, 1968). However, concentrations (500 ppm) of CEPA which promote citrus fruit abscission cause defoliation and gumming and dieback of defoliated twigs results (Cooper *et al.*, 1968, 1969). Although these trials with exogenous ethylene, when used either as a gas or in water solution, have been more or less unsuccessful in solving the practical problem of regulating citrus fruit abscission, they do illustrate the effectiveness of exogenous ethylene in promoting abscission.

Unrelated chemicals such as ascorbic acid, citric acid, cycloheximide, and iodoacetic acid injure the rind of citrus fruit and cause the rind to produce ethylene and accelerate abscission (Cooper *et al.*, 1968). Ethylene produced at the site of the injury is more accessible to the abscission zone than ethylene (in CEPA) in water solution sprayed on the fruit. We, therefore, hypothesize that the solution to the problem lies in making the plant generate "hyperethyleneism."

Measurement of ethylene has now proven to be a reliable indicator of fruit-abscission processes. Improved methods of measuring ethylene by high-sensitivity gas chromatography have been a great asset. Direct physical injury to produce ethylene would probably work, but it is nearly impossible to produce and control on a practical basis. The most practical method is to find a chemical that will induce excess ethylene production. Several chemicals have been found to work. The best so far is cycloheximide. Because only 20 ppm of cycloheximide is required to loosen fruit, the cost per acre is approximately $35.00, or approximately 35¢ per tree or 3.5¢ per box. The maximum acceptable cost is about 25¢. The Food and Drug Administration has granted an experi-

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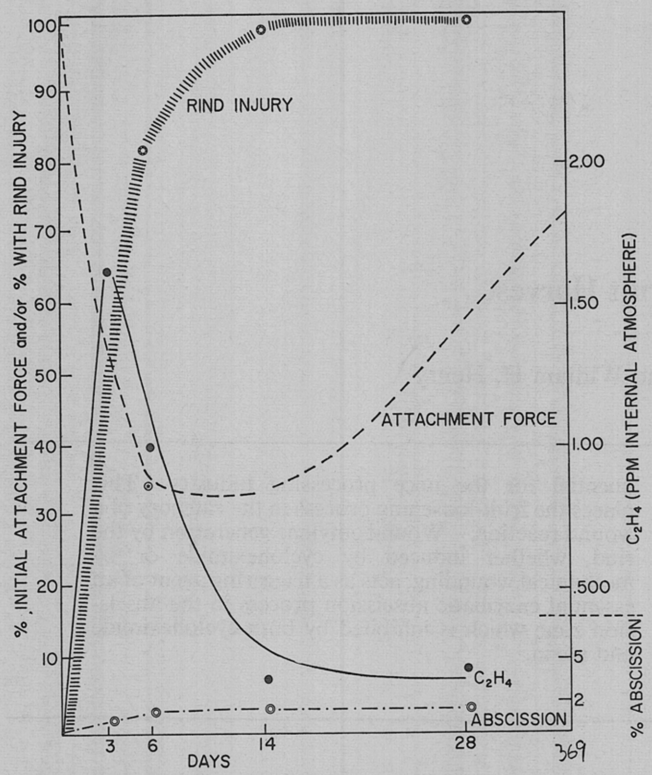

Figure 1. Levels of ethylene under the rind of mature fruit, attachment force of fruit and development of rind injury during 35 days following application of 10 mg/l. CHI to Valencia orange trees on 3/15/70. The initial attachment force was 8.0 kg

mental permit for the spraying of 50 acres of oranges with 20 ppm cycloheximide in Florida during 1970.

RECENT EXPERIMENTATION

Effects of Chemical *vs.* Physical Injury on Ethylene Production. The data in Figure 1 show the effects of cycloheximide on the mature fruit of Valencia orange [*Citrus sinensis* [L.] Osbeck] trees in full bloom when sprayed on March 15. The ethylene content of the air under the rind rises sharply from 0.02 to 1.5 ppm within 3 days, and then drops to near the initial low level within 7 to 10 days. Rind injury and a loss in attachment force of the treated fruit develop concomitantly with the rise in ethylene content of the fruit. The extent of these reactions varies with the concentration of cycloheximide used, season of year, and cultural practices. The surge in ethylene production in the fruit is usually more for 10 mg/l. cycloheximide than for 5, and more for 20 than 10. Likewise, rind lesions are less extensive and slower developing for a 5 mg/l. solution than for higher concentrations (Table I).

