J. Amer. Soc. Hort. Sci. 102(5):580–583. 1977.

Abscission Response and Color Changes of 'Valencia' Oranges[1]

T. Adair Wheaton
University of Florida, Institute of Food and Agricultural Sciences, Agricultural Research and Education Center, P.O. Box 1088, Lake Alfred, FL 33850
W. C. Wilson
Florida Department of Citrus, Institute of Food and Agricultural Sciences, Agricultural Research and Education Center, P.O. Box 1088, Lake Alfred, FL 33850
R. E. Holm
Diamond Shamrock Corporation, Painesville, OH 44077

Additional index words. ethylene, 5-chloro-3-methyl-4-nitro-1H-pyrazole, regreening, *Citrus sinensis*

Abstract. **Mature Florida 'Valencia' oranges [*Citrus sinensis* (L.) Osbeck] go through a period of reduced response to abscission chemicals. This nonresponsive period is characterized by both a reduction in the amount of ethylene induced by abscission chemicals and by decreased sensitivity of the abscission process to exogenous ethylene. During the nonresponsive period, application of the abscission chemical 5-chloro-3-methyl-4-nitro-1H-pyrazole (Release) induced less ethylene formation and less reduction in fruit removal force than at times either prior to, or following, the nonresponsive period. Experiments with radioactive Release showed that uptake was not a factor in the reduced effectiveness of this material during the nonresponsive period. Abscission of explants induced by exogenous ethylene was slower during the nonresponsive period. Mature 'Valencias' also go through a period of regreening. Regreening and the nonresponsive period may begin at about the same time, but regreening continues after the nonresponsive period is over. In addition, color changes induced by exogenous ethylene remain similar throughout the regreening and nonresponsive periods, but abscission of explants induced by exogenous ethylene is reduced only during the nonresponsive period. These differences in time-course and ethylene sensitivity between regreening and the nonresponsive period allow differentiation of these two processes.**

Efforts to find suitable abscission chemicals for 'Valencia' oranges to facilitate either manual or mechanical harvesting in Florida have encountered various problems. At the beginning of the harvest season, new vegetative growth and small immature fruit of the following year's crop are developing on the tree and are very sensitive to most abscission chemicals (2). However, a selective abscission material, Release (trademark, Abbott Laboratories), has been found that is effective in inducing abscission in mature fruit without damaging new growth and immature fruit (13). All effective abscission agents to date appear to act either by releasing ethylene directly, or by causing ethylene production by injury of the tissue (6). Release when used at rates up to 250 ppm does not damage or induce ethylene production in leaves or young fruit but does induce ethylene production by injury to mature fruit (13).

Release promotes fruit loosening when applied to 'Valencia' trees early in the harvest season. Then there is a period of several weeks, generally in early May, when response to abscission chemicals is poor (1, 2, 5, 7). After this period, response is again good. This nonresponsive period has frequently been referred to as "retightening," with the implication that the fruit undergoes loosening during early maturity but then becomes more firmly attached. However, there has been no data to support retightening (1, 3, 7, 13) and we provide evidence in this paper that retightening does not occur. Therefore, we will refer to the period of poor response to abscission chemicals as the nonresponsive period.

'Valencia' oranges in Florida generally reach maturity and good external color in March and April but then go through a period of regreening due to an increase in chlorophyll in May and June during the peak of the harvest season (8). Fruit of a number of citrus cultivars as well as 'Valencia' fruit early in the harvest season may be degreened with ethylene. However, regreened 'Valencia' fruit do not respond to ethylene degreening treatments. It has been suggested that regreening and nonresponse problems may be closely related and result from changes in hormone balance associated with the spring period of flowering and vegetative growth (7, 9).

The work reported here attempts to answer 2 questions: 1) Is the nonresponsive period due to ineffectiveness of abscission chemicals in inducing ethylene production, or due to decreased sensitivity of abscission to ethylene? 2) Are regreening and nonresponse closely related, or are they unrelated events that happen to occur at about the same time? This paper presents information on fruit bonding force, ethylene production induced by Release, uptake and distribution of radioactive Release in relation to ethylene production, abscission response to exogenous ethylene, and color changes during the 1976 'Valencia' season.

