THE EFFECTS OF SOME SPECIAL TREATMENTS IN THE DEGREENING OF FLORIDA ORANGES AS MEASURED BY RESPIRATION RATE

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(WITH ONE FIGURE)

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Introduction

For many years it has been customary to treat certain varieties of citrus fruits, especially early oranges, with ethylene gas for the purpose of removing the green color from the rinds. This process is referred to by the trade as "coloring" but should more properly be termed "degreening." That the term "coloring" is ill-chosen may be seen from the fact that though the maximum amount of yellow rind pigments (carotenoids) is not reached by the time that oranges are mature green, present degreening methods serve only to remove the green pigments (chlorophylls) and do not increase the yellow pigments already present (**20**). (A special process of adding natural vegetable dyes to the peel is commonly used today and fruits so treated must be stamped "color added.") The procedure now followed for degreening (**28**) is to stack field crates of oranges in rooms which are equipped with means for heating, introducing ethylene, maintaining proper humidity, and circulating fresh air. The fruit is kept at approximately 85° F (29.4° C), 80 to 100% relative humidity and is treated with ethylene in low concentrations. Under these conditions a fruit which is quite green may require as much as 60 hours to degreen whereas a fruit in which chlorophyll has begun to decompose while on the tree requires proportionately less time. This method is essentially the same as it was 20 years ago, with improvements being found only in construction of rooms and mechanical equipment.

The need for degreening arises solely from a consumer preference for "orange" colored oranges, for in Florida (**29**) and in other regions having semitropical and subtropical climates some of the early orange varieties mature before losing their green color. Citrus fruits of any variety growing as an "inside" crop on densely foliated trees often retain much of their greenness long after maturity. It is common knowledge (**12**) that "maturity" refers to a stage of development and "ripening" refers to the process by which a mature fruit becomes edible or desirable as food. Apples, bananas, and pears, which contain a starch reserve, may become mature before harvest but may not ripen until the action of enzymes and life processes have hydrolyzed the starches to sugars, produced a certain amount of softening, and effected other changes such as rendering bitter principles insoluble.

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Oranges are different in that they contain practically no starch, so that ripening processes are not accompanied by changes such as those just mentioned. Instead, while on the tree, fruits ripen by increasing the volume of juice and per cent. of total soluble solids (mainly sugars) and by decreasing the per cent. of acids. This process is stopped by picking. Thus, maturity and ripeness in oranges is synonymous and oranges must be of desirable eating quality at time of harvest in order to be regarded as mature. In other words, they must always be "tree-ripened" if they are ripe at all.

Although the ethylene treatment does not appreciably affect the sugars and acids in the orange, the conditions under which the fruits are held in the degreening rooms tend to accelerate some of the biological processes which may shorten the period of marketability. If the time of treatment with ethylene could be shortened it would be of advantage to producers and would result in fruit with better flavor and longer keeping quality. However, it was felt that before any marked changes can be made in the commercial practices it will be necessary to learn more regarding the fundamental physiological processes involved during the degreening of the oranges. This in brief, is the reason for undertaking these investigations. Because of the known stimulating effects of ethylene on the respiratory processes of fruits it seemed desirable to approach the present problem by making observations on the effects of various treatments on the rate of respiration in oranges and correlating the results with degreening.

Review of literature

Considerable research has been done on the precursors of the chlorophylls, the factors influencing the development of these pigments, and the mechanisms whereby photosynthesis occurs. However, very little has been said about the process by which the chlorophylls are destroyed in plants. GARDNER (**8**) demonstrated the presence of a chlorophyll-decomposing enzyme in the rinds of Satsuma oranges and NOACK (**21**) concluded that decomposition of the chlorophylls is a process involving oxidative catalysis. ROTHEMUND (**23**), on the other hand, in discussing autumnal decomposition of the chlorophylls in leaves, states that "chemical details of this process are not known."

