Plant Physiol. (1970) 45, 604–607

Effects of Iron and Copper Ions in Promotion of Selective Abscission and Ethylene Production by Citrus Fruit and the Inactivation of Indoleacetic Acid[1]

Received for publication July 8, 1969

S. Ben-Yehoshua[2] and R. H. Biggs
Department of Fruit Crops, University of Florida, Gainesville, Florida 32601

ABSTRACT

Application of Cu^{2+} ($<10^{-2}$ M) and Fe^{3+} ions as aqueous solutions of chloride salts promoted fruit abscission, erratic rind damage, and ethylene production of various citrus species with little to no defoliation. Mixing of 10^{-5} M Cu^{2+} or Fe^{3+} ions with equimolar indole-3-acetic acid resulted in a reduction of the ultraviolet absorption at 220 nanometers, and an increase at 245 nanometers. Ultraviolet irradiation accelerated the change by Fe^{3+} and Cu^{2+} ions in the absorption of indole-3-acetic acid. Pretreatment of indole-3-acetic acid with Fe^{3+} and Cu^{2+} ions for 6 hours resulted in more than 90% reduction in its growth-promoting activity in the Avena bioassay, even when cations were removed by chromatography. Acceleration of abscission by Fe^{3+} and Cu^{2+} ions could be related to both promotion of ethylene production and direct inactivation of auxin.

Various chemicals are capable of accelerating abscission of fruit and leaves (1, 2, 7, 16). Addicott (2) cited various reports showing that excessive application of Fe and Zn, as well as their deficiencies, causes defoliation. Morgan *et al.* (12) reported that $MnSO_4$ accelerated defoliation of cotton. Wilson and Hendershott (16) found that immersing the stem of detached orange fruit in $FeSO_4$, $FeCl_3$, or Cu_2SO_4 accelerated the abscission process. However, later, Wilson and Coppock (15) reported that ferric ammonium citrate was ineffective as an additive to ascorbic acid in accelerating abscission of fruit in the grove. Cooper *et al.* (7) demonstrated that addition of CuEDTA or FeEDTA to ascorbic acid enhanced the abscission-inducing effect of ascorbic acid with citrus fruit. They further reported that CuEDTA by itself, but not FeEDTA, accelerated fruit abscission but also caused marked defoliation. This work reports that inorganic salts of copper and iron accelerated abscission of orange fruit at certain concentrations with little to no defoliation. It was suggested that this acceleration was related both to enhancement of ethylene production and to a probable direct inactivation of auxin.

MATERIALS AND METHODS

Abscission studies were conducted with fruit explants composed of fruit with an attached stem piece 7 cm long and branch explants 25 cm long with leaves as described by Ben-Yehoshua and Eaks (3). Explants were from Hamlin, Valencia, Pineapple, and Washington navel oranges (*Citrus sinensis*, Osbeck). Fruit was clipped from the tree, washed in running water, and placed inside containers. Also, several small branches bearing a total of 100 leaves were placed in 10-liter containers with the cut end of the stems inmersed in water. Water was replaced daily, and stems were recut twice a week. All treatments on explants were applied by dipping fruit or leaves for 30 sec in the test solutions and then allowing them to drain for about 1 min before placing them in containers. Some of the containers were continuously ventilated with water-saturated air at a flow of 5 to 30 liters/hr. Others used for the study of rates of abscission were ventilated at a rate that would allow less than 0.01 μl/liter of ethylene in the effluent air, to prevent it from affecting the rate of abscission. The inflowing air was freed of ethylene and other impurities by brominated charcoal filters. For ethylene measurements, rate of flow through the containers was adjusted to allow between 0.02 and 0.1 μl/liter of ethylene in air. If ethylene evolution was below the level of detection in a continuously ventilated system, the containers were sealed until a sufficient quantity accumulated for analysis. The nonventilated containers were provided with a small beaker containing 50% KOH solution to absorb the CO_2 produced by the plant material.