Materials and Methods

Weekly samples of fruit were collected during the regreening and nonresponsive periods from a block of mature 'Valencia' oranges on Rough Lemon rootstock located at Lake Alfred. For the on-tree evaluations, 250 ppm Release was applied in 0.1% X-77 to branches containing 20 to 30 mature fruit with a pressurized hand sprayer using 500 ml per branch. Treatments consisted of 3 branches located on different trees. Fruit drop and fruit removal force (FRF) measurements were recorded on 10 fruit per branch 7 days following treatment (Fig. 1A). In some expt, Release was applied to small trees with a back-pack sprayer using 7.57 liters per tree with 2 trees per treatment. For internal ethylene determinations (Fig. 2A), samples of 20 fruit were collected daily. A gas sample was removed by syringe from the center of the fruit and ethylene determined by gas chromatography.

In addition, fruit samples from untreated trees were harvested at weekly intervals and randomly selected for laboratory evaluation of Release-induced ethylene production, sensitivity of abscission of explants to exogenous ethylene, and color responses to ethylene.

The production of ethylene induced by treatment with Release was determined on 2 lots of 6 detached fruit. These fruit were dipped in a 250 ppm solution of Release containing 0.1% X-77 as a wetting agent, air dried, and placed in a closed container in a humidified flowing air stream at 25°C. Ethylene production was monitored by sampling the exiting air stream at

[1]Received for publication March 26, 1977. Florida agricultural Experiment Stations Journal Series No. 451.

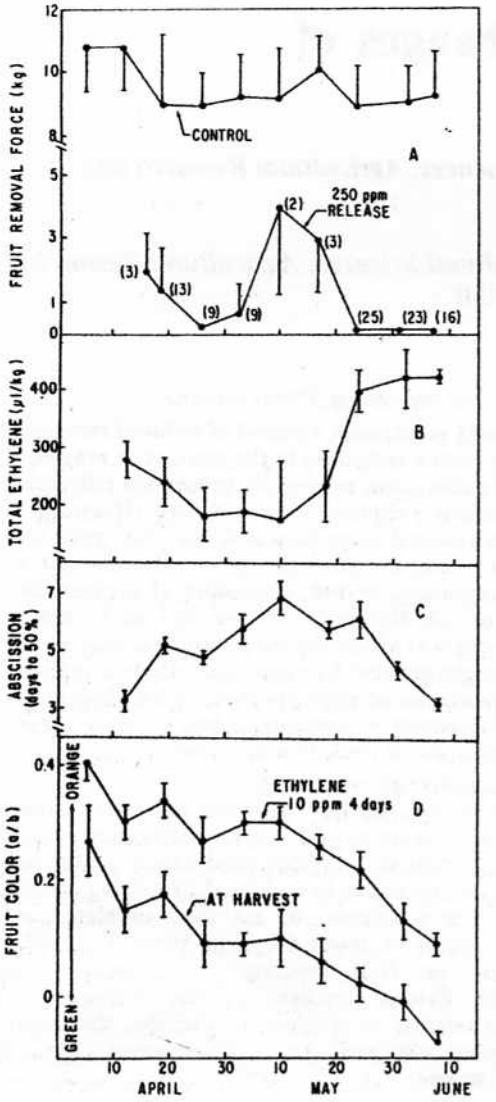

Fig. 1. Trends in FRF, ethylene production, abscission response, and color changes of mature fruit during the 'Valencia' harvest season. Mean and standard deviation are plotted for each date. A. FRF 7 days after treatment. Values in parentheses ( ) are number of fruit abscised per branch. B. Total ethylene production by harvested fruit during 4 days following treatment with 250 ppm Release. C. Explants treated with 1 ppm ethylene. D. Initial color, and color after 4 days at 10 ppm ethylene, of fruit harvested at weekly intervals.

