Table 3. Water pollution generated during juice and molasses evaporation (based on processing 1 ton of fruit).

References	Juice evaporator		Molasses evaporator		
	Condensate	Barometric	'Clean' condensate	'Scrubber' condensate	Barometric
		Volume in lb/ton			
7[z]	788		278	278	
8	871.3		362.8	362.8	
14		8713			
		Load ppm			
8 COD	10,500		11,000	3,050	
BOD	3,930		8,334	1,224	
14 COD	500	60			
9 BOD (range of values)	35-3,930	20-150	495-1,220	614-1,224	150-200

[z]Refer to references in Literature Cited section.

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MODELING THE EFFECTIVENESS OF RELEASE®[1] AS A CITRUS HARVEST AID FOR 'VALENCIA' FRUITS[2]

R. H. Biggs
University of Florida, IFAS, Fruit Crops Department, Gainesville, FL 32611

S. V. Kossuth
USDA Forest Service SE Experimental Station, P. O. Box 70, Olustee, FL 32072

Additional index words. Citrus sinensis (L.) Osbeck, abscission, growth regulators, regreening, ethylene, uptake, absorption.

Abstract. **There is a significant interaction that occurs between temperature and uptake of Release®[1], between temperature and metabolism of Release and both differ with the physiological state of the fruit. Mature-green fruit have greater uptake and rates of metabolism of Release than orange-mature fruit. Release is an effective agent for stimulating peel tissue to produce ethylene and this production is very dependent on concentrations of Release in the peel. A critical level of Release** per se **in the peel tissue is required for ethylene production at a concentration and a duration to cause fruit loosening and abscission. Thus, the differential response between green-mature and orange-mature fruit is highly dependent on the rate of metabolism of Release and is a critical factor controlling ethylene production by the tissue. A model is presented to demonstrate the interrelations between environmental factors, particularly temperature; physiological state of the fruit; metabolism of Release; ethylene production and fruit abscission.**

[1]Release is a trademark registered by Abbott Laboratories.

[2]Florida Agricultural Experiment Stations Journal Series No. 2907.
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A technique used in biological sciences is the conceptualization of complex systems into organizational levels to identify the reactive components controlling a process and then to characterize each component as to the involvement in the process on the basis of a direct or indirect control (hierarchic levels) of a connective link in the overall process. The merits of such a systems analysis is four-fold. (a) It allows identification of primary mechanisms controlling the process; (b) identification of secondary indirect mechanisms; (c) possible quantitation of each element of both the direct and indirect components by physiological studies; and (d) parametization of each element so models for further testing and for decision making can be made. This latter becomes extremely important in making an application of research to solving a problem whether it is one related to agriculture, industry, or some other phase of human endeavor. A good review of systems analysis as related to crop production can be found in references (5) and (14).

We have taken the systems approach in this report of analyzing the application of a chemical to aid in citrus fruit harvesting. The specific chemical used is Release (5-chloro-3-methyl-4-nitro-1H-pyrazole) on a specific citrus cultivar, namely, 'Valencia', but the method can be applied to other chemicals and other cultivars. By necessity, because of time-space limitations, each element of the analysis can only be briefly described. Reviews are used to document concepts and references limited to data for parametizing the elements of the analysis.

Materials and Methods

Techniques of modeling physiological systems as reviewed by Loomis, et al. (13) were used to establish the interactive components. All components for parametizing the model can be documented from previous reports except the metabolism of Release as related to physiological state of 'Valencia' fruit and the effectiveness of the chemical in causing fruit abscission. The method used to parametize this element is as follows.

'Valencia' fruit from 34-year-old trees were harvested with 8-inch stems early in the morning and handled carefully to avoid any damage. After the stems were trimmed under water, they were inserted into Aqua Pics containing water and then placed in controlled environmental conditions of a 12-hour day of light intensity of 250 $\mu Em^2 \cdot s^{-2}$, designated temperatures, and a 55 $\pm$ 5 percent relative humidity. Green-mature 'Valencia' fruit were harvested in mid-March and orange-mature 'Valencia' fruit were harvested in late April.

