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Respiratory Response, Ethylene Production, and Response to Ethylene of Citrus Fruit during Ontogeny

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ABSTRACT

The initial respiratory rates at 20 centrigrade of detached oranges (Valencia and navel), grapefruit, and lemons decreased during ontogeny. Small attached oranges respired at the same rate as detached fruits of the same weight, and cutting the pedicel produced no shock or injury stimulus to the respiratory rate. Small oranges and grapefruit (average weight about 15 grams) showed pseudoclimacteric respiratory patterns and produced ethylene. The height of the respiratory rise and the amount of ethylene produced decreased as the fruit increased in weight until the September 4th harvest, when the fruit weights were 120, 64, and 87 grams for grapefruit, Valencia, and navel oranges, respectively; at that time no respiratory rise or ethylene production was observed. The pattern for all subsequent harvest revealed no postharvest rise in the respiratory rates. Lemon fruit, in contrast, had a continuously decreasing respiratory rate at all stages of ontogeny. Exposure to 20 microliters of ethylene per liter induced an increase in the respiratory rate of all varieties at every stage of ontogeny; this was true also in young oranges and grapefruit following their respiratory rise and decline.

Evidence is presented that citrus fruits are nonclimacteric fruits.

The postharvest respiratory response of citrus fruits is a subject of considerable controversy. Biale (3, 4) classified citrus as nonclimacteric based on the respiratory pattern of mature fruit. Trout *et al.* (22) reported evidence of a climacteric rise for oranges at 4.4 to 10 C, especially for fruit picked early in the season. Recently, Aharoni (1) presented respiratory patterns for small oranges and grapefruit that were similar to those displayed by typical climacteric fruit; in addition, the production of ethylene by small oranges paralleled the respiratory pattern of climacteric fruit. However, as the fruit approached maturity, no climacteric-like respiratory pattern was observed. The initial respiratory rates of citrus fruit, during their development showed a rapid decline in the early stages of fruit growth followed by a gradual decline as the fruit became larger (1, 2, 21); but determinations were delayed after harvest, and so the rates may not represent rates of those exhibited while fruit was on the tree or immediately after harvest. The occurrence of the climacteric on the plant, by determination of the respiratory rate immediately after fruit detachment, has been reported for apples (14, 15), tomatoes (11), apricots (5), and olives (18). It appears that true climacteric fruits demonstrate this phenomenon on the plant; therefore, the classification of citrus is still undetermined.

Reported here are respiratory responses of orange, grapefruit, and lemon fruits, also the response of attached orange fruits, and the effect of detachment of orange fruits. The results are discussed relative to the changes during growth and development and in relation to the problem of the climacteric or nonclimacteric nature of citrus fruit.

MATERIALS AND METHODS

The fruits used in the experiments were obtained from the Citrus Research Center groves: orange, *Citrus sinensis* (L.) Osbeck, cultivars Valencia and navel; grapefruit, *Citrus paradisis* Macf., Marsh-Seedless; and lemon, *Citrus limon* (L.) Burn, Eureka. In the study to determine the response of attached fruit, and the influence on respiration of detaching the fruit, Valencia orange fruits grafted to seedlings, as described by Erickson (9), were used. The plant with a growing fruit attached was brought from the greenhouse to the constant temperature laboratory at 20 C. The fruits were placed in respiratory chambers in the dark, and the opening through which the stem passed was sealed with silicon grease. The respiratory rate was determined for 24, 48, and 72 hr before the pedicel was cut and for several days afterward. Prior to cutting the pedicel a continuous record was obtained for 1 hr before cutting and for 3 hr afterward.