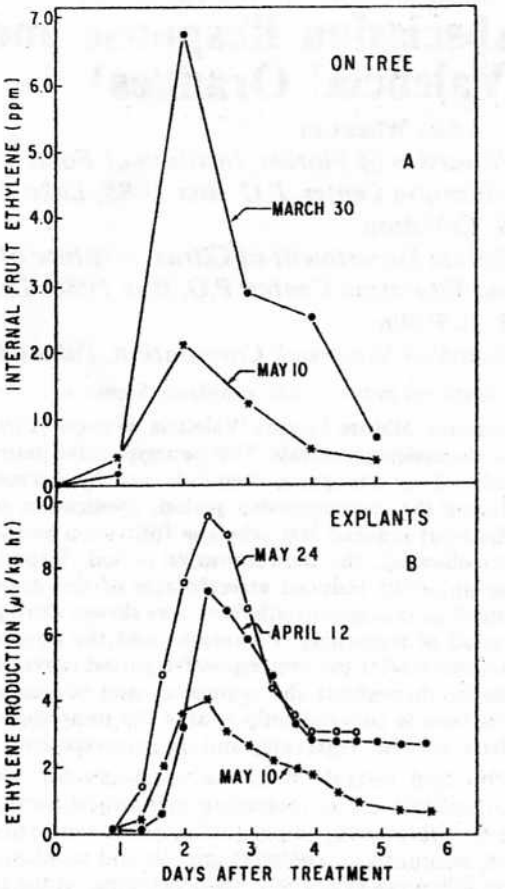

Fig. 2. Response of mature 'Valencia' oranges to application of 250 ppm Release during responsive (April 12, March 30, May 24) and nonresponsive (May 10) periods. A. Internal ethylene concentrations resulting from tree application. B. Ethylene production rates under controlled conditions following application of Release to harvested fruit.

8-hr intervals and measuring ethylene concn by gas chromatography. Flow rates were adjusted so that ethylene concn did not exceed 5 ppm at peak production. In addition to ethylene production rates (µl/kg-hr shown in Fig. 2B), total ethylene production over a 4-day period (µl/kg shown in Fig. 1B) was calculated by integrating production rates over the time period.

The uptake and distribution of ^{14}C-Release in relation to fruit ethylene production was determined using additional fruit from the same grove. Mature regreened fruit on the tree were treated on May 18, 1976 by wetting the fruit surface with 0.35 ml of a 250 ppm Release solution containing 227,958 dpm (1.92 µg) of ring-labelled ^{14}C-Release. Fruit were harvested 2 days after chemical application and sampled for internal ethylene. Fruit were grouped into classes of low, medium, and high internal ethylene levels before extraction and measurement of ^{14}C distribution (Table 1). The purpose of this grouping was to determine the relationship between Release uptake or distribution and the amount of ethylene formed. Immature green fruit (3.8–5 cm diam) were treated on June 15 with 0.2 ml of a 250 ppm Release solution containing 229,946 dpm (1.94 µg) of ^{14}C-Release and harvested after 3 days. Distribution of radioactivity in a surface water wash, acetone-water wash, and in the flavedo and albedo was determined. The flavedo and albedo were separated using an apple peeler. The tissue was ground and extracted in 80% acetone-0.01N HCl using a VirTis tissue homogenizer. This procedure removed 98% of the tissue radiotracer as verified by tissue digestion.

Sensitivity of the abscission response to exogenous ethylene was determined using mature fruit explants with 4 cm stem attached. These fruit (20 per sample) were placed in closed containers in a flowing stream of humidified air or humidified air containing 1 ppm ethylene and maintained at 25°C. Abscission was checked daily by determining if the fruit would abscise due to its own weight when lifted by the stem. The time required for 50% abscission was determined.

Additional samples of harvested mature fruit (8 fruit per treatment) were used to determine color initially and daily thereafter during exposure to 0, 1, or 10 ppm ethylene. Color was measured using a Hunterlab Color Difference Meter (12).