Five 1 x 4 cm rectangles were outlined on each orange using a stamp and India ink. Twenty μl of 300 ppm ^{14}C-ring labelled Release containing 36,454 dpm was applied evenly over the entire surface of each scribed rectangle using a 20 μl automatic pipette. A total of 10 oranges with 5 sections per fruit, or 50 sections, were used for each treatment. Sections were vigorously washed with a sponge and rinsed twice in water after the designated uptake period of 6 or 24 hr. To examine total uptake, each section was cut from a fruit and combusted in a Packard auto-oxidizer to yield water and carbon dioxide. Radioactive $^{14}CO_2$ was collected in a carbosorb, phenethylamine scintillation mixture, and radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer, model 3385. The pyrazole ring on Release is stable through this uptake period (Biggs and Kossuth, unpublished). Uptake was considered the amount of the chemical which was not removed by vigorous sponge washing and rinsing in water.

To determine the amount of Release in the tissue, samples of peel were extracted with methanol and isolated as described previously.[3] Briefly, 10 g of treated peel tissue is extracted 3 times with 100, 30 and 30 ml of methanol, respectively; extract centrifuged and filtered through 0.45 μm pore membranes; concentrated to near dryness; subjected and eluted from C^{18} reverse phase preparative column chromatography; fraction containing parent ^{14}C-labelled compound then subjected to high-performance liquid chromatography using an analytical C^{18} reverse-phase column system and a linear gradient of 20 to 90 percent high purity methanol in H_2O. Detection of fractions was by radioactivity tracers and ultraviolet absorption. Identity of Release was verified by gas chromatography:mass spectrometry. The radioactivity in the isolated samples was determined by scintillation techniques on a Tri-Carb, model 3385.

Data from all tests were subjected to an analysis of variance.

Results and Discussion

There is a two-stage process involved in citrus fruit abscission much the same as has been outlined for leaf abscission (1). This is illustrated in the scheme portrayed in Fig. 1. As far as the bonding force of fruit to stem is concerned, there is an intermediate stage that can be characterized as the stage where the decreased bonding force can be reversed. The range of this attachment force in mature 'Valencia' fruit is from approximately 10 to 3 kg. Generally, when the bonding strength between fruit and peduncle decreases below 3 kg, the abscission processes are non-reversible (1).

There are two distinct ways of grouping factors that modify the abscission processes, namely, environmental and physiological. As shown in Table 1, the primary limiting

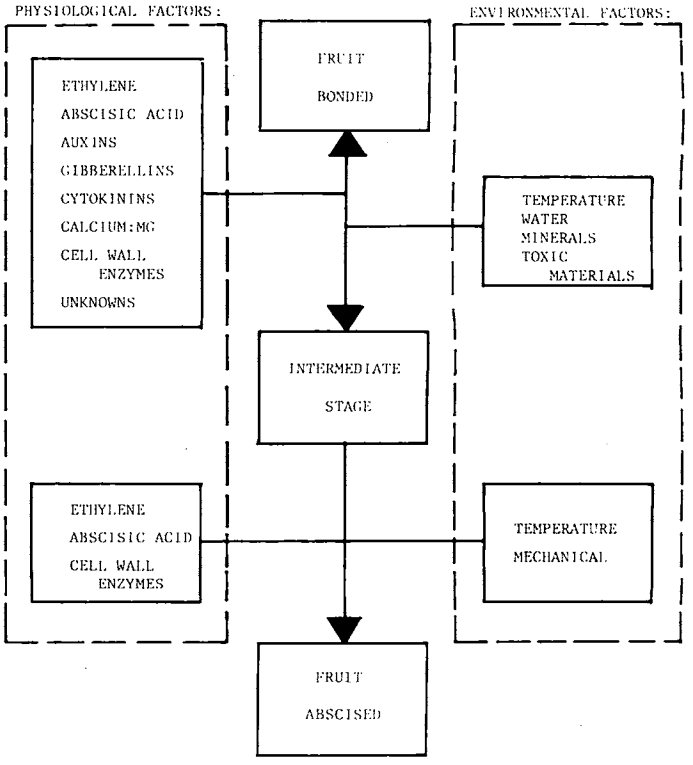

Fig. 1. General scheme for Citrus fruit abscission.