Cycloheximide rind injury usually consists of small necrotic spots which are associated with a localized accumulation of the chemical solution in droplets at the blossom end of the fruit (Figure 2). The lesions caused by treatment with either ascorbic acid or erythorbic acid are more or less the same as cycloheximide-induced lesions (Figure 2).

Vines *et al.* (1968) found that ethylene production in detached oranges is enhanced by rough handling, such as bouncing the fruit on the floor. In our experiments, we found that rubbing the blossom end of Valencia oranges with emery paper caused the fruit to produce more ethylene than cycloheximide-injured fruit (Table II). Cycloheximide treatment of emery paper-injured fruit caused further ethylene

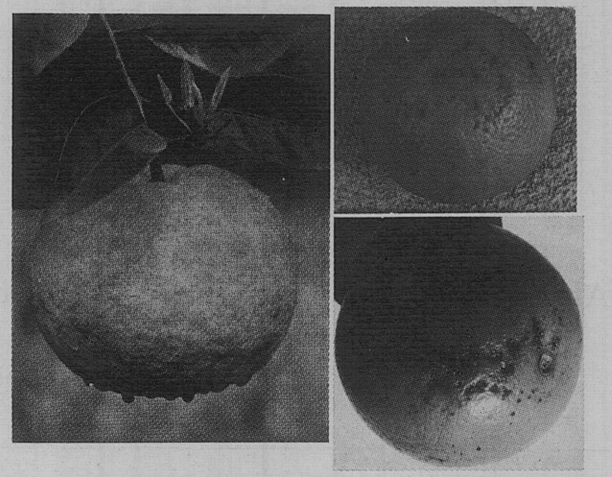

Figure 2. Left: Localized accumulation of abscission chemical in droplets at the blossom end of a satsuma fruit (*C. reticulata* Blanco). Upper right: Cycloheximide injury on the blossom end of Valencia orange fruit. Lower right: Ascorbic acid injury on the fruit of Pineapple orange

Table I. Development of Rind Injury on Mature Valencia Oranges when Treated with Cycloheximide on Four Different Dates[a]

Date Treated and Concentration (mg/l.)	% Fruit Showing Rind Injury		
	After 3 days	After 6 days	After 14 days
3/15 Control	0	0	0
5	0	60	70
10	50	80	95
20	95	100	100
4/15 Control	0	0	0
5	0	100	100
10	0	100	100
20	0	100	100
5/15 Control	0	0	0
5	0	45	25
10	0	70	75
20	0	90	100
7/15 Control	0	0	0
5	0	40	30
10	40	90	100
20	65	100	100

[a] Fruit fully orange on 3/15 and 4/15; considerable regreening of fruit on 5/15. Increased susceptibility to injury on 7/15 associated with over-ripe condition. Three trees used per treatment and 10 random fruit examined from each tree on each date. Fruit showing single pit classified as rind pitting.

Table II. Effect of Physical Rind Injury and the Combination of Physical Rind Injury and Cycloheximide Treatment on the Production of Ethylene by Valencia Oranges[a]

Kind of physical injury	Portion of fruit treated with 20 mg/l. CHI[b]	C_2H_4 under rind (ppm)	
		at stem end	at blossom end
Uninjured control	None	0.020	0.020
	Blossom end—CHI	0.160	0.250
	Whole fruit—CHI	0.180	0.200
Emery paper at blossom end	None	0.650	1.115
	Whole fruit—CHI	1.935	1.335
Detached peel	None	18.000	22.000

[a] Three samples of two fruits each used for each treatment. Fruit given respective physical and chemical injury treatments on 8/5/70 and held in the laboratory free of other fruit and sources of ethylene for 5 days, at which time the ethylene content of the air under the rind was measured. [b] CHI indicates cycloheximide.

production by the fruit. Peeling the rind from oranges stimulated the production of tremendous quantities of ethylene by the rind. We obtained similar results in experiment with Redblush grapefruit (*C. paradisi* Macf.). Tearing a piece of rind from the stem end of the fruit, as often occurs when fruit is harvested without abscission chemicals, caused a 35-fold increase in ethylene production of the fruit as compared with controls.