In addition to the treatments with explants, various solutions were sprayed on separate branches or on whole trees in the grove. The fruit and branch explants were removed from the tree just before the breaking-force test or the analysis of ethylene production.

A surfactant, Multifilm (a product of Colloidal Products, Inc. California), was added to the iron solution for the over-all tree spray at a concentration of 0.05% (v/v). At the concentration used, this surfactant had no effect on abscission, nor did it enhance the iron effects.

Abscission of fruit and leaves was evaluated, with an Instron model TM stress and strain analyzer for measuring force, by applying a straight pull at a constant rate of movement of 2.5 cm/min. Bonding force of fruit to stem was measured by placing the fruit in a stainless steel cup with the stem projecting through a slit and held by an iron-bar clamp. In order to prevent crushing the petiole, the clamps were equipped with a rubber tape cover. The breaking force needed to separate leaf from its petiole was affected greatly by the size and age of leaf; accordingly, leaves of similar age and of equal size were used. Reduction in the bonding force of leaves or fruit at the abscission zone was considered to be an indication of the induction and progression of the processes leading to abscission.

Abscission of leaves from branch explants was counted daily, after gently passing the limb through the hands to remove loosened leaves. In addition, the influence of treatments under field conditions upon preferential abscission of leaves and fruit

[1] Florida Agricultural Experiment Station Journal Series 3332.

[2] Present address: Volcani Institute of Agricultural Research, Rehovot, Israel.

was checked by gently shaking a branch bearing both fruit and leaves.

Ethylene measurement was performed with an Aerograph 600D gas chromatograph equipped with a hydrogen flame ionization detector and a 2-ft × ⅛-inch activated alumina column (5). This system could detect 0.005 μl/liter in a 1.0-ml gas sample.

Auxin activity was assayed biologically with the sensitized *Avena* coleoptile test (14). Changes in the absorption spectra of IAA were followed with a Beckman model DU spectrophotomomter.

For the interaction studies between Fe^{3+} and Cu^{2+} and ultraviolet light on destruction of IAA, the ultraviolet source was a small mercury lamp (110 v, 9 w) equipped with a filter transmitting at 2537 A. The radiation output by the lamp was approximately 50 μw/cm² at the surface level of a continuously stirred solution.

RESULTS

Acceleration of Abscission and Ethylene Production. Application of copper and iron chloride salts markedly accelerated abscission and ethylene evolution (Tables I and II and Fig. 1). These observations were made by dipping explants of branches or fruit (Table I, Fig. 1), or by spraying branches or whole trees in the orchard (Tables II and III). Fe^{3+} and Cu^{2+} affected leaf and fruit abscission differently. Copper ions were the most effective accelerant of ethylene production and abscission of both leaves and fruit (Tables I and II and Fig. 1). The effect was concentration-dependent, and 10^{-4} M $CuCl_2$ was still slightly effective on fruit explants (Fig. 1). $CuCl_2$ at 0.1 M caused complete defoliation, and the treated branch subsequently died. However, at concentrations between 10^{-2} and 10^{-3} M, the force required to separate fruit from stem was reduced with little to no defoliation.

Table I. *Effect of 0.01 M Cu and Fe Chloride at pH 2.5 upon Abscission and Ethylene Production of Fruit Explants and Defoliation of Branch Explants of Valencia Orange*

All values were obtained 7 days after treatment, except ethylene production data which represent the means of 4 daily measurements during the 1st week after treatment.

Treatment	Ethylene Production	Defoliation[1]	Breaking Force	Plugging[2]	Area of Rind Injured
	μl/kg·hr	%	kg	%	%
Untreated	0.023 ± 0.001	3	5.6 ± 0.6	80	0
$CuCl_2$	1.000 ± 0.137	78	2.2 ± 0.6	0	20
$FeCl_3$	0.438 ± 0.168	3	3.7 ± 1.0	0	5

[1] Defoliation was determined after the limb was gently passed through the hand.

[2] Fruit that separated at points other than the abscission zone.