[3]Biggs, R. H. and S. V. Kossuth. 1981. Metabolism of Release® by citrus peel. In preparation for *Proceedings of Plant Growth Regulator Working Group.*

factors in these two groups are quite different for the pre- and post-intermediate reactions leading to abscission. The scheme in Fig. 2 expands on environmental and physiological factors as related to the pre- and post-intermediate stage. The factors related to these two schemes have been periodically reviewed in general as related to citrus (1, 3, 4). Two obvious factors become apparent from the hierarchic levels portrayed in the systems of Table 1 and 2. The natural systems can be altered primarily by changing the endogenous chemical regulator and the most successful attack point has been to alter the metabolism of ethylene and possibly that of abscisic acid (ABA). Secondly, the entire system is very temperature dependent. Ethylene biogenesis and tissue sensitivity to ethylene is temperature dependent (1) so are enzymic reactions (10, 11). In the case of healthy trees in good citrus producing areas, other environmental factors either are of secondary importance, can be readily identified, i.e., rain, or can be modified if they become a limiting factor, i.e., water as related to humidity in the grove that may influence uptake of chemicals used to stimulate abscission (9, 20).

From an analysis of the hierarchic physiological processes affecting abscission that result from integration of sublevel processes as influences by the external environment, it is possible to construct a model for decision making in relation to the use of a chemical to stimulate the abscission processes. A schematic for such a simplified model is presented in Fig. 3 and is discussed as related to 'Valencia' fruit for each cultivar responds differently to chemical-accelerants of abscission (8, 17, 18, 20).

There are several features to this model. (a) Economics becomes prominent in the decision to use or not to use a chemical aid for harvest (2, 19). (b) Temperature can have a major influence on the success of a chemical at four main points: a. uptake of the chemical (6, 9, 12, 13, 14), b. metabolism of parent compound (efficacy) (Biggs and Kossuth, unpublished), c. biogenesis of ethylene[4] and, d. tissue sensitivity to ethylene (1, 16). (c) The physiological state of the fruit has a major affect on the capacity of the fruit to respond (12, 16). There is both a qualitative and a quantitative aspect to this capacity of fruit to respond to Release (12). The quantitative characteristic can be modulated by changing the concentration of Release (20) to some extent and adding adjuvants to the formulation (7, 12) and is strongly influenced by temperature (9). (d) Computation of concentration of Release to apply depends on a knowledge of the tissue levels of parent compound expected and rate of detoxification, physiological state of fruit as related to chemical regulators (12) and temperatures expected during and post-application (0 to 90 hrs.) (9).

[4]Evensen, K. B. 1978. The physiology of ethylene production by citrus peel tissue. *Ph.D. dissertation.* University of Florida.