The observations show that wounded (either by chemical of physical means) cells of the citrus rind generate a wound ethylene. Possibly the first detailed study of the products of damaged cells was that of Haberlandt (1913). He found that diffusible substances, set free from damaged cells, induced cell division in neighboring cells leading to healing of the wound. For effective periderm formation to occur, two sources of diffusible substances were required: one from phloem tissue and the other from damaged cells. He called the latter a wound hormone. Current notions suggest that cytokinins are the products of the damaged cells (Kefford and Goldacre, 1961). It now appears, however, that damaged cells produce wound ethylene in addition to cytokinin. We have found that wound ethylene is generated not only from citrus rind tissue but also from citrus stem tissue when the bark is peeled from the wood. In this instance the injury occurs in the cambium region separating the bark from the wood. Macerating the isolated bark or wood further enhances the generation of wound ethylene. The generation of ethylene has been reported in plugs of apple tissue (Burg and Thimann, 1959), in black rot-infected sweet potatoes (Stahmann *et al.*, 1966), in cut tissue discs of sweet potatoes (Imaseki *et al.*, 1968), and with a host of diseased tissues too numerous to mention. Goeschl *et al.* (1966) has also shown a relationship of ethylene production by tissue under stress where wounding is not involved. Obviously most of what has been accomplished with wound ethylene is descriptive. There now arises a need to investigate the fundamental aspects of production of wound ethylene. In this paper we refer to endogenous ethylene as that produced normally by fruit; and wound ethylene as the additional ethylene produced by damaged cells.

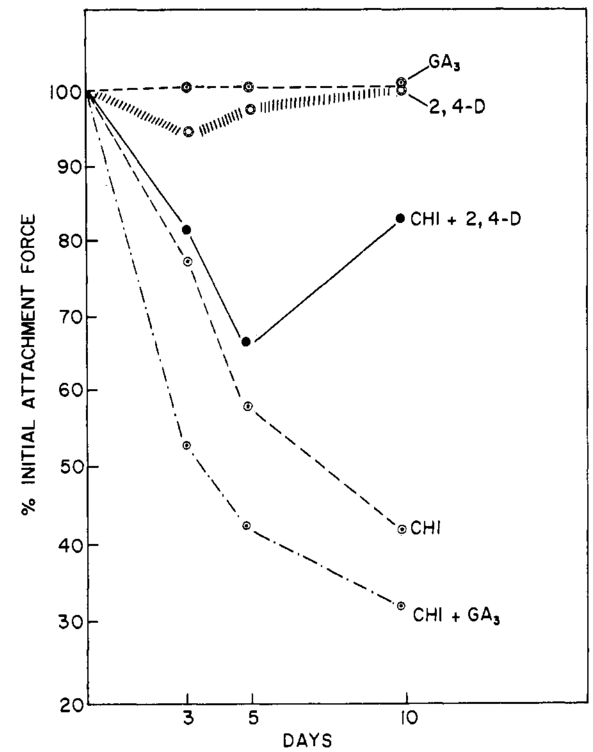

Figure 3. Attachment force of mature Valencia oranges for 10 days after application to trees on 7/7/69 of 25 mg/l. cycloheximide, 25 mg/l. gibberellic acid, 25 mg/l. 2,4-D, 25 mg/l. cycloheximide + 25 mg/l. gibberellic acid, 25 mg/l. cycloheximide + 25 mg/l. 2,4-D. Initial attachment force was 7.3 kg

Table III. Effect of Various Growth Regulators, Sprayed on Valencia Orange Trees, on Ethylene Production, and Fruit Abscission Activity[a]

