Cellulase Activity and Ethylene Concentration in Citrus Fruit Sprayed with Abscission-inducing Chemicals[1]

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Abstract. **Cellulase activity in separation zones of 'Orlando' tangelo and 'Valencia' orange fruits was associated with the ethylene concn in their internal atm. The pull force required to separate the fruit from its stem decreased when ethylene increased cellulase activity.**

Cell wall-degrading enzymes are associated with abscission of fruits and leaves of many plants. Cellulase activity increases during leaf abscission and is localized in the leaf separation zones of kidney beans (5,7), coleus (2), tobacco (17), citrus (12) and citrus fruit (8). The cellulase activity in these separation zones is stimulated by ethylene. However, Ridge and Osborne (13) found no cellulase stimulation by ethylene during lateral expansion of pea cells.

Morre (6) reported that pectinase increased during abscission of bean petiole explants and other enzymic changes during abscission have been found by others. Pectin methylesterase (5) was high when abscission was delayed by treatment with 2,4-dichlorophenoxyacetic acid, but when abscission was accelerated by ethylene, pectin methylesterase was reduced. Yager (17) reported similar results with tobacco and Rasmussen and Bukovac (11) reported wall changes suggesting similar enzyme changes in aging bean explants.

The hypothesis that ethylene regulates enzyme synthesis is supported by reports that ethylene stimulates RNA and protein synthesis (1,2) and increases the activity of L-phenylalanine ammonialyase in flavedo of grapefruit peel (14). Since protein synthesis, in general, is stimulated by ethylene, it is quite likely that several enzymes are associated with loosening of cell walls. At least, anatomical changes during development of separation zones in citrus (16), and other plants (15), suggest complex enzyme reactions. This report indicates an increase in cellulase activity in separation zones of citrus fruit sprayed with ethylene-producing chemicals.

Two-year-old 'Orlando' tangelo (*Citrus paradisi* Macf. X *C. reticulata* Blanco) trees that had 10 to 20 mature fruit each were sprayed with an H_2O solution of 20 ppm 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyl-ethyl] glutarimide (cycloheximide) containing 0.01% Triton X-100[3]. Trees sprayed with 0.01% Triton X-100 served as controls. One, 4, and 7 days after spraying, ethylene content and pull force was measured by previously described methods (4,9,10), and cellulase was determined by methods similar to those reported by Abeles (2).

Each sampling day, two 10-fruit samples were clipped from the control and 2 from treated trees. Two mm sections transverse to the axis of the fruit that include the separation zones, of each set of 10 fruit were chopped and homogenized in 10 ml of 0.05M potassium phosphate buffer (pH 7.0). The homogenate was filtered through miracloth. Then, 1 ml of the homogenate (enzyme solution) and 1 ml of 1.5% carboxymethyl cellulose (CMC) solution were incubated at 40°C in viscometer heads. Readings were taken at 10 and 30 min, and the results are presented as % change in viscosity (%$\Delta\eta$/hr), compared to blanks that contained 1 ml of CMC plus 1 ml of buffer without enzyme.

Ethylene and cellulase activity were also determined in 'Valencia' orange fruit from field trees 5 days after they had been sprayed with abscission-inducing chemicals on May 5, 1970. Cellulase was determined in these fruits by the same method as in the 'Orlando' tangelo fruits, except that the separation zones were frozen as soon as collected and stored until homogenized in buffer. Spray and sampling methods of field trees have been described (3).

Effect of ethylene on cellulase in separation zones of 'Orlando' tangelo fruit. The fruit from trees sprayed with 20 ppm cycloheximide always contained more ethylene than that from unsprayed trees. Cellulase activity increased in the separation zones of treated fruit as ethylene in the internal atm increased. Therefore, the greatest cellulase activity occurred in the sprayed fruit 4 days after spraying when the ethylene content was highest (Table 1). At the same time, the pull force of the sprayed fruit was 1.9 lb. compared to 7.7 lb. for the unsprayed fruit. After 7 days, even with these mature 'Orlando' tangelos, some "retightening" of the fruit to their stems occurred as the ethylene and cellulase activity decreased. Similar observations have been made, mainly with 'Valencia' oranges. The pull force is decreased by abscission chemicals in about 5 days; then in about 14 days it begins to increase, depending on a number of climatic factors (3,10).

Tests were carried out in a greenhouse. Therefore, the possible reason for increased ethylene in control fruits may have been due to ethylene contamination. Ethylene contamination observed in similar tests in growth chambers increased cellulase activity. Also cellulase increases much slower in separation zones as fruit mature in the field, than in these 'Orlando' tangelo fruit kept in the greenhouse.