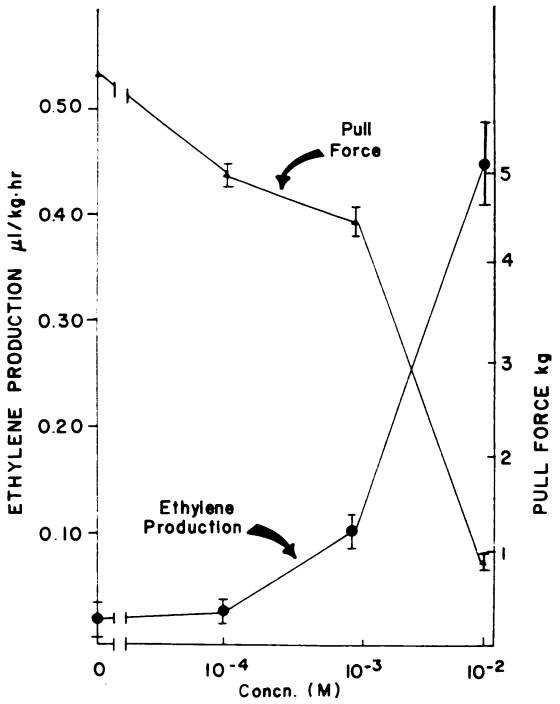

FIG. 1. Effect of a 30-sec dip in various concentrations of $CuCl_2$ upon ethylene evolution and bonding force of Valencia fruit explants kept at 20 C. Fruits were pulled 3 days after treatment.

All iron salts tested preferentially increased abscission of fruit more than leaves. Application of 0.01 to 0.1 M Fe^{3+} reduced the bonding force in the abscission zone and the percentage of fruit with the point of separation not at the abscission zone. The effective concentration depended on fruit maturity, temperature, and other factors. Abscission of fruit and leaves from explants occurred at lower concentrations than that of organs on intact plants (compare Tables I and II).

The physiological effects were ascribed to the Fe and Cu cations and not to the Cl anions since KCl at comparable concentrations did not induce abscission or lessen the bonding force and sulfate salts of Fe and Cu had about the same effect as the chloride salts.

The abscission responses of fruit and leaf to the Fe^{3+} treatment varied with time (Table III). During the first 6 weeks after treatment, the breaking force required to separate Pineapple fruit from its stem slightly declined as time elapsed. However, leaves were able to restrengthen their force of attachment. During the first week after treatment, the bonding force of leaves declined, and a gentle shake of the limb caused a small percentage of the leaves to abscise. However, no difference existed between treated and control leaves a month after treatment.

Iron and copper salts, but particularly copper, caused phytotoxic damage; however, this was limited in low concentrations only to young growth, if available, and to rind damage. Moreover, the enhancment of abscission and ethylene production did not seem to be necessarily related to visible injury, as both oc-

TABLE II. *Effect of Various Concentrations of Cu and Fe Chloride, Sprayed on Separate Branches, upon Bonding Force and Ethylene Evolution of Fruit and Defoliation of Hamlin Orange*

Salts solutions at pH 2.0 were applied to 10-year-old trees by spraying to run-off. Ethylene values represent the means of two daily measurements of fruit explants harvested 3 days after treatment. Defoliation was determined 7 days after treatment after a branch was hand-shaken gently.

Treatment	Concn	Ethylene Production	Defoliation	Breaking Force[1]	Plugging[2]	Area of Rind Injured
	M	μl/kg·hr	%	kg	%	%
Untreated		0.006 ± 0.001	0	9.1 ± 0.5	100	0
$FeCl_3$	0.1	0.583 ± 0.136	0–2	3.4 ± 0.2	0	0–10
$FeCl_3$	0.01	0.082 ± 0.045	0	9.8 ± 0.6	30	0
$CuCl_2$	0.1	3.082 ± 0.354	100[3]	0	0	10–50
$CuCl_2$	0.01	0.744 ± 0.225	2–10	5.6 ± 0.2	0	2–12
$CuCl_2$	0.001	0.025 ± 0.005	0	8.4 ± 0.4	80	0

[1] Fruit harvested and pulled 25 days after treatment.