Growth Regulator Applied 8/5/70 (mg/l.)	C_2H_4 under Rind after 5 Days ppm	Attachment force after 7 Days (%)	Rind Injury after 7 Days (%)
Control	0.025	100	0
10 CHI[b]	2.660	30	75
5 2,4-D	0.025	100	0
20 2,4-D	0.040	100	0
5 GA_3[b]	0.125	100	0
20 GA_3	0.015	90	0
5 Kn[b]	0.025	100	0
20 Kn	0.110	85	0
20 ABA[b]	0.005	75	0
100 ABA	0.025	90	0
100 DSA	0.005	100	0
1000 DSA	0.005	100	0
5000 DSA	0.025	50	0
100 CCC	0.010	100	0
1000 CCC	0.008	100	0
5000 CCC	0.005	100	0

[a] Each treatment applied to three separate branches of Valencia trees in July. Two fruits collected from each branch for ethylene measurements after 5 days. Attachment force measurements and rind injury observations made on 10 fruit from each branch after 7 days. The initial attachment force of the fruit was 7.3 kg. An acceptable range for attachment force is 25 to 50%. [b] CHI indicates cycloheximide, GA_3 is gibberellic acid, Kn is kinetin, and ABA is abscisic acid.

Various Chemical Agents. The growth regulators abscisic acid, gibberellic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin, and 2-chloroethyltrimethylammonium chloride (CCC) did not, when applied alone, show any striking promotion of fruit abscission when sprayed on trees (Table III). Only the growth retardant, *N*-dimethylaminosuccinamic acid (DSA), showed any abscission activity, and this occurred only at a concentration of 5 g/l. on Valencia oranges in early August. The treated fruit showed little to no increase in ethylene evolution and no rind injury. CCC included in the same test showed no abscission activity.

In an experiment conducted in late September 1967 on Valencia oranges 18 months after the March 1966 bloom, 200 mg/l. of abscisic acid induced a large reduction in attachment force. However, no abscission activity was obtained with abscisic acid applications to trees during the normal harvesting season for Florida citrus, November through June. Even in early August 1970, abscisic acid applications to Valencia orange trees induced only slight abscission activity.

When gibberellic acid was added to cycloheximide and applied to mature Valencia oranges in July, greater abscission activity resulted than when cycloheximide was used alone (Figure 3). Similar results (not shown) were obtained for abscisic acid additions to cycloheximide.

The data for 2,4-D, shown in Figure 3, confirm the generally held view that the dominant role of 2,4-D in abscission is to retard it. When 2,4-D + cycloheximide is applied to the rind, ethylene production is enhanced over that produced by cycloheximide alone, and this ethylene apparently moves more

Table IV. Effect of Growth Regulators, Absorbed through Pedicles of Fruit Explants, on Ethylene Production, and Abscission Activity

Growth Regulator Applied 8/15/70 (mg/l.)	C_2H_4 under the Rind at AZ after 4 Days ppm	Attachment force after 4 Days[a] (%)	Rind Injury after 4 Days (%)
Initial control	0.065	100	0
4-day water control	0.070	71	0
10 CHI[b]	1.940	109	30
5 2,4-D	0.115	100	0
20 2,4-D	0.750	140	0
20 2,4-D + CHI	2.520	112	30
20 ABA[b]	0.080	40	0
100 ABA	0.215	10	0
20 ABA + 20 2,4-D	0.173	100	0
20 ABA + 10 CHI	2.815	88	0
5000 DSA	1.115	53	0
5000 DSA + 20 2,4-D	0.085	81	0
5000 DSA + 10 CHI	2.270	100	0
5 GA_3[b]	0.140	84	0
20 GA_3	0.170	84	0
5 Kn[b]	0.224	84	0
20 Kn	0.024	92	0

[a] The initial attachment force for the controls was 7.3 kg. An acceptable range for attachment force is 25 to 50%. [b] CHI indicates cycloheximide, GA_3 is gibberellic acid, Kn is kinetin, and ABA is abscisic acid.

rapidly through the fruit to the abscission zone than does the 2,4-D. At least the initial effect of 2,4-D + cycloheximide applied to the rind is a weakening of the attachment force of the fruit, but the fruit soon regains its strong attachment force (Figure 3).