Despite the lack of information regarding the fundamental nature of chlorophyll decomposition, progress has been made in the practical aspects of degreening oranges. DENNY (**4**) was among the early contributors to the subject, showing that ethylene was the active agent in the fumes of the kerosene stoves which were used for degreening citrus fruits at that time. Since then many studies on the effects of ethylene have been made but few have been concerned with the destruction of chlorophyll in citrus fruits. DUFRENOY (**7**) observed cytological changes in cells of orange peel degreened by treatment with ethylene gas. He noted a "fatty degeneration" of the chloroplasts with the formation of oil drops in which the yellow pigments dissolved.

One of the first noticed effects of ethylene on fruits was the stimulating effect on the respiratory rate. BAIER, RAMSEY, and collaborators (**1**), in a very comprehensive study of the whole process of degreening citrus fruits, have contributed valuable information on means for improving the construction and equipment of degreening rooms. However, data they obtained on rates of respiration were slightly lower than those found in the work now being reported and no shortening of degreening time was apparent in their experiments, possibly because fruits which they observed were in an atmosphere gradually increasing in carbon dioxide concentration. In a preliminary report, STAHL and CAIN (**24**) described equipment for measuring respiration rates of degreening oranges but stated only that oranges "showed marked increase in respiration while exposed to ethylene gas." The studies of HALLER, ROSE, LUTZ, and HARDING (**10**) were made in an atmosphere containing no CO_2, but the respiration rates found during ethylene treatments were considerably lower and required much longer to reach a maximum than those found in the present experiments. The response that the above-mentioned investigators obtained might be explained by the fact that their fruit was probably post-climacteric and had undergone certain changes prior to the experiment while degreening on the tree.

Materials and methods

The following three lines of approach were made in the development of this investigation: (a) treatment of the fruits with growth-modifying compounds; (b) treatments with certain volatile organic compounds prior to or during exposure to ethylene gas; and (c) exposure of the fruit to radio-frequency radiation with and without concurrent treatment with ethylene. In these experiments the fruits under test were placed in standard glass aquaria ($10 \times 12 \times 20$ inches). These aquaria were fitted with double-weight bottoms and ends in which holes were drilled for inlet and outlet tubes. The respired CO_2 was absorbed by the KOH in a specially-designed plexiglass (methylmethacrylate) funnel. A capillary tube of plexiglass was bent into the shape of a ring and placed over the above-mentioned funnel to serve both for flushing out the used KOH solution and for adding a fresh amount of this material.

Lids to the aquaria consisted of double-weight glass. These were held in place by recess frames which in turn were fitted to the metal flanges of the aquaria. Glass junctions were made gas-tight by use of a pliable, plastic weather-stripping called "Mortite." Wooden blocks were cemented onto the glass lids to permit removal when changing samples of fruit. In addition to this the aquaria joints received several coats of spar varnish.

No control of humidity in the chambers was considered necessary since it was observed to range between 95 and 100%, which is approximately that found in many commercial degreening rooms.

The apparatus used for supplying gases at atmospheric pressure was a modification of that first described by MAGNESS and DIEHL (**18**) and subsequently improved by other workers (**5, 9, 27**). The essential principles involved were as follows. (See figure 1.)

The oxygen to replace that used in each respiration chamber (D) was supplied from two glass cylinders (B) approximately 2½ inches in diameter and 28 inches in length. Each cylinder contained a smaller glass tube. Water was maintained at the tops of these smaller tubes by a siphon connected to the constant-level water source (A). When the CO_2 in the respiration chamber was absorbed in the KOH there occurred a momentary reduction in pressure. Oxygen then moved into the chamber to replace the absorbed CO_2. This caused water to be siphoned over to replace the oxygen, thus restoring equilibrium to the system. Constant level was maintained in "A" by allowing water to drop slowly into the funnel shown in

