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Respiration during Postharvest Development of Soursop Fruit, *Annona muricata* L.

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ABSTRACT

Fruit of soursop, *Annona muricata* L., showed increased CO_2 production 2 days after harvest, preceding the respiratory increase that coincided with autocatalytic ethylene evolution and other ripening phenomena. Experiments to alter gas exchange patterns of postharvest fruit parts and tissue cylinders had little success.

The respiratory quotient of tissue discs was near unity throughout development. 2,4-Dinitrophenol uncoupled respiration more effectively than carbonylcyanide *m*-chlorophenylhydrazone; 0.4 millimolar KCN stimulated, 4 millimolar salicylhydroxamic acid slightly inhibited, and their combination strongly inhibited respiration, as did 10 millimolar NaN_3. Tricarboxylic acid cycle members and ascorbate were more effective substrates than sugars, but acetate and glutarate strongly inhibited.

Disc respiration showed the same early peak as whole fruit respiration; this peak is thus an inherent characteristic of postharvest development and cannot