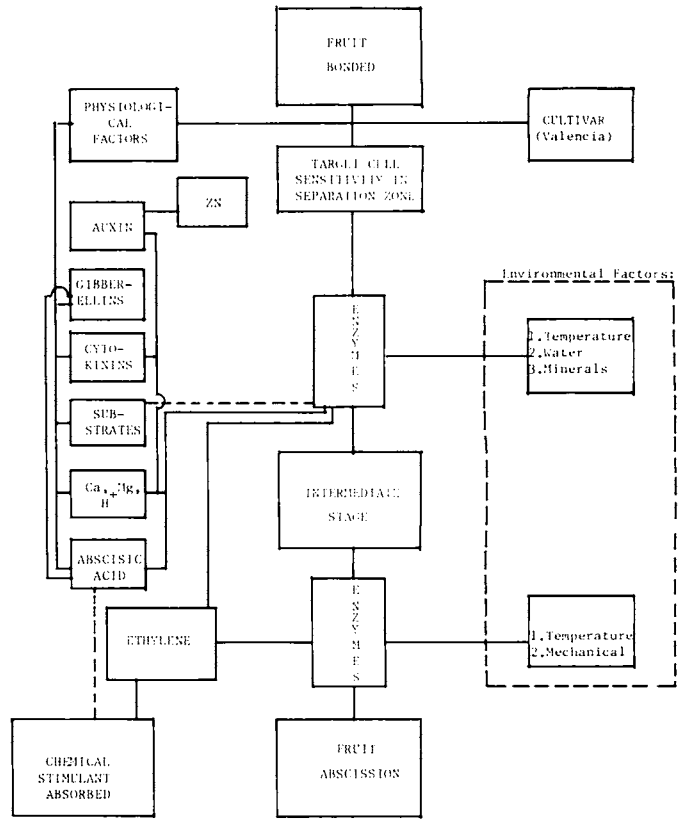

Fig. 2. Scheme of the critical interactive components affecting citrus fruit abscission. The lower left component is the modification made by the addition of a chemical aid to harvest, i.e., Release®.

Since metabolism of Release becomes a critical element in the predictive nature of the model, several quantitative aspects of this parameter are presented in Tables 1 and 2. As shown in Table 4, green-mature and orange-mature fruit respond very differently in the amount of chemical absorbed as discussed previously (12), rate of detoxification of the parent compound, the quantity of ethylene internal to the fruit and percent fruit abscised. Calculations based on duration of tissue exposure to parent compound as related to ethylene production and fruit abscission indicates that a half-life ($Y_{1/2}$) of tissue absorbed Release has to be at least 16 hours with the amount of parent Release in the tissue at $Y_{1/2}$ of 1.5 μg/gm of peel tissue for it to be effective. Critical to both the metabolism of Release and to ethylene production is temperature as illustrated in Table 2. The quantitative aspects of the interaction of temperature to effectiveness of Release is very critical and is being studied further in our laboratory and others (Wilson, personal communication).

Table 1. Effect of the amount of Release® in Valencia peel tissue of green-mature and orange-mature fruit on ethylene production and fruit abscission.[z]

Time from application (hrs)	Parent Compound (%)[yx]		Ethylene produced (ppb)[x]		% Fruit abscised[x]	
	green	orange	green	orange	green	orange
6	76	67	10	10	0	0
24	23	37**	23	80**	0	0
48	12	22**	31	265**	0	0
72	8	14*	28	1,180**	0	20**
96	6	8	36	10,400**	0	100**

[z]One hundred μl of 300 ppm of ^{14}C – ring labelled Release® containing 36,454 dpm was applied to a 20 cm^2 area.
[y]Amount initially absorbed in green-mature fruit was 84.1 percent and in orange-mature fruit 68.6 percent. The uptake period was 6 hours.
[x]A single asterisk indicates significants at the 5 percent level and a double asterisk at the 1 percent level.