Table 1. Ethylene in fruit, cellulase activity in stems, and pull force of 'Orlando' tangelos sprayed with 20-ppm cycloheximide.

Elapsed time (days)	CHI[x] (ppm)	C_2H_4 (ppb)	Cellulase (%$\Delta\eta$/hr[y])	Pull force (lb.)
1	0	18	24	11.5
	20	111	60	9.6
4	0	81	40	7.7
	20	336	84	1.9
7	0	95	32	7.6
	20	284	56	2.8

[x]CHI = cycloheximide.
[y]%$\Delta\eta$/hr is % decrease in viscosity (centipoises) per hr for slices 2 mm thick through 10 separation zones.
[z]Pull force equals lb. of force required to separate fruit from their stems in a straight pull.

Effect of ethylene on cellulase activity in separation zones of 'Valencia' oranges. On April 16, the separation zones of untreated fruit showed practically no cellulase activity and only 50 ppb of ethylene in their internal atm. The pull force was high (Table 2), even though the fruit were mature as measured by soluble solids, acid, and juice content.

Table 2. Cellulase activity of separation zones related to the ethylene in the internal atm of 'Valencia' oranges 4 days after being sprayed on April 16, 1970.

Treatment	Cellulase (%$\Delta\eta$/hr)	C_2H_4 (ppb)	Pull force (lb.)
H_2O	1.5	50	17
1% EA + FeAC[y]	12.0	440	10
20 ppm CHI[z]	15.0	480	10

[y]1% EA + FeAC equals 1% erythorbic acid plus 0.1% ferric ammonium citrate
[z]cycloheximide.

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Ethylene content and cellulase activity were increased by the abscission chemicals and pull force was decreased (Table 2). However, the changes were not nearly so great as they were on May 5, 1970, in fruit from another site (Table 3).

The unsprayed fruit from this site had about 3X as much cellulase activity as the 'Valencias' had on April 16, 1970. Ethylene was a little higher and the pull force a little lower. The 20 ppm cycloheximide spray increased ethylene and cellulase activity and decreased the pull force more on this date than 3 weeks earlier. Even 5 ppm cycloheximide was effective (Table 3).

Chlorobenzilate, oil and CuO were added to 5 ppm cycloheximide because of an observation made by us that these chemicals increased ethylene and lowered the pull force of 'Valencia' oranges when they were applied shortly after cycloheximide was sprayed for fruit abscission. Chlorobenzilate, plus 5 ppm cycloheximide, stimulated more ethylene production than 20 ppm cycloheximide alone, but cellulase activity and pull force were the same. Oil and CuO had little if any effect on cellulase activity when sprayed with 5 ppm cycyloheximide, even though the ethylene was higher than when 5 ppm cycloheximide was applied alone. Cu, in combination with ascorbic acid, increases ethylene production (9); and oil sprays (unpublished), in some instances, increase ethylene production by leaf tissue similar to increases noted here in fruit tissues. The increase in ethylene production above that produced by fruit treated with 20 ppm cycloheximide has no influence on abscission, since no appreciable difference in cellulase or pull force was observed from the other additives.

Table 3. Cellulase activity in separation zones and ethylene in the internal atm of 'Valencia' oranges 5 days after being sprayed on May 5, 1970.

Spray treatment	Cellulase (%$\Delta\eta$/hr)	C_2H_4 (ppb)	Pull force (lb.)
H_2O	4.4	70	15
CHI 20 ppm	27.0	520	8
CHI 5 ppm	24.0	175	10
CHI 5 ppm + 200 ppm oil	22.5	395	9
CHI 5 ppm + 50 ppm chlorobenzilate	27.0	770	8
CHI 5 ppm + 1000 ppm CuO	21.0	440	8
CHI 5 ppm + 3 additives[z]	31.5	720	7

[z]100-ppm oil, 50-ppm chlorobenzilate, 100-ppm CuO.

Since 10 separation zones were required for each cellulase determination, we were unable to examine the correlation between cellulase activity and ethylene content on a fruit-by-fruit basis. Although exact correlations were not found when average values were used, the data show that treatments that increased ethylene accumulation also increased cellulase activity in separation zones.