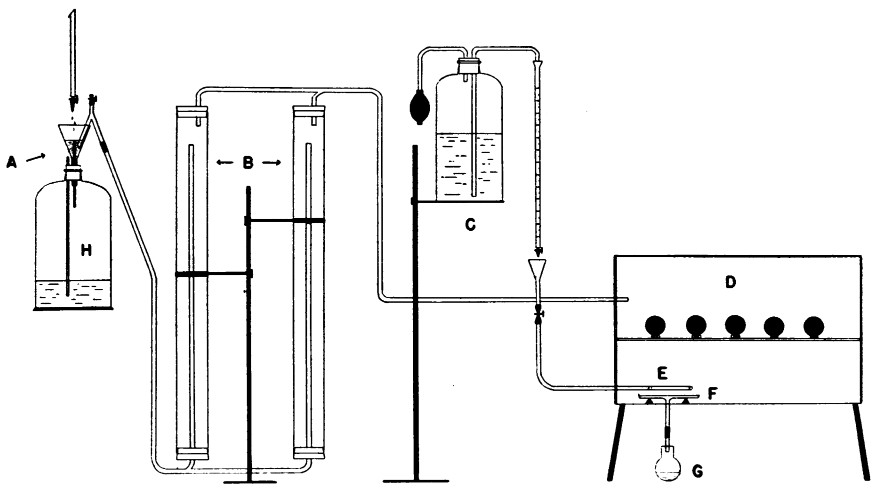

FIG. 1. Apparatus employed for studying respiration in oranges: A. Constant-level water source; B. Glass cylinders containing oxygen; C. Standard KOH; D. Respiration chamber; E. Sprinkler for flushing out old alkali and introducing a new supply; F. Plexiglass funnel for holding alkali; G. Flask for collecting spent alkali; H. Overflow water reservoir.

the diagram; when the level rose too high it drained into the overflow reservoir (H).

The procedure followed in most of the "runs" is described at this point and any deviations from this practice will be noted in the section concerned with the special treatment. The oranges were of the Parson Brown variety grown on sour rootstock near Ferndale, Florida. Fruits in Bruce box or bushel basket containers were shipped by Railway Express from Winter Garden, Florida, soon after picking. They were received four days later in Pittsburgh and immediately placed in a "walk-in" type refrigerator kept at 6° C (42.8° F). Twelve hours prior to the start of respiration measurements, oranges were removed to a covered container (not airtight but containing KOH pellets) in the constant temperature room, maintained at

32.2° C (90° F), so that a temperature equilibrium might be reached. Before starting the "run," fresh air was circulated into the chambers for several minutes by an electric fan. Ten oranges were then arranged in five rows of two each on glass racks and the chamber was closed and sealed. Immediately after closing the chamber, standard alkali was added and flushed in with about 10 ml. of water.

For the 0 to 3-, 3- to 6-, and 6- to 9-hour periods 25 ml. of approximately 2 *N* KOH was used. The amount added for the 9- to 24-hour period varied from 50 to 85 ml., depending upon the response found during the first nine hours. Several times in the course of the experiment the concentrations of the O_2 and CO_2 in the chambers were determined by analytical methods. Oxygen did not vary more than 1% from that in the outside air (average 20.85%) and only in one instance was CO_2 found in a measurable amount. At the end of a definite period of time, the absorbent was drained into a 100-ml. volumetric flask and made nearly to volume by water added through the "sprinkler," giving the inside funnel a thorough rinsing. The partially neutralized alkali was made up to volume and titrations were run in triplicate with 5-ml. aliquots. These aliquots were added to Erlenmeyer flasks which contained 50 ml. of distilled water and some barium chloride. The titrations were made using a phenolphthalein indicator and 0.242 *N* (later 0.247 *N*) sulphuric acid. The acid was standardized with potassium acid phthlate. Calculations were made as follows:

ml. KOH blank – ml. KOH "run" = ml. of KOH neutralized by CO_2 in aliquot
ml. KOH neut. × 20 = total ml. KOH neut.
total ml. KOH neut. × 0.242 = milliequivalents KOH neut.
m.e. KOH neut. / 2 = m.e. of CO_2 formed in chamber
m.e. CO_2 × 44 = mg. CO_2 formed in chamber
mg. CO_2 / (kg. fruit × hours) = CO_2 mg. per kg.-hr.