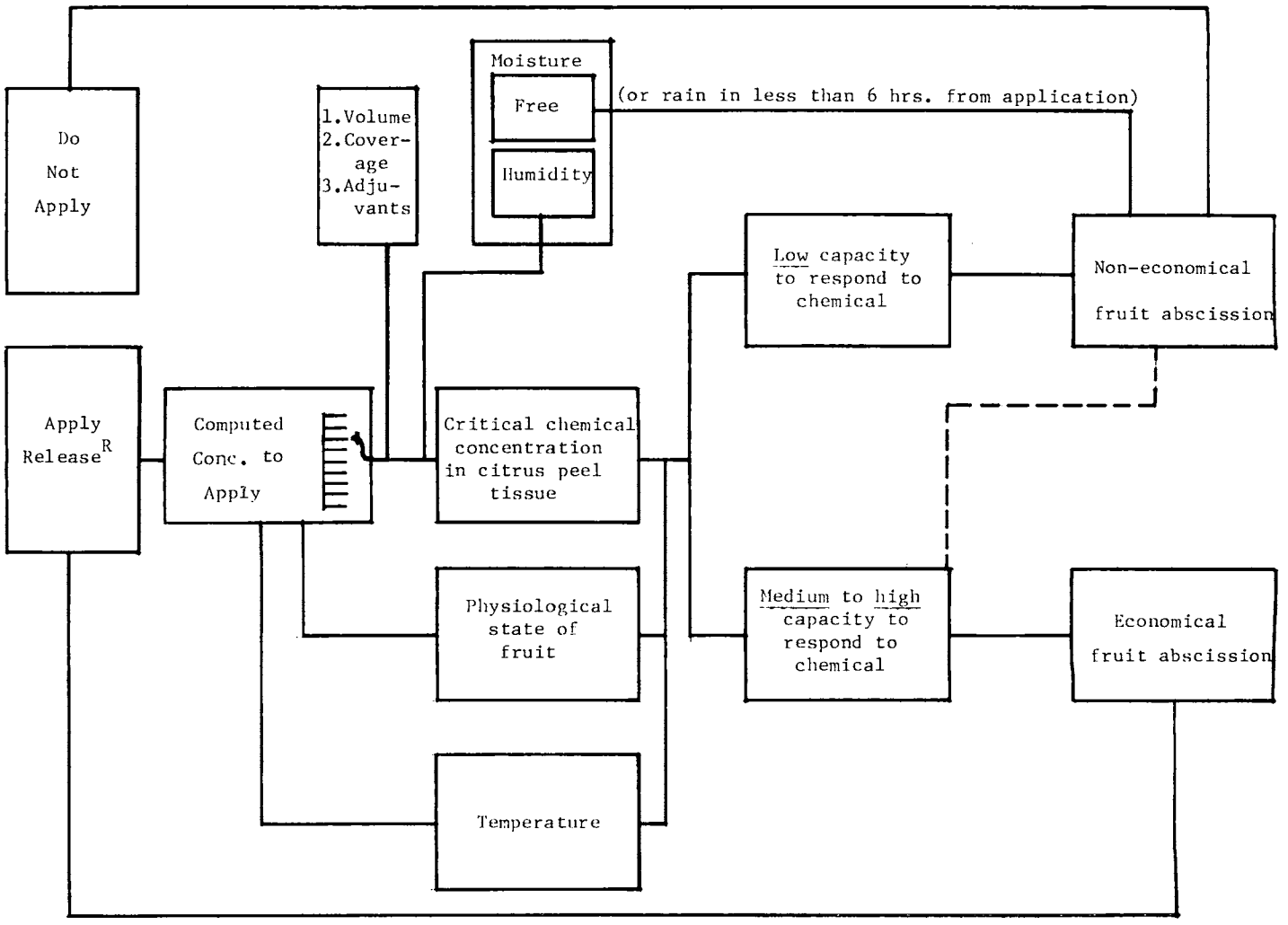

Fig. 3. Model for a decision to either use or not use Release® toharvest 'Valencia' fruit.

Table 2. Effect of temperature on uptake of ^{14}C—Release®, metabolism and ethylene production by 'Valencia' fruit peel.[z]

Measurements	Temperature[y] 10°C	20°C
Release absorbed[x] (% of applied)	26	38**
Parent compound[x] (% of applied)		
24 hrs	27	35**
48	23	24
72	22	19.5*
96	20	16*
Ethylene internal to fruit (ppb)		
24 hrs	10	32**
48	10	120**
72	10	740**
96	10	903**

[z]'Valencia' fruit sampled April 16, 1980.
[y]A single asterisk indicates significant differences at 5 percent level and a double asterisk at 1 percent level.
[x]Uptake period was 24 hrs.

From the data base, it is now possible to develop a scenario where Release will probably induce 90 percent of 'Valencia' fruit to abscise, namely, average daily temperature greater than 12°C from time of application until 72 hours later with the temperature during application being above 16°C; concentration of Release being in the range of 250 to 300 ppm if fruits are in the medium to high capacity for response category, volume of spray to give run-off from tree canopy with complete coverage and with the proper adjuvant; and relative humidity is within the range of 45 to 85 percent from time of application until 6 hours later. A heavy dew or rain within this 6-hour period decreases drastically the effectiveness of Release (9).