Differences in Citrus Varieties. The sensitiveness to abscission chemicals of the three principal sweet orange [*C. sinensis* (L.) Osb.] varieties in Florida (Hamlin, Pineapple and Valencia) varies from year to year. Generally, the Pineapple variety is the most sensitive, and 5 mg/l. of cycloheximide or 10 g/l. of erythorbic acid will effectively loosen mature fruit of this variety. During the 1970 season, 10 mg/l. of cycloheximide or 10 g/l. of erythorbic acid + 1 g/l. ferric ammonium citrate were required. Because of the phytotoxic effects of the abscission chemicals on the bloom and immature fruitlets of the Valencia orange, these chemicals should not be used on Valencia oranges until early May. During the 1969 and 1970 seasons, 20 mg/l. cycloheximide effectively loosened Valencia oranges in May, whereas 20 g/l. erythorbic acid was effective in 1969, but not in 1970.

During 1969, we investigated the effect of cycloheximide on the Robinson, Lee, Osceola, and Nova tangerine hybrids [*C. reticulata* × (*C. reticulata* × *C. paradisi*)]. The initial attachment force of the varieties varied from 9 kg for Nova to 4 kg for Osceola. Cycloheximide induced rind injury and caused a reduction in attachment force for all varieties. All varieties, when treated, remained attached to the tree, except Lee. The Lee fruit fell to the ground. All treated fruit, except Nova, showed a decrease in tearing of the rind on harvesting; the treated Nova fruit in contrast showed 100% tearing of the rind, whereas untreated fruit showed none.

Reversibility of the Abscission Process. There is a tendency after any treatment of the rind, including cycloheximide and erythorbic acid, for the fruit to reverse the abscission process during the third and fourth weeks, if the attachment force had not been weakened below a threshold level of about 2 kg. Thus, while some fruit abscise, other fruit retighten (Fig. 1). The data in Figure 3 show that the fruit will retighten faster if 2,4-D is applied. In other experiments where gibberellic acid was added to cycloheximide, we observed both an enhanced lowering of the attachment force during the first week and an enhanced retightening during the third and fourth weeks. Presumably, the synthesis of cell wall degradation enzymes is blocked by a return to the normal supply of auxins and gibberellins and possibly cytokinins. Thus, we believe that during the course of normal orange harvest from November through July, the fruit separation layer has not aged in the sense used by Abeles (1968) and Osborne (1968); and the abscission process is readily halted by 2,4-D, even after the abscission process has been initiated by abnormal levels of wound ethylene.

Experiments with Abscised Fruit Explants. When DSA was applied directly to the abscission zone through pedicle (woody stem attached to the fruit) absorption by fruit explants, the treatment caused the same abscission response as when the material was applied to the rind. However, in this instance an enhancement in ethylene production occurred (Table IV).

When abscisic acid was applied to the abscission zone by pedicle absorption by fruit explants, it induced a slight stimulation in ethylene production and a large reduction in attachment force of the fruit. These results confirm the findings of Craker and Abeles (1969) that abscisic acid has more abscission activity than is accounted for by ethylene production alone.

When gibberellic acid and kinetin are applied directly to the abscission zone, it induces a slight increase in ethylene production, but actually tightens the fruit to the pedicle as compared with the water control.

2,4-D, applied directly to the abscission zone, prevented abscission in the presence of abscisic acid, DSA, cycloheximide, and ethylene.

When cycloheximide is placed directly on the target (the abscission zone), it inhibits abscission in the presence of ethylene (Table IV). Cycloheximide caused injury of the cells of the abscission zone, and produced wound ethylene just as it does when applied to the rind.

DISCUSSION

The data show that there is a wound ethylene-producing system in the citrus peel which is not activated under normal ripening conditions. However, following chemical and physical injury to the rind, wound ethylene is generated by the fruit. The ability of cycloheximide, when applied to the outer surface of the rind, to accelerate citrus fruit abscission appears to be in chemical injury to the rind. This results in the production of wound ethylene which moves readily to the abscission zone, where it triggers the essential enzymatic abscission process. If, however, cycloheximide is placed directly on the abscission zone (through stem absorption), it inhibits the enzymatic abscission process even in the presence of wound ethylene. We believe that when cycloheximide is sprayed on the fruit, only a minute amount of the active inhibitor penetrates into the interior of the fruit and not enough of it moves to the abscission zone to inhibit abscission.

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