The detached fruits were brought to the laboratory, weighed, measured, and placed in respiratory chambers at 20 C within 30 min of harvest. When field temperatures varied significantly from 20 C, the initial respiratory rates were influenced, but temperature determinations with thermocouples and the respiratory pattern indicated that small fruit equilibrated within 1 hr and larger fruit equilibrated within 3 hr. The evaluation of the initial respiratory rate and the pattern of Valencia oranges and lemon fruits began with fruit weighing less than 1 g and continued through to mature fruit for a total of 179 lemon fruits and 171 Valencia oranges. The determinations were made for a 23-hr period on individual fruits. The data for this portion of the study were summarized by averaging the weights and respiratory rates during the 23-hr experimental period for fruits in various weight categories as follows: less than 1 g, 1 to 4.9 g, 5 to 9.9 g; for 10-g intervals between 10 and 69.9 g, 70 and 89.9, 90 and 119.9; and all those above 120 g. Each group contained 14 to 15 fruits.

The postharvest respiratory pattern and ethylene production of Valencia and navel oranges, grapefruit, and lemons were determined for a 21-day period. Sampling began in June when the fruit weighed about 15 g and continued throughout the season. Six individual fruits of each variety, of nearly the same weights, were evaluated at each sampling date.

The rate of CO_2 production was determined by a model 215 Beckman infrared nondispersion CO_2 analyzer connected to a

Leeds and Northrup recorder. Glass jars of appropriate size, fitted with rubber stoppers, were used as the respiratory chambers. The jars were covered with black cloth to prevent possible photosynthetic activity of the fruit (21). The air stream, metered by calibrated capillaries through the chambers, was freed of CO_2 by bubbling it through a fritted gas dispersion tube into 2 N NaOH and then through water to humidify it. Anhydrous $CaCl_2$ was used to dry the gas stream before it entered the analyzer. The rate of flow and instrument sensitivity were adjusted to accommodate the CO_2 production of fruits of varying sizes. The rapid air flow and containers with small free volumes provided equilibrium in the system within 2 to 4 min, and thus responses could be determined with a minimum lag period. A switching device was used to direct the air stream from each of 12 fruit samples and the air background in sequence each hour to the analyzer. The readings taken hourly or at other convenient intervals were converted to microliters of CO_2 per liter from standard curves, and the respiratory rate was calculated as milliliters of CO_2 per kg per hr or milliliters of CO_2 per 1000 cm^2 per hr; fruit measurements were converted to surface area according to the tables compiled by Turrell (23).

Ethylene production was evaluated on individual fruit placed in chambers that were closed daily for 1 to 4 hr. An Aerograph Hi Fi model 600-C flame ionization gas chromatograph, equipped with a 6-ft $\times$ 1/8-inch column packed with 60 to 80 mesh activated alumina, was used to analyze 1-ml gas samples from the chambers for the presence of ethylene. Verification was made by using columns of various Poropak materials and cochromatography with authentic ethylene. In addition, gas samples were analyzed for CO_2 and O_2 in a dual column thermal conductivity gas chromatograph to insure against adverse conditions influencing the fruit response. The method of Pratt *et al.* (19) was used to treat citrus fruits with ethylene.

RESULTS

Effect of Detachment. The respiratory rates for fruits detached 24 hr after initiation of the experiment are given in Table I. The initial high rates were due to the higher greenhouse temperatures and possibly handling manipulations necessary to enclose the fruit in the respiratory chamber. After 6 to 12 hr a steady state was reached. Detaching the fruit caused no change in the respiratory rate immediately after the pedicel was cut nor in the trend for the next 24 to 48 hr. Fruits kept for 48 and 72 hr before detachment exhibited a slowly declining respiratory rate; no deviation in the pattern due to cutting was observed. Fruits attached to the plant in the dark showed a slowly decreasing respiratory pattern during a 21-day period. Most fruits abscissed after 15 to 20 days with no change in the respiratory rate associated with abscission.