[2] Fruit that separated at points other than the abscission zone.

[3] Treated branch died about 2 weeks after treatment.

Table III. *Effect of 0.03 M $FeCl_3$ Applied as an Over-all Tree Spray on Bonding Force of Fruit and Leaves and Defoliation of Pineapple orange*

Treatment	Breaking Force				Defoliation[1]	
	Fruit		Leaves			
	7 days	47 days	7 days	47 days	7 days	47 days
	kg				%	%
Untreated	9.5 ± 0.5	8.0 ± 0.5	2.9 ± 0.1	2.4 ± 0.1	0	0.25
0.03 M $FeCl_3$ + 0.05% Multifilm[2]	5.1 ± 0.4	4.2 ± 0.4	1.6 ± 0.1	2.3 ± 0.1	2.5	0.25

[1] Defoliation was counted after applying a gentle shake to a branch. This shake resulted in 50 to 100% abscission of fruit of the treated branch and 0 to 10% of untreated branches on both dates.

[2] Young growth and flower buds of treated plant had light to severe injury.

Table IV. *Effects of Cupric and Ferric Ions in Presence and Absence of Ultraviolet Light on Absorbance of IAA at 220 and 245 nm*

Concentrations of chemicals were equimolar at 3×10^{-5} M, and reactions were conducted at 35 C, pH 2.5, and in darkness or ultraviolet light.

Treatment	Incubation	Absorbance	
		220 nm	245 nm
	hr		
IAA	6	0.960	0.051
IAA + ultraviolet	6	0.745	0.268
IAA + Fe^{3+}	0	0.970	0.050
IAA + Fe^{3+}	6	0.952	0.137
IAA + Fe^{3+} + ultraviolet	0	0.979	0.041
IAA + Fe^{3+} + ultraviolet	6	0.441	0.333
IAA + Cu^{2+}	0	1.050	0.047
IAA + Cu^{2+}	6	0.975	0.168
IAA + Cu^{2+} + ultraviolet	0	1.030	0.049
IAA + Cu^{2+} + ultraviolet	6	0.688	0.392

Table V. *Influence of Cupric and Ferric Ions on the Response of Avena Coleoptile Sections to IAA*

Concentrations of the chemicals were equimolar at 10^{-5} M. Solutions were subjected to incubation for 90 min followed by cation exchange with a resin of Dowex 50-8X in the H^+ form.

Treatment	Ion Exchange	Length of Section
		mm
Control − IAA	−	6.1 ± 0.1
Control − IAA	+	6.2 ± 0.1
Control + IAA	−	9.8 ± 0.2
Control + IAA	+	10.1 ± 0.3
IAA + Fe^{3+}	−	7.2 ± 0.2
IAA + Fe^{3+}	+	7.6 ± 0.2
IAA + Cu^{2+}	−	5.6 ± 0.1
IAA + Cu^{2+}	+	6.8 ± 0.2

curred at times without any visible injury. The enhanced ethylene production evoked by the Cu^{2+} and Fe^{3+} treatments was accompanied by a faster rate of degreening of early season Hamlin and Washington navel.

Alteration by Fe^{3+} and Cu^{2+} of the Absorption Spectra and Growth-stimulating Properties of IAA. Auxin plays an important role in regulating the abscission process (1, 2), and so the effects of Fe and Cu were studied on IAA. Studies were conducted in solution without plant tissue in order to rid the system of the effect of these metals in enhancing ethylene production by the plant tissue, as ethylene itself is known to reduce auxin content (5).

Incubation of IAA with equimolar concentration of either Fe^{3+} or Cu^{2+} for 6 hr in darkness caused a pronounced change in the spectrophotometric absorption properties of IAA (Table IV). These changes were accelerated by ultraviolet light. Changes in the absorbance at 220 and 245 nm are presented in Table IV since these points on the absorption spectra were altered to the greatest extent.