Results

Growth-modifying compounds applied as a spray

Since it has been claimed that the action of ethylene on plants is similar to that of auxins (**3**), it seemed logical in this work to attempt to substitute growth-modifying compounds for ethylene in degreening oranges.

The concentration of material sprayed on plants is known to be a large factor in determining the modifications caused. Since no recommendations concerning the concentrations most likely to increase respiratory rates of citrus fruits were available, sprays in four different concentrations were used. The growth-modifying compounds were dissolved in melted "carbowax" and this mixture was diluted with enough distilled water to make the final volume 2% carbowax. An aerosol type sprayer was used to apply the materials to the fruits just before the beginning of the respiration measurements. The treatments were made using 10, 100, 500, 1000 parts per million of the compounds while the control lot was sprayed with a 2% solution of carbowax only. Respiration rates were measured only on the control and the lot treated with 500 p.p.m. spray. The remaining three lots were held in a covered aquarium containing an evaporated dish filled with KOH pellets to prevent accumulation of carbon dioxide. Observations were made on their rate of degreening.

Results with 2,4,6-trichlorophenoxyacetic acid, 3-indolebutyric acid, beta-naphthoxyacetic acid, and alpha-naphthaleneacetic acid showed that these compounds applied in this manner offered no promise as degreening agents, for at no concentrations of spray was there any color difference between the treated and control lots. The respiration measurements indicated that no major change in metabolic activities was caused by the treatments. In only one instance (during the first 3-hour period) did the control and treated lots differ by more than 6.5 mg. of CO_2 produced per kg.-hr. (10%). These data are found in table I.

TABLE I

EFFECT OF GROWTH-MODIFYING COMPOUNDS ON RESPIRATORY RATE OF IMMATURE FLORIDA ORANGES. EACH FIGURE REPRESENTS A SINGLE OBSERVATION ON 10 FRUITS.

Treatment	Mgs. of CO_2 per kg. per hr. during:				Total mgs. of CO_2 during 24 hrs.
	0–3 hrs.	3–6 hrs.	6–9 hrs.	9–24 hrs.	
(500 p.p.m. applied in aerosol spray)					
Control	46.6	55.4	55.4	53.9	1280.7
2,4,6,-Trichlorophenoxyacetic acid	47.8	57.3	60.6	58.9	1380.6
Control	62.8	58.9	60.1	56.2	1388.4
3-Indolebutyric	48.1	58.0	58.9	51.9	1273.5
Control	54.2	55.8	60.1	56.3	1354.8
Beta-naphthoxy-acetic acid	52.3	....	57.3	50.6	....
Control	66.7	61.8	64.3	58.9	1461.9
Alpha-naphthalene-acetic acid	64.6	57.5	57.8	52.9	1333.2

GROWTH-MODIFYING COMPOUNDS APPLIED AS A VAPOR

Increased activity of the growth-modifying compounds was sought by introducing them into the chamber as a vapor at the start of a "run." To do this, a small amount of the material to be tested was placed in a vial fitted with a rubber stopper containing two capillary tubes. Through these tubes, air was forced over the compound into the respiration chamber. Increased volatility was obtained by heating the vial on a small hot plate, but even this produced only a slight loss of weight by the compounds (1 to 10 mg.). Data on the effect of the vapors of these compounds are summarized in table II.

Methyl alpha-naphthalene acetate produced almost no change in respiration rate and no color difference from that of the controls. With alpha-(*o*-chlorophenoxy)-propionic acid, the respiration rate was found to be slightly greater than the control lot but no color difference with fruits could be determined. Likewise cinnamic acid produced no effects upon the respiratory rate and no noticeable effect on degreening. However, the cinnamic acid was not treated with ultraviolet light to convert it from the trans to cis form as suggested by ZIMMERMAN and HITCHCOCK (**30**).