Initial Rates and Trends. The average respiratory rates (ml CO_2/kg·hr) during the first 20 hr after harvest for 8 of the 12 weight categories of Valencia oranges are illustrated in Fig. 1; rates for 6 of the 12 weight categories of lemons are shown in Fig. 4. Fruits weighing less than 1 g had exceedingly high initial respiratory rates which decreased to a slowly declining rate in about 10 hr (Figs. 1 and 4). As the fruits increased in weight, the initial respiratory rates decreased and the rate of change with time was less accentuated. Figure 2 and 5 show for Valencia oranges and lemons, respectively, the average respiratory rates (ml CO_2/kg·hr and ml CO_2/fruit·hr) for the various weight categories: (*a*) 3 hr after harvest, representing an initial rate after temperature equilibrium had occurred; (*b*) the average of the rates from 3 to 12 after harvest (this corresponds to data obtained in other investigations when absorption or other techniques required a period of time to make the determination); and (*c*) 20 hr after harvest representing steady state conditions and data where the first determination was made about 1 day after harvest. Note that the extremely rapid decrease in milliliters of CO_2 per g per hr during the initial growth phase followed by a slow decline as the fruit increased in weight; also notice the differences between the rates shown for 3 and 20 hr after harvest for the small fruits. The milliliters of CO_2 per fruit per hr increased throughout the sampling period represented here, which included the normal commercial harvest season. Additional samplings of Valencia oranges were made as they became senescent. The milliliters of CO_2 per kg per hr continued to decline with age, but the fruit did not increase in weight; therefore, the milliliters of CO_2 per fruit per hr began to decline as the fruit became senescent. The respiratory rate expressed on the surface area basis (ml CO_2/1000 cm^2·hr) (Figs. 3 and 6) for Valencia oranges and lemons, respectively, follows essentially the same pattern as that when expressed on a weight basis (Figs. 2 and 5).

Table I. *Effect of Detachment on Respiratory Rate of Valencia Oranges*

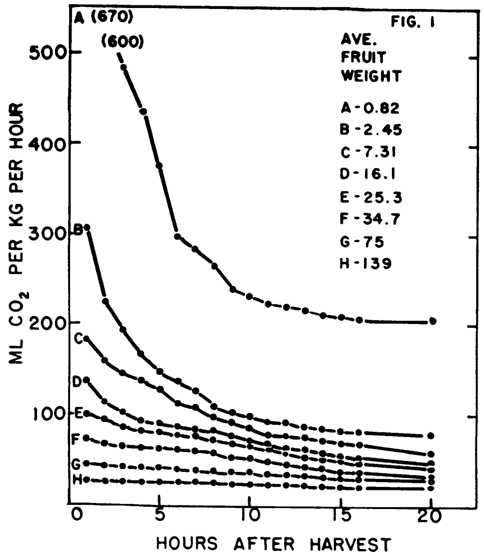
Pedicel cut outside the respiratory chamber 24 hr after the plant, with fruit attached, was brought from the greenhouse to 20 C laboratory and fruit was placed in a respiratory chamber. Except for the 1- and 3-hr averages, the coefficient of variability was less than 2.0%.

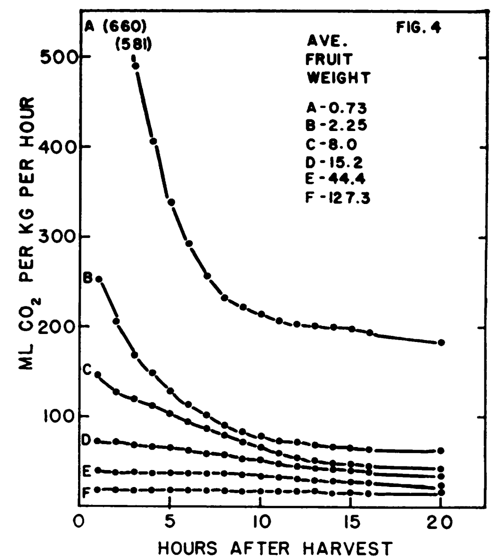
Time	Respiratory Rate		
	21-g fruit	53-g fruit	78-g fruit
hr	*ml CO_2/kg·hr*		
1	41.0	31.2	28.3
3	28.1	20.9	19.2
6	24.5	19.2	16.9
12	23.6	18.9	16.1
18	23.1	18.7	16.0
21	22.8	18.5	16.0
24[1]	22.9	18.5	16.1
25	23.0	18.4	16.3
26	22.8	18.6	16.1
27	22.7	18.4	16.0
30	22.1	18.1	15.8
36	22.0	17.8	15.3
42	21.5	17.7	15.0
48	21.2	17.8	15.1