Results

Fruit removal force. The FRF of control mature fruit remained relatively constant throughout the season at about 10 kg (Fig. 1A). FRF decreased dramatically as a result of field application of Release at 250 ppm. FRF of treated fruit was extremely low initially, increased substantially for treatments applied early in May, and then decreased again for later appli-

Table 1. Uptake of ^{14}C-Release and ethylene production in mature regreened and immature 'Valencia' oranges.

Variable	Mature regreened fruit			Immature fruit
	Ethylene level			
	Low	Medium	High	
Group-ethylene	8	2	2	4
Fruit number	0.06 ± .03	0.41 ± .03	0.96 ± .14	0.009 ± .003
Internal fruit ethylene (ppm)				
	Distribution of ^{14}C-Release (% recovered radioactivity)			
Fruit surface				
A. Water wash	19.6 ± 1.4	20.3 ± 0.5	19.3 ± 0.8	1.8 ± 0.1
B. Acetone-water wash	13.9 ± 0.8	15.5 ± 0.5	14.4 ± 0.5	1.3 ± 0.1
Flavedo	63.4 ± 1.9	61.3 ± 0.5	63.1 ± 0.8	64.3 ± 2.4
Albedo	3.1 ± 0.2	2.9 ± 0.1	3.2 ± 0.3	31.7 ± 1.9
Total recovery as % applied dose	95.2 ± 1.9	95.0 ± 1.7	94.6 ± 2.3	93.1 ± 1.9

cations. Substantial fruit drop occurred during periods of low FRF. The increase in FRF for treatments applied in May is an indication of the time period during which the fruit is less responsive to abscission chemicals; i.e., the nonresponsive period.

Ethylene production. Applications of Release to fruit on the tree resulted in a peak of ethylene concn internally in the fruit 2 to 3 days following application (Fig. 2A). Internal ethylene values rose to a much higher value as a result of the March 30 application than an application on May 10.

Ethylene production of harvested fruit treated with Release and held under controlled conditions also peaked 2 to 3 days after application (Fig. 2B). The amount of ethylene production varied throughout the season, being higher before and after the nonresponsive period. The time-course of ethylene production remained similar for all harvest dates, but total ethylene production decreased during the nonresponsive period. Total production during 4 days following harvest and Release treatment was lowest in early May during the nonresponsive period and increased substantially following this period (Fig. 1B).

^{14}C-Release uptake. Three days after application of ^{14}C-Release to mature regreened 'Valencia' oranges, 95% of the radioactivity could be recovered from water and acetone washes of the surface and from the flavedo and albedo (Table 1). About 35% of the recovered radioactivity was in the washes, 62% in the flavedo, and 3% in the albedo. Prior to extraction, internal ethylene concn of the treated fruit was determined and the fruit grouped according to the ethylene levels. Although ethylene concn varied over a substantial range, no relationship was found between uptake or distribution of radioactivity from ^{14}C-Release and internal ethylene levels. In fact, distribution of radioactivity was very similar for all levels of internal ethylene.

Two days after application of ^{14}C-Release to immature green 'Valencia' oranges, 93% of the applied radioactivity could be recovered from washes, the flavedo and albedo. Only 2% of the recovered radioactivity was found in the washes, 64% in the flavedo, and 32% in the albedo. These results indicate more rapid uptake, and more rapid movement from the flavedo into the albedo for immature fruit.

Abscission response to exogenous ethylene. Stems attached to harvested fruit which were exposed continuously to 1 ppm ethylene abscised more rapidly than those maintained in air. Time required to reach 50% abscission was 3 to 5 days early in the season, increased to 7 days in early May, and then decreased to shorter times again (Fig. 1C). Time to 50% abscission in air (data not shown) ranged from 10 to 15 days with no pattern evident.

Color changes. Substantial regreening of fruit occurred early in April and this trend continued throughout the season. During this period, fruit changed from orange color (a/b = 0.25) to a condition where the shoulders of the fruit were definitely green (a/b <0). Color measurements were also made at the equator of the fruit. These values were generally about 0.15 a/b units higher (orange color) than on the shoulder. However, the pattern of regreening was parallel to that at the shoulder (data for equator not shown).