TREATMENT WITH CERTAIN VOLATILE ORGANIC COMPOUNDS

The effects of ethylene upon plant metabolism have been compared to those of the common anaesthetics (**13**). The next logical step in these studies therefore appeared to be a comparison of ethylene with other organic compounds, some of which are used as anaesthetics. Accordingly, experiments were set up in which three representative compounds, ethylene chlorohydrin, carbon tetrachloride, and chloroform, were tested for their effect upon degreening and respiration. The compounds were applied either as a pre-treatment prior to the respiration studies or as a supplemental treatment during respiration measurements. In the pre-treatment, the fruits were enclosed in a 50-pound lard can (capacity approximately 25 liters) and exposed to 0.2 to 0.5 ml. of the specific organic compound as it volatilized from a cheese cloth suspended from the sealed lid. When the volatile organic compound was applied directly to the respiration chamber the compound was likewise permitted to volatilize from cheese cloth. Ethylene gas was introduced into the chambers through the "sprinklers" used to add the alkali. The amount of gas was determined by adjusting the time required to displace the desired amount of water (about 8 ml.) from an inverted graduated cylinder and then allowing the gas to flow into the chamber for this length of time.

TABLE II

EFFECT OF VAPORS OF GROWTH-MODIFYING COMPOUNDS ON RESPIRATORY RATE OF IMMATURE FLORIDA ORANGES. EACH FIGURE REPRESENTS A SINGLE OBSERVATION ON 10 FRUITS.

Treatment	Mgs. of CO_2 per kg. per hr. during:				Total mgs. of CO_2 during 24 hrs.
	0–3 hrs.	3–6 hrs.	6–9 hrs.	9–24 hrs.	
(Vapors introduced into chamber)					
Control	68.7	65.6	67.3	54.9	1428.3
Methyl alpha-naphthalene acetate	70.7	66.1	64.3	53.8	1410.3
Control	48.4	55.8	50.5	48.9	1197.6
Alpha-(o-chlorophenoxy)-propionic acid	62.0	57.4	59.0	50.8	1297.2
Control	56.5	62.2	58.7	52.5	1319.7
Cinnamic acid	57.8	58.9	60.5	49.9	1280.1

The increase in respiration rate of lots treated with ethylene was striking when compared to the rate of untreated controls. An ethylene-treated lot compared with a control lot showed that 53% more CO_2 was produced per kg. during the whole 24-hour period by the treated than by the controls and that respiration rate was 78% higher during the 9- to 24-hour period. If an average of the 9- to 24-hour periods of the eight control lots is taken as normal and compared to the average of the six ethylene treatments, an overall increase of 116% in respiration rate is noted. In every instance ethylene effected excellent degreening while the control fruit remained almost unchanged.

Ethylene chlorohydrin, a compound which has previously been shown to be physiologically active (**6**), was used in three different types of treatments. When this compound was introduced into the chamber in concentration of approximately 200 p.p.m. at the start of respiration measurements, a negligible increase in rate was seen and no degreening was produced. Ethylene chlorohydrin and ethylene were introduced simultaneously at the beginning of the respiration measurements on another lot and an increase in respiration rate during the first nine hours was observed. However, no color difference could be detected between fruits with this type of treatment and the ethylene controls. A 12-hour pre-treatment with ethylene chlorohydrin, followed by ethylene treatment, produced a 36% increase in the respiration rate during the first nine hours but the total CO_2 produced per kg. in the 24-hour period was only about 3% greater than the controls. The color of the ethylene controls was slightly better than the pre-treated lot.

Pre-treatments with the non-polar compounds, carbon tetrachloride and chloroform, were not effective in changing the rate of metabolism as measured by CO_2 production. Data for this series of experiments are summarized in table III.