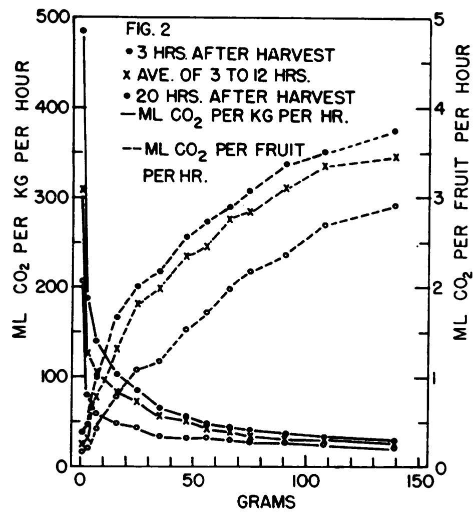
[1] Fruit detached after 24-hr determination.

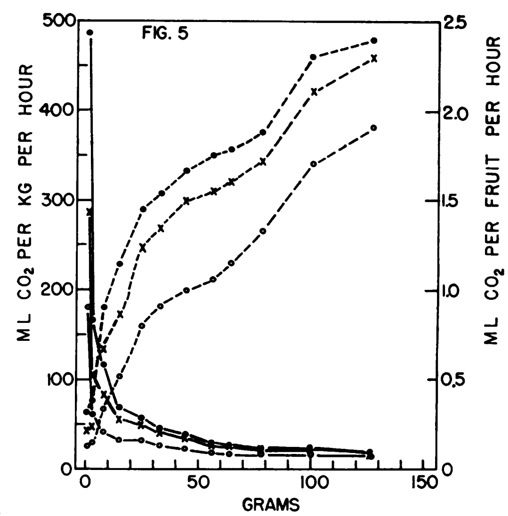
Respiratory Pattern and Ethylene Production. The average respiratory rates at 20 C of grapefruit, Valencia and navel oranges, and lemon fruits, 20 days after harvest, are presented in Figures 7 to 11. The initial rates and trends for Valencia and lemon fruits are similar to those presented previously (Figs. 1 and 3). However, 3 to 4 days after harvest the respiratory rates for small grapefruit, Valencia oranges, and navel oranges began to increase, rising to a maximum by the 5th to 8th day after harvest and then decreasing to a relatively steady state (Figs. 7–10). The actual height of the maximum and the ratio of the maximum to the pre rise minimum decreased as the fruit increased in size until no rise was observed for fruit sampled September 4 (Fig. 11); on that date the fruit weights were 120, 64, and 87 g for grapefruit, Valencia and navel oranges, respectively. The subsequent sampling of these varieties at monthly intervals revealed no postharvest rise in the respiratory rates. It will be noted that lemon fruit, in contrast, had a continually declining respiratory rate at all stages of development.

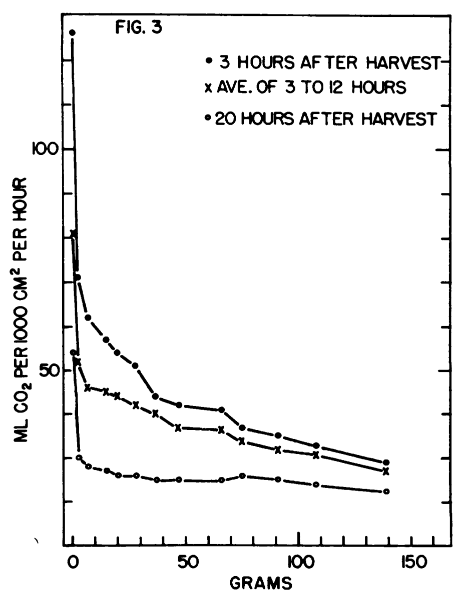
During the first 2 days after the June 3 harvest, the small fruits (respiratory rates shown in Fig. 7) produced no detectable ethylene. Starting on the 3rd day, the grapefruit, Valencia and navel oranges began producing ethylene at increasing rates, paralleling