Exposure of fruit to 10 ppm ethylene at 25°C resulted in substantial degreening or color improvement. Color changes were rapid for the first 4 or 5 days and then leveled off. Exposure to 0 or 1 ppm ethylene did not induce color changes. Color values after 4 days at 10 ppm ethylene increased by 0.15 to 0.20 a/b units throughout the regreening period. Fruit remained responsive to ethylene throughout this time and the amount of degreening remained similar although regreening was continuing.

Discussion

The 'Valencia' oranges in this study went through a period of reduced response to an abscission chemical, Release (Fig. 1A). This nonresponsive period occurred in early May and lasted about 2 weeks. The bonding force of control fruit during this period did not change significantly. Thus, there was no indication of "retightening," or of any increase in FRF of nontreated fruit during this time. Retightening of control fruit was not observed in a previous study (7) or during 5 years of observations by Wilson (unpublished data). Therefore, we recommend that the terminology "retightening" be discontinued and that this period of reduced response to abscission chemicals be referred to as a *nonresponsive* period.

Changes in both ethylene production rates and responses to exogenous ethylene occurred during this nonresponsive period. Field applications of Release during the nonresponsive period resulted in much lower internal ethylene levels than an application made earlier (Fig. 2A). Measurement of Release-induced ethylene production of fruit harvested at weekly intervals throughout the season showed lower production rates (Fig. 2B) and lower total production during the nonresponsive period (Fig. 1B). It can be concluded then, that during the nonresponsive period changes occur in the fruit that reduce ethylene production induced by Release.

Experiments with application of radioactive Release to mature regreened fruit demonstrated rapid uptake of radioactivity into the flavedo and failed to show any relationship between uptake and levels of internal ethylene (Table 1). Uptake of radioactivity into immature fruit was even more rapid although Release applied at rates up to 250 ppm is ineffective in inducing ethylene formation or causing abscission of immature fruit. These experiments rule out differential uptake

of Release as a possible explanation of the reduced mature fruit loosening during the nonresponsive period and the lack of response of immature fruit. Other possible explanations for reduced ethylene production during the nonresponsive period include morphological or chemical changes limiting access of Release to the active injury site, inhibition of injury-induced ethylene biosynthesis, or altered metabolism of Release. Release may be rapidly detoxified by chemical alteration or, alternatively, a metabolite of Release rather than the parent compound may be effective in inducing ethylene formation. Future studies should include a comparison of Release metabolism in responsive and nonresponsive mature fruit to supplement the radioactive uptake data provided here.

Not only is injury-induced ethylene production reduced, but the sensitivity of the abscission process to exogenous ethylene is also lowered during the nonresponsive period. Time to abscission of stems from fruit harvested at weekly intervals and exposed to 1 ppm ethylene substantially increased during the nonresponsive period (Fig. 1C). Since various kinds of hormone pretreatments influence the sensitivity of the abscission zone to ethylene (4), probable explanations for the reduced sensitivity during nonresponse must include changes in endogenous hormones in the tissue during this period. Furthermore, the lack of response to field application of abscission chemicals must be interpreted both in terms of reduced ethylene production and reduced sensitivity of the abscission zone to ethylene.

Regreening was also observed in the fruit used in this experiment, but regreening appeared to be poorly related to nonresponse (Fig. 1D). Rapid regreening occurred before the period of nonresponse began. During the nonresponsive period, regreening temporarily leveled off, and increased again later in the season. This last period of substantial regreening occurred during a period when response to abscission chemicals in the field was excellent and during a period when highest rates of Release-induced ethylene production occurred and sensitivity to exogenous ethylene was high.