Radio frequency radiation

Since the experiments thus far described gave no indication of the mechanism whereby degreening was being facilitated, another approach to the problem seemed desirable. Radiations have been shown to affect plant respiration by influencing many vital processes (**25**). It was hoped that, by applying energy of this type, a certain stimulation of the degreening mechanism of oranges might be obtained. The equipment employed in these experiments consisted of a Model CMD–4 Raytheon "Microtherm" which operated at a frequency of 2450 megacycles, had a maximum output of 125 watts, and was fitted with director C to produce an elliptical power pattern. The director was moved along the outside of the chamber (approximately four inches from the center of the oranges) and held opposite each fruit for one to two minutes per hour during the 0 to 9-hour period. Another type treatment was given by placing aluminum plates above and below the area occupied by the oranges in the chamber and "driving" them at 27.32 megacycles for 20 minutes per hour during the 0 to 9-hour period. This was done by using a Model No. 1000 "Meditherm" short wave diathermy unit

TABLE III

EFFECT OF VARIOUS ORGANIC COMPOUNDS ON RESPIRATORY RATE OF IMMATURE FLORIDA ORANGES. EACH FIGURE REPRESENTS A SINGLE OBSERVATION ON 10 FRUITS.

Treatment	Mgs. of CO_2 per kg. per hr. during:				Total mgs. of CO_2 during 24 hrs.
	0–3 hrs.	3–6 hrs.	6–9 hrs.	9–24 hrs.	
Control	46.7	65.4	70.4	64.2	1510.5
Ethylene	43.2	68.5	87.8	114.5	2316.0
Ethylene control	43.4	54.6	77.4	119.9	2324.7
Ethylene chlorohydrin	57.7	60.3	61.2	63.5	1490.1
Ethylene control	50.7	75.5	99.5	126.8	2579.1
Ethylene plus ethylene chlorohydrin	69.8	94.0	105.7	108.3	2433.0
Ethylene control	65.4	69.1	91.9	124.4	2545.2
Ethylene after 12 hours pretreatment with ethylene chlorohydrin	90.2	105.8	112.5	113.3	2625.0
Ethylene control	58.4	75.1	91.0	118.2	2446.5
Ethylene after 12 hours pretreatment with carbon tetrachloride	60.3	77.3	96.1	110.5	2358.6
*Ethylene control	57.8	74.3	95.1	119.5	2474.1
*Ethylene after 12 hours pretreatment with chloroform	66.6	88.0	102.4	115.1	2497.5

*Note: This last run was continued for 24 to 28 hours and the rate during this period was 101.1 for the ethylene control lot and 103.6 for the chloroform pretreated lot.

produced by the Medical Quartz Products Company. In each case, the power and current recorded were those indicated by the meter mounted in the equipment. After the first run was made, a 60-watt light bulb wired into the control chamber produced a rise in temperature equal to that caused by r-f energy inside the treatment chamber. The fruit itself showed a 1.5 to 2.0° C rise in temperature while under micro-wave treatment and a 3 to 6° C increase while under short-wave treatment, though surface temperature may have been even higher in the latter case.

The effect of microwaves on respiration during the 0 to 9-hour period was rather consistent. An increase in respiration of 33% was produced by an exposure of 10 minutes (one minute per orange) per hour at the 100% power level and a 20% increase was produced by 20 minutes per hour at the 65% power level. In neither of these experiments was degreening apparent, and it should be noted that, for the 24-hour period, the total respiration was only 14 and 8% above that of the controls. The treatment consisting of ethylene and microwaves produced a 27% greater respiration during the 0 to 9-hour period than did ethylene alone. However, the total respiration for the 24-hour period was only 7% above that of the controls and no difference in amount of degreening could be detected.

The data obtained from experiments using 11-meter treatments were inconsistent. The exceptionally large increase in respiration of 81% during the 0 to 9-hour period in one lot was caused by a 20-minute per hour treatment at four amperes r-f current. The rate dropped rapidly after treatments were stopped so that the 24-hour average increase was only 53%. Since in one such experiment, fruits were severely burned (at 5 to 6 amps.) and results had to be discarded, it is likely that the tough dark green spots around the stem end of three oranges in this lot indicated that tissue damage had occurred and results were questionable. Also, ethylene plus a 20-minute per hour treatment at 2 amperes r-f current gave only a 10% increase in respiration during the 0 to 9-hour period and a total increase of only 4% for the 24-hour measurements. In neither case was any difference in amount of degreening in treated and control lots apparent. Data for this series of experiments are summarized in table IV.