Pretreatment of IAA with the equimolar concentrations of Fe^{3+} or Cu^{2+} markedly reduced the growth-promoting activity of the auxin on *Avena* coleoptile sections (Table V). Removing the exchangeable cations from the solution by ion exchange failed to restore the activity of IAA.

DISCUSSION

Acceleration of abscission by Cu and Fe chloride salts could be related to the enchancement of ethylene evolution, to the direct inactivation of IAA, or both. The change in the absorption spectra of IAA as a result of treatment by Fe and Cu ions coupled with a loss of 90% of the growth-promoting activity of IAA on *Avena* coleoptile sections demonstrated that these cations can cause direct inactivation of IAA. The effects of these cations on the auxin levels *in vivo* is under investigation, but it might be difficult to separate the direct effect of the metals on auxin from indirect effects of enhancing ethylene production by the treated tissues which by itself is known to reduce auxin content (5). This difficulty did not exist in the direct chemical analysis or the *Avena* bioassay as elongation of *Avena* coleoptile does not seem to respond to low levels of ethylene (5).

During these studies, Cooper *et al.* (7) reported that CuEDTA defoliated various citrus species. Our data confirmed their observation but extended it to show that lower concentrations of $CuCl_2$ accelerated fruit abscission with only little defoliation. However, our findings contradicted their conclusion that Fe salts by themselves did not induce abscission or ethylene production by calamondin or orange plants. Various iron salts used in this study increased ethylene production and lessened the bonding force of fruit to stems of various citrus species including calamondin. The discrepancy might be related to the iron salt used, to the age of the fruit (immature green fruit is much more resistant to iron effect), and to techniques of application. In our experience too, Valencia fruit, which is known to resist loosening treatments (7, 15), responded erratically to iron spray. Only in one experiment out of three, fruit were loosened adequately by 0.1 M $FeCl_3$.

The comparative effect of Cu and Fe ions upon abscission of

leaves and fruit cannot be conclusively explained. It appears that these metals weakened the bonding force of both organs, but the leaf was better able to restrengthen the force of attachment. Thus, a gentle shake of a branch at the proper time resulted in a selective abscission of fruit without defoliation. Another possible explanation for the preferential abscission of fruit is that the bulky fruit serves as a reservoir for accumulating ethylene whereas the leaf with its inherent better gas exhange cannot accumulate or maintain the endogenous ethylene level needed to induce abscission (3). The effect of Fe in reducing the bonding force was more consistent on different experiments with fruit than with leaves.

Our studies suggest that iron spray and possibly also copper at low concentration could serve as a new method of loosening fruit attachment to the tree in order to facilitate both hand and mechanical picking. Additional advantages found in these sprays are the acceleration of coloration of green fruit and provision of essential nutrient to the plant (13). However, as these ions might exert phytotoxic damage, such as: possible toxicity due to excess of Fe or Cu, defoliation injury to rind and particularly to the young growth, more data should be accumulated before proper evaluation of this new method can be done.

Basic cellular reactions leading to stimulated ethylene production by the Cu and Fe ions are unknown. Metals are known to catalyze various reactions by virtue of the formation of a metal-substrate complex (13). Lieberman *et al.* (10) showed that Cu^{2+}, H_2O_2, and ascorbic acid could mediate ethylene production from methionine in a model reaction. Since enzymes related to ethylene production are supposed to contain Fe or Cu (11, 17) or both, these metal effects may relate to becoming a portion of a metalloprotein catalyst. However, it appears more probable that the enhanced ethylene production is due to the marked stress caused by the large exogenous supply of Fe and Cu, bringing abnormal increases in the levels of these ions in the tissue.