Literature Cited

1. Abeles, F. B. 1968. Abscission: Role RNA and protein synthesis. *Plant Physiol.* 43:1577-1586.
2. ———. 1969. Abscission: Role of cellulase. *Plant Physiol* 44:447-452.
3. Cooper, W. C., and W. H. Henry. 1968. Field trials with potential abscission chemicals as an aid to mechanical harvesting of citrus in Florida. *Proc. Fla. State Hort. Soc.* 81:62-68.
4. ———, G. K. Rasmussen, and D. J. Hutchison. 1969. Promotion of abscission of orange fruits by cycloheximide as related to site of treatment. *BioScience* 19:443-444.
5. Horton, R. F., and D. J. Osborne. 1967. Senescence abscission and cellulase activity in *Phaseolus vulgaris*. *Nature* 214:1086-1088.
6. Morre, D. J. 1968. Cell wall dissolution and enzyme secretion during bean leaf abscission. *Plant Physiol.* 43:1545-1559.
7. Osborne, D. J. 1968. Hormonal mechanisms regulating senescence and abscission. *In* Biochemistry and Physiology of Plant Growth Substances. p. 815-840. F. Wightman and G. Setterfield, ed. The Runge Press Ltd., Ottawa, Canada.
8. Pollard, J. E., and R. H. Biggs. 1970. Role of cellulase in abscission of citrus fruits. *Proc. Amer. Soc. Hort. Sci.* 95:667-673.
9. Rasmussen, G. K., and W. C. Cooper. 1968. Abscission of citrus fruits induced by ethylene-producing chemicals. *Proc Amer. Soc. Hort. Sci.* 93:191-198.
10. ——— and ———. 1969. Influence of temperature and humidity on cycloheximide-induced abscission and ethylene content of citrus. *Proc. Fla. State Hort. Soc.* 82:81-84.
11. Rasmussen, H. P., and M. Bukovac. 1969. A histochemical study of abscission layer formation in the bean. *Amer. J. Bot.* 56:69-76.
12. Ratner, A., R. Goren and S. P. Monselise. 1969. Activity of pectin esterase and cellulase in the abscission zone of citrus leaf explants. *Plant Physiol.* 44:1717-1723.
13. Ridge, Irene, and D. J. Osborne. 1969. Cell growth and cellulases: Regulation by ethylene and indole-3-acetic acid in shoots of *Pisum sativum*. *Nature* 223:318-319.
14. Riov, J., S. P. Monselise, and R. S. Kahan. 1969. Ethylene-controlled induction of phenylalanine ammonia-lyase in citrus fruit peel. *Plant Physiol.* 44:631-635.
15. Webster, B. D. 1968. Anatomical aspects of abscission. *Plant Physiol.* 43:1512-1544.
16. Wilson, W. C., and C. H. Hendershott. 1968. Anatomical and histochemical studies of abscission of oranges. *Proc. Amer. Soc. Hort. Sci.* 92:203-210.
17. Yager, R. E. 1960. Possible role of pectin enzymes in abscission. *Plant Physiol.* 35:157-162.

1,1,5,5-Tetramethyl-3-dimethylaminodithiobiuret, A Promising New Peach Thinner[1]

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Abstract. **Sprays of 1,1,5,5-tetramethyl-3-dimethylaminodithiobiuret (MATB), when applied to peach trees during cytokinesis, thinned fruits without causing excessive leaf chlorosis or defoliation during 3 years of testing. The concn that thinned fruits satisfactorily in the cultivars tested varied between 100 and 300 ppm. MATB at 100 ppm thinned 'Ranger' fruits without excessive damage to leaves while at 200 and 300 ppm it produced comparable results in normal and vigorous trees, respectively, of the 'Earlired' cultivar. 'Rio Oso Gem' was thinned satisfactorily by 300 ppm. MATB appears to be a promising peach fruit-thinner for such commercially important, hard-to-thin cultivars as 'Earlired', 'Rio Oso Gem', 'Redskin', and 'Redhaven'.**

Thinning peaches chemically is a major unsolved problem of the fruit industry. Several compounds have been tried extensively, but none has consistently thinned hard-to-thin peach cultivars. Concn that thin the fruit often cause excessive chlorosis, defoliation, or both. During routine greenhouse screening studies for control of *Xanthomonas pruni* (E. F. Smith) Dows, we discovered that MATB[3], when applied to 'Sunhigh' peach seedlings, caused injury similar to that caused by chemicals used for thinning in the orchard. These observations led us to test it as a peach fruit-thinner. Preliminary trials in 1968 of foliar sprays to bearing 'Sunhigh' and 'Earlired' peach trees at Beltsville, Maryland, were encouraging and led to subsequent tests in 1969 and 1970 (4).

We applied the sprays to individual limbs, half trees, and whole trees, with a

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