TABLE IV

EFFECT OF RADIO-FREQUENCY RADIATIONS ON RESPIRATORY RATE OF IMMATURE FLORIDA ORANGES. EACH FIGURE REPRESENTS A SINGLE OBSERVATION ON 10 FRUITS.

Treatment	Mgs. of CO_2 per kg. per hr. during:				Total mgs. of CO_2 during 24 hours
	0–3 hrs.	3–6 hrs.	6–9 hrs.	9–24 hrs.	
Control	48.4	56.6	62.1	56.8	1353.3
10 min. of 12 cm. r-f per hr. for 0–9 hrs. at 100% power	60.8	81.0	81.0	58.3	1542.9
Control	72.4	85.7	76.9	61.1	1621.5
20 min. of 12 cm. r-f per hr. for 0–9 hrs. at 65% power	78.8	105.4	97.8	60.6	1755.0
Ethylene control	56.8	81.8	103.4	121.7	2551.5
Ethylene plus 20 min. of 12 cm. per hr. for 0–9 hrs. at 65% power	75.9	106.0	126.0	120.0	2723.7
Control	42.8	62.9	58.4	43.5	1144.8
Ethylene plus 20 min. of 11 meter r-f per hr. for 0–9 hrs. at 4 amps.	71.4	123.5	102.0	57.6	1754.7
Ethylene control	55.3	71.5	90.2	109.6	2295.0
Ethylene plus 20 min. of 11 meter r-f per hr. for 0–9 hrs. at 2 amps.	44.0	85.8	109.5	111.6	2391.9

Discussion

The conclusions to be drawn from the data gathered in this series of experiments are largely concerned with the mechanisms whereby degreening occurs in oranges and the way in which it is influenced by ethylene, for in no instance was any compound or treatment discovered which would produce a color change in less time than would ethylene alone. However, the oranges in these experiments showed a tendency to degreen with ethylene in somewhat less time than is normally required under commercial conditions.

It is possible that the O_2 and CO_2 content of the atmosphere was more accurately controlled under the experimental conditions than it is in commercial degreening rooms. Furthermore, a certain amount of delay between picking and degreening occurred as a result of the transportation between Florida and Pittsburgh and it is likely that degreening mechanisms were initiated by the fruits themselves during this period.

The experiments using growth-modifying compounds did not indicate that this type of treatment would be of use commercially nor did they produce any effects which could be correlated with degreening processes in fruits. However, it has been suggested that plant hormones are made more active by exposure to ethylene (**19**) and the influence of the growth-modifying compounds studied in these experiments should be further investigated by applying them in conjunction with ethylene treatments. This is especially true in the case of vapor treatments, since in this form the material should be better able to penetrate the cells of the plant and to reach the pigmented subepidermal layers. Since it was shown that compounds known to increase permeability did not alter the speed of degreening, one may conclude that ethylene alone diffuses into the plant parts rapidly enough and increases permeability sufficiently to allow gaseous exchange to occur as fast as is necessary for optimum degreening. The increase in respiration caused by ethylene chlorohydrin pre-treatment might be ascribed to its effect on enzymes, pH, or other metabolic factors. However, in describing the effects of ethylene chlorohydrin, CROCKER (**2**) says that because of the very multiplicity of factors influenced by this compound it is practically impossible to formulate a satisfactory explanation of the exact mechanism whereby it forces plant responses.