The alteration of the absorbance of IAA solution by the Cu and Fe ions in light was much greater than that exerted by the metals in darkness or by the ultraviolet light itself (Table IV). Effects of ultraviolet light on molecular structure of IAA were shown before by Brauner (4). As metal ions are capable of catalyzing photooxidations (8), it is suggested that the Cu and Fe may accelerate IAA destruction by such a reaction, but in addition these cations are able to react with IAA even in darkness. The nature of the reaction was not studied in detail, but the change in the absorption spectra cannot be totally ascribed to one of chelation, although chelation might contribute to some of the change, as shown by Cohen *et al.* (6). Chelation would not be expected to cause the pronounced change in absorbance at 245 nm. Furthermore, the activity of IAA should have been restored by ion exchange since IAA is at best only a weak chelating agent (6). The change in spectrophotometric absorption at 245 nm is more in line with the changes noted by Hinman and Frost (9) on oxidation of IAA by peroxidases.

Acknowledgments—Special thanks are due to J. Peter and N. Vakis for their capable technical assistance, to Dr. R. C. Fluck for the use of the Instron, and to Clemens and Williams, Inc., for the use of their orchard. Acknowledgment is happily made to the University of California in Riverside in which the original observation on the effects of metals on selective abscission of citrus fruit was made while the senior author served as a visiting researcher.

LITERATURE CITED

1. Abeles, F. B. 1968. Role of RNA and protein synthesis in abscission. Plant Physiol. 43: 1577–1586.
2. Addicott, F. T. 1968. Environmental factors in the physiology of abscission. Plant Physiol. 43: 1471–1479.
3. Ben-Yehoshua, S. and I. L. Eaks. 1969. Mode of action of ascorbic acid in inducing selective abscission of citrus fruit *Citrus sinensis*, Osbeck. J. Amer. Soc. Hort. Sci. 94: 292–298.
4. Brauner, L. 1953. Untersuchungen über die Photolyse des Heteroauxin. Int. Z. Bot. 41: 291–341.
5. Burg, S. P. 1968. Ethylene, plant senescence and abscission. Plant Physiol. 43: 1503–1511.
6. Cohen, D., B.-Z. Ginzburg, and C. Heitner-Wirguin. 1958. Metal-chelating properties of plant-growth substances. Nature 181: 686–687.
7. Cooper, W. C., G. K. Rasmussen, B. J. Rogers, P. C. Reece, and W. H. Henry. 1968. Control of abscission in agricultural crops and its physiological basis. Plant Physiol. 43: 1560–1576.
8. Ellis, C. and A. A. Wells. 1941. The Chemical Action of Ultraviolet Rays. Reinhold Publishing Corporation, New York.
9. Hinman, R. L. and P. Frost. 1961. A model chemical system for the study of the oxidation of indole-3-acetic acid by peroxidase. *In:* Plant Growth Regulation International Conference, 4th, Yonkers, N.Y. pp. 205–219.
10. Lieberman, M., A. T. Kunishi, L. W. Mapson, and D. A. Wardale. 1965. Ethylene production from methionine. Biochem. J. 97: 442–449.
11. Mapson, L. W. and D. A. Wardale. 1968. Biosynthesis of ethylene. Enzymes involved in its formation from methional. Biochem. J. 107: 433–442.
12. Morgan, P. W., H. E. Joham, and J. V. Amin. 1966. Effect of manganese toxicity on the indole-acetic acid oxidase system of cotton. Plant Physiol. 41: 718–724.
13. Nason, A. and W. D. McElroy. 1963. Modes of action of the essential mineral elements. *In:* F. C. Steward, ed., Plant Physiology, Vol. III. Academic Press New York. pp. 451–536.
14. Nitsch, J. P. and C. Nitsch. 1956. Studies on the growth of coleoptile and first internode sections. A new, sensitive, straight-growth test for auxins. Plant Physiol. 31: 94–111.
15. Wilson, W. C. and G. E. Coppock. 1968. Chemical abscission studies of oranges and trials with mechanical harvesters. Proc. Fla. State Hort. Soc. 81: 39–43.
16. Wilson, W. C. and C. H. Hendershott. 1967. The effect of various chemical treatments on the rate of abscission of "Hamlin" orange explants. Proc. Amer. Soc. Hort. Sci. 90: 123–129.
17. Yang, S. F. 1967. Biosynthesis of ethylene. Ethylene formation from methional by horseradish peroxidase. Arch. Biochm. Biophys. 122: 481–487.