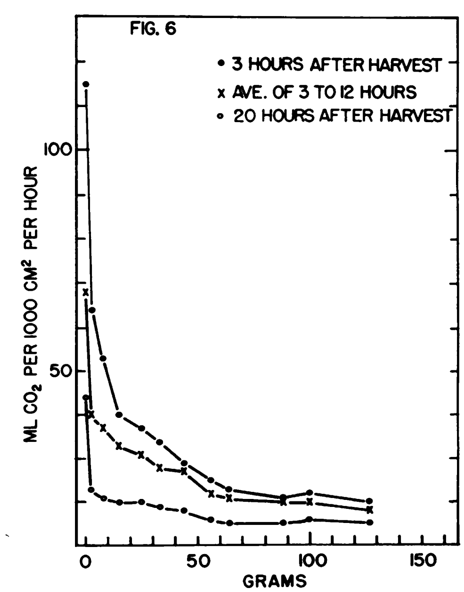


FIG. 1. Average respiratory rates (ml of CO_2/kg·hr) during the first 20 hr after harvest for 8 of the 12 weight categories of Valencia oranges.

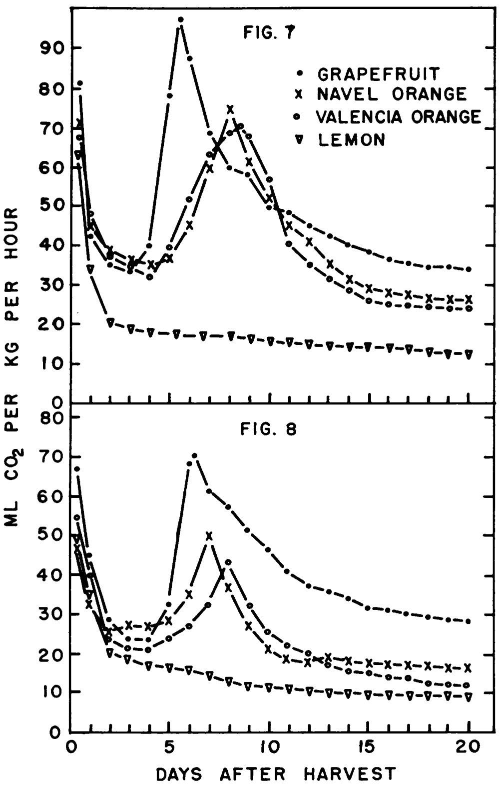
FIG. 2. Average respiratory rates (ml of CO_2/kg·hr and ml of CO_2/fruit·hr) for the various weight categories 3 hr after harvest, average of rates from 3 to 12 hr after harvest, and 20 hr after harvest of Valencia oranges.

FIG. 3. Average respiratory rates (ml of CO_2/1000 cm^2·hr) 3 hr after harvest, average of rates from 3 to 12 hr after harvest, and 20 hr after harvest of Valencia oranges.

FIG. 4. Average respiratory rates (ml of CO_2/kg·hr) during the first 20 hr after harvest for 6 of the 12 weight categories of lemon fruits.

FIG. 5. Average respiratory rates (ml of CO_2/kg·hr and ml of CO_2/fruit·hr) for the various weight categories 3 hr after harvest, average of rates from 3 to 12 hr after harvest and 20 hr after harvest. Identification of data given in Figure 2.

FIG. 6. Average respiratory rates (ml of CO_2/1000 cm^2·hr) during the first 20 hr after harvest of lemon fruits.