Although ethylene has been employed commercially for over 25 years the exact mechanism by which it induces ripening or coloring is still not entirely understood. Both E. M. HARVEY (**13**) and R. B. HARVEY (**14**) have reported that ethylene causes the simple soluble substances in plants to increase at the expense of the higher, soluble and insoluble forms. Increased activities of hydrolytic enzymes in pineapples (**22**), pears, peaches, and lemons (peel) (**11**), have also been demonstrated, so that this effect of ethylene is generally known. IVANOV, PROKOSHEV, and GABUNYA (**15**) went so far as to state that "in our opinion the role of ethylene may be reduced to the acceleration of the internal disintegration or partial autolysis of the living cells." However, they also showed that catalase activity was reduced in fruits treated with ethylene while the amount of peroxidase was increased by this compound. These effects on hydrolytic and oxidative enzymes are seen to be extremely important when associated with NOACK's (**21**) explanation of the biological decomposition of chlorophyll. He proposed that the first step in degreening is the proteolysis of the plastid stroma and this would seem to be confirmed by Dufrenoy's cytological observations (**7**). Thus it would seem that the increased activity of proteolytic enzymes is the first process speeded up by ethylene. This hastens the breakdown of the protective lipo-protein complex (**26**) of the stroma and enables chlorophyllase to form the phyllins more rapidly. Also by causing the starch present in the plastid (**7**) to be hydrolyzed, material is supplied for the increased respiration which occurs, possibly because of the reduction in catalase. Noack further suggested that decomposition of chlorophyll occurs as a result of the oxidative action of hydrogen peroxide on the products of chlorophyll hydrolysis which are present in the form of their soluble alkali salts. Their oxidation is catalyzed by colloidal ferric hydroxide which is formed from the iron of the chloroplast. Since oranges have been shown not to contain oxidase (**17**) it seems likely that increased respiration is necessary to produce the H_2O_2 required for this oxidation, for the peroxidase present in oranges is probably more than sufficient to utilize all the H_2O_2 produced at normal respiration rates. However, it seems logical that when the respiration of fruits in a normal atmosphere was increased by irradiation during the 0 to 9-hour period H_2O_2 was not the limiting factor, but in this instance the increased respiration did not speed degreening because the hydrolytic enzymes had not broken down the plastids sufficiently to allow hydrolysis and oxidation of the chlorophylls.

From these observations the following hypothesis might be offered. The actions of ethylene and enzymes serve to hydrolyze the plastid stroma and produce materials which can be used in respiration. By this process, the chlorophylls are left unprotected, they are acted upon by chlorophyllase, and subsequently oxidized by H_2O_2 in the presence of a $Fe(OH)_3$ (or possibly a combination of $Fe(OH)_2$, $Cu(OH)_2$) catalyst. In this series of reactions the internal quality of the fruit is left unaltered because the activities are centered in the sub-epidermal layers of the peel.

The exact mechanism whereby ethylene acts upon the enzymes is not known. The idea of a co-enzyme relationship between ethylene and the oxidases as expressed by LYNCH (**16**) seems most feasible but should be extended to include the hydrolytic enzymes. It has been proposed by HARVEY (**14**) that the function of the ethylene double bond is to take on H^+ and OH^- ions and yield them easily to anhydrides. He also noted that the surface tension effects, solubility in aqueous and lipoid phases, as well as low molecular weight give this double bond compound properties not possessed by other compounds found in plants. Thus the reactions which cause degreening to occur are natural ones and the addition of ethylene is the most effective means known to make these processes occur at the maximum rate possible.

Summary

Using specially designed chambers, studies were made on the effects of various treatments on "degreening" and respiration of Florida oranges.

Several growth-modifying substances, applied either as an aerosol spray or as a vapor, did not appreciably affect the rate of degreening nor the rate of respiration. When certain volatile organic compounds were applied either as a pre-treatment or in conjunction with ethylene, no effect on degreening was noted, but there appeared to be a slight increase in respiration rate over that caused by ethylene alone. However this increased rate of respiration

was observed only during the 3-, 6-, and 9-hour determinations and did not appear in the 24-hour readings.

Treatments with radio-frequency radiations definitely accelerated respiration without affecting the rate of degreening.

An attempt was made to explain the observations recorded in these experiments by assembling what has been reported on the effects of ethylene on the physiology of the plant cell and especially the chloroplasts, thus postulating a theory which presents a comprehensive picture of the mechanisms involved in the loss of chlorophyll during the degreening of oranges.

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