Sensitivity analyses such as presented in Table 3 can also pinpoint areas of research needed to improve predictability of applying a chemical to cause fruit abscission. Answers are needed to the following questions: What are factors involved in the rates of metabolism of Release? Capacity of tissue to produce ethylene? Sensitivity of the abscission processes to ethylene? And the interaction of these three elements with temperature?

Another factor becomes very evident in our attempt to devise a chemical aid to assist in fruit harvest—another attack point is the cell wall hydrolyzing processes *per se*. That is, at the present all chemicals that have been successful in the field seem to operate via either stimulating the tissues to produce ethylene by wounding or are ethylene generating compounds *per se*, and it is the ethylene affecting the abscission processes. The next generation of chemical accelerants should be sought to operate directly on the enzymic components in the separation zone. (Note the prominent location of these elements in Fig. 2).

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SOFT ROT OF CITRUS FRUIT CAUSED BY PENICILLIUM DIGITATUM AND P. ITALICUM[1]

CHARLES R. BARMORE
University of Florida, IFAS, Agricultural Research and Education Center, P. O. Box 1088, Lake Alfred, FL 33850

G. ELDON BROWN
Florida Department of Citrus, Agricultural Research and Education Center, P. O. Box 1088, Lake Alfred, FL 33850

Additional index words. green mold, blue mold, postharvest decay.

Abstract. **Peel tissue of citrus fruit rotted by** Penicillium digitatum **Sacc. and** P. italicum **Wehmer was similar in degree of softness and relative water content. Pectolytic enzymes produced by these fungi degraded pectin of the cell wall middle lamella resulting in loss of cell coherence. This process is considered important in aiding hyphal penetration. Rate of development of the decay was not determined by the macerating ability of the pectolytic enzymes but apparently due to the growth rate of each organism.**

Penicillium digitatum Sacc. (green mold) and *P. italicum* Wehmer (blue mold) are 2 important postharvest fungal pathogens of citrus fruit. In Florida, green mold is by far the more important decay of the 2 types. Infection of citrus fruit by these 2 organisms requires an injury which is usually incurred at some time during harvesting and/or during postharvest preparation (4). Rot caused by the 2 organisms is similar in appearance and texture and may spread to contiguous healthy fruit in packed cartons.

The importance of pectolytic enzymes produced by various pathogens in the development of soft rots of other fruits is well documented (3). The enzymes degrade pectic substances of the cell wall middle lamella causing loss of tissue cohesiveness and death of cells.

The following discussion reports similarities in properties of the 2 decays and the identity of pectolytic enzymes produced by each pathogen.

Materials and Methods

Inoculations. Mature 'Valencia' oranges (*Citrus sinensis* (L.) Osbeck) were washed and inoculated through a puncture, 5 mm deep, into the albedo with spores (approx. 10^6 spores/ml) of either *P. digitatum* or *P. italicum* in water containing 0.01% Triton X100. The fruit were incubated at near 100% relative humidity and 25°C.

Pectolytic enzyme analysis. Methods used in the extraction and characterization of the pectolytic enzymes in the decayed tissues have been published (1, 2).

Organic acid analysis. The organic acids in the decayed tissue were extracted according to the procedure of Fernandez-Flores et al. (8) as modified by Ting (unpublished, Florida Dept. of Citrus, Lake Alfred). The decayed peel was homogenized in water, centrifuged and the supernatant was adjusted to 80% ethanol and centrifuged again. Saturated lead acetate solution was added to the supernatant, centrifuged and the pellet was serially washed with ethanol, acetone, diethyl ether and then vacuum dried. The organic acids in the pellet were silylated with Tri-Sil (Pierce Chemical Co., Rockford, IL 61105) and analyzed with a Hewlett-Packard gas chromatograph, Model 5736, equipped with a flame ionization detector and an SE 30 column. A temperature program of 70 to 210°C at a rate of 4°C per minute was used.

[1]Florida Agricultural Experiment Stations Journal Series No. 2708.