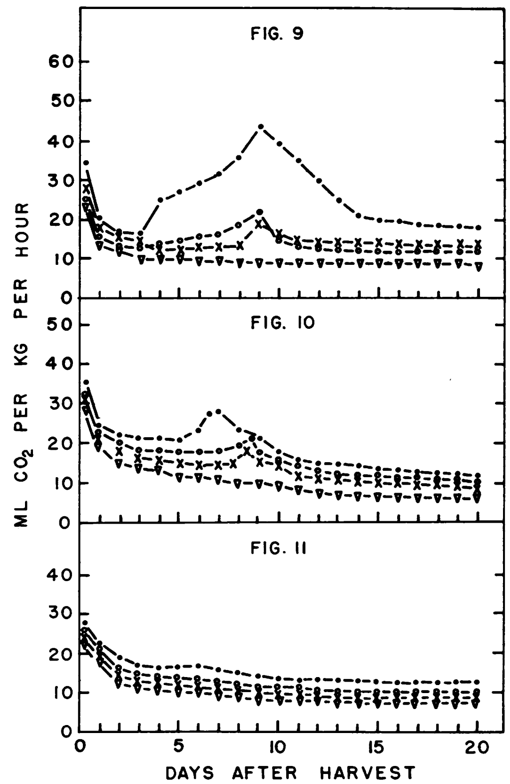


FIG. 7. Respiratory rates of citrus fruit harvested June 13. Fruit weight: grapefruit, 15 g; navel orange, 17 g; Valencia orange, 15 g; lemon, 14 g.
FIG. 8. Respiratory rates of citrus fruit harvested July 1. Fruit weight: grapefruit, 31 g; navel orange, 25 g; Valencia orange, 25 g; lemon, 24 g.
FIG. 9. Respiratory rate of citrus fruits harvested July 22. Fruit weight: grapefruit, 40 g; navel orange, 34 g; Valencia orange, 33 g; lemon, 31 g.
FIG. 10. Respiratory rate of citrus fruit harvested Aug. 16. Fruit weight: grapefruit, 62 g; navel orange, 70 g; Valencia orange, 50 g; lemon, 37 g.
FIG. 11. Respiratory rate of citrus fruit harvested Sept. 4. Fruit weight: grapefruit, 120 g; navel orange, 87 g; Valencia orange, 64 g; lemon, 61 g.

the rise in respiratory rates to a maximum of 5.1, 3.2, and 3.8 µl of C_2H_4/kg·hr, respectively, and coincident with the respiratory peak. Similar ethylene production patterns were observed for these varieties on successive harvest, but the peak rates decreased as the fruit increased in weight until the Aug. 16 harvest (respiratory rates shown in Fig. 10) when the peak rates were 0.4, 0.3, and 0.2 µl of C_2H_4/kg·hr for grapefruit, Valencia and navel oranges, respectively. Grapefruit, Valencia and navel oranges harvested Sept. 4 (Fig. 11) and those of all subsequent harvests produced no detectable ethylene (less than 0.01 µl/kg·hr). The lemon fruits of all weights failed to show a rise in the respiratory rate and produced no ethylene or only trace amounts (less than 0.01 µl/kg·hr). During the period that the chambers were closed to determine ethylene production, the CO_2 concentration therein did not exceed 1.0%, and the oxygen concentration remained above 20.0%.

Grapefruit, Valencia and navel oranges, which displayed a rise in the respiratory rates (Figs. 7–10) and produced ethylene in more than trace amounts, lost their green color, becoming yellow; abscission of the buttons also occurred. The color change began midway during the respiratory rise and was complete about 2 days after the respiratory maximum. The color change was less marked in the fruit harvested Aug. 16; it did not occur, during the 21-day experimental period, in fruit of these varieties harvested Sept. 4, or in subsequent harvests until the fruit was mature and began to show color break in the field. Also, the buttons normally remained attached on fruit sampled Sept. 4 and on all subsequent samples. Color changes were not observed in lemons until they neared commercial maturity.

Exposure to 20 µl of ethylene per liter induced a rise in the respiratory rate, color changes, and abscission of the buttons in all varieties at every stage of growth and development. Also, the respiratory rates of small fruits were stimulated again by exposure to ethylene after the rise shown in Figures 7 to 10. In fact, the respiratory rates of citrus fruits can be repeatedly stimulated by intermittent exposures to ethylene.