Treatment of fruit with 10 ppm ethylene substantially improved fruit color throughout the harvest season. Although severely regreened fruit did not revert to a desirable external color for marketing, the rate of color change due to ethylene treatment remained similar throughout the season. There was no indication of any reduction of color response to ethylene during the nonresponsive period, as opposed to the significant reduction in abscission response to applied ethylene during this period. On the basis of the poor relationship between the onset and development of regreening and the nonresponsive period, as well as the distinct changes that occur in sensitivity of abscission to ethylene but not of color to ethylene, we conclude that regreening and the nonresponsive period should be considered distinctly separate phenomena and suggest that the use of "regreening" to define the nonresponsive period be discontinued.

Various authors have suggested that the nonresponsive period may be due to changes in endogenous gibberellins resulting from the resumption of active growth in the spring (7, 9). However, the spring growth flush is completed by the time of the nonresponsive period with almost complete cessation of vegetative growth in early May (10). Early or middle May typically is the beginning of the period of "June drop" of small citrus fruit of the next year's crop (11). The rapid development of young fruit at this time and abscission associated with "June drop" correlate more nearly in time with the nonresponsive period than does the spring growth period. This is also the optimum period for applying auxin or ethylene releasing chemical thinning sprays to citrus cultivars in Florida (11). The circumstantial evidence relating the nonresponsive period to the "June drop" period along with the known effects of auxins on the sensitivity of abscission processes suggest that changes in endogenous auxin levels should be given as much credence as the previously suggested changes in gibberellin levels as a possible explanation of the nonresponsive period of 'Valencia' oranges.

Literature Cited

1. Clark, R. K. and W. C. Wilson. 1975. The effect of several abscission adjuvants on the abscission activity of Release® with 'Valencia' orange. *Proc. Fla. State Hort. Soc.* 88:100-103.
2. Cooper, W. C. and W. C. Wilson. 1971. Abscission chemicals in relation to the harvest of 'Valencia' oranges. *Proc. Fla. State Hort. Soc.* 84:70-76.
3. _____ and W. H. Henry. 1972. Effect of growth regulators on the response of citrus fruit to cycloheximide-induced abscission. *Proc. Fla. State Hort. Soc.* 85:29-32.
4. _____ and _____. 1973. Chemical control of fruit abscission, p. 475-524. *In* T. T. Kozlowski (ed.) Shedding of plant parts. Academic Press, N.Y.
5. Davies, F. S., W. C. Cooper, and F. E. Galena. 1975. A comparison of four abscission compounds for use on 'Hamlin', 'Pineapple', and 'Valencia' oranges. *Proc. Fla. State Hort. Soc.* 88:107-113.
6. _____, _____, and R. E. Holm. 1976. The effect of four abscission chemicals on orange fruit and leaf ethylene production. *J. Amer. Soc. Hort. Sci.* 101:651-653.
7. Holm, R. E. and W. C. Wilson. 1976. Loss in the capacity of 'Valencia' oranges treated with abscission chemicals to produce ethylene and fruit loosening during the regreening period. *Proc. Fla. State Hort. Soc.* (In press)
8. Jahn, O. L. and Roger Young. 1976. Changes in chlorophyll a, b, and a/b ratio during color development in citrus fruit. *J. Amer. Soc. Hort. Sci.* 101:416-418.
9. Rasmussen, G. K. 1973. The effect of growth regulators on degreening and regreening of citrus fruit. *Acta Hort.* 34:473-478.
10. Simanton, W. A. 1970. Seasonal variation in magnitude of foliage growth in Florida citrus groves. *Proc. Fla. State Hort. Soc.* 83:49-54.
11. Wheaton, T. A. and Ivan Stewart. 1973. Fruit thinning of tangerines with naphthaleneacetic acid. *Proc. Fla. State Hort. Soc.* 86:48-52.
12. _____ and _____. 1973. Optimum temperature and ethylene concentrations for postharvest development of carotenoid pigments in citrus. *J. Amer. Soc. Hort. Sci.* 98:337-340.
13. Wilson, W. C. 1973. A comparison of cycloheximide with a new abscission chemical. *Proc. Fla. State Hort. Soc.* 86:56-60.