DISCUSSION

The respiratory rate of Valencia oranges is not significantly altered by detaching the fruit from the plant (Table I). Todd *et al.* (21) did not find significant differences between attached and detached Valencia oranges, but they gave single values for fruits of various sizes without indicating the time period involved, which is an important factor. In the present investigation data were obtained on fruits 1 to 3 days before the pedicel was cut and a continuous record was obtained of the respiratory rate for 1 hr before fruit detachment to 3 hr after; these data revealed no shock or injury stimulus as a result of cutting the pedicel. Therefore, fruits sampled from trees in the field give a true respiration response of the fruit during its growth and development on the tree. However, fruit temperatures at the time of harvest do influence the initial readings, as previously outlined. Also, the fruits must be carefully handled because citrus fruit respiration is sensitive to bruising or pressure damage (7, 24, 25).

The respiratory rate, immediately after harvest, decreased during ontogeny of the citrus fruits (Figs. 1–6); this is in accord with previous reports by Aharoni (1), Bain (2), and Tood *et al.* (21). However, Luthra and Chima (17) reported decreasing respiratory rates for Malta oranges during growth, from 75.56 mg of CO_2/100 g·hr 5 days after fruit set to 6.05 mg of CO_2/100 g·hr for 200-day-old green fruit. These rates were determined by CO_2 absorption during a 24-hr period after harvest at 30 C. Samples taken 230 and 245 days after setting, when the fruits were changing color from green to orange, respired at 10.00 and 15.00 mg of

CO_2/100 g·hr, respectively. Samples taken 260 and 290 days after setting (full color having developed) respired at 4.50 and 3.75 mg of CO_2/100 g·hr, respectively. This is a characteristic pattern of typical climacteric fruits (3, 5, 11, 14, 15). However, this response cannot be considered typical for citrus, especially since no other report in the literature confirm this response nor does the present study.

The rise in the respiratory rate of small oranges and grapefruit is considered a pseudoclimacteric, not a true climacteric. Similar rises were observed in small immature olives (18), apricots (5), peaches and plums (20), and apples (14, 15); the increase tended to disappear as the fruit grew larger and, except for apples, did not reappear when the fruit reached horticultural maturity. The responses observed in oranges and grapefruit, reported here, appear to be due to the ethylene produced and are similar to those reported by Aharoni (1). However, in this study, unlike the present one, the respiratory rise persisted in larger navel oranges and grapefruit (110 and 180 g, respectively). Further evidence that this is not a typical autogenous climacteric is seen in the fact that after the respiratory rise and decline these small fruits responded to externally applied ethylene and could be repeatedly stimulated by ethylene.

No explanation can be given for the rather large quantities of ethylene produced by the small fruits but not by the larger ones. The response of lemon fruits is an anomaly. The respiratory response following application of ethylene to oranges, grapefruit, and lemons at all stages of growth and development is typical of nonclimacteric fruits; even senescent citrus fruit respond. The application of ethylene to postclimacteric apples (13), pears (12), and bananas (10) did not stimulate the respiratory rate. The "climacteric" rise reported by Trout *et al.* (1960) for oranges at 4.4 to 10 C may be, as the authors indicated, a response to chilling injury. Sweet potato roots (16) and cucumber fruits (8), both nonclimacteric products, displayed similar respiratory stimulations when held in the temperature range used by Trout *et al.* (22). In addition, respiratory rates of oranges, sweet potatoes, and cucumbers at 20 C after exposure to 0, 5, or 10 C were considerably greater than fruits exposed directly to 20 C, whereas the respiratory rate at 20 C following exposure to 0 or 10 C of carrots, a crop not subject to chilling injury, was not greater than that of roots placed directly at 20 C (6). Furthermore, the extrapolation made by Trout *et al.* (22) leading to the conclusion that the climacteric maximum would be reached in less than 1 day at 20 C and, therefore, possibly would not be detected by other investigations does not describe a typical climacteric response; no such evidence was found in the present study for fruits of comparable size and maturity.

Citrus fruits should be classified as nonclimacteric, considering the evidence presented regarding their respiratory response during ontogeny, their response to ethylene, and the absence of physical and chemical changes normally associated with fruit ripening. The respiratory rise observed for small immature oranges and grapefruit by Aharoni (1), as well as in the present study, is a response to an unexplained stimulation of ethylene production by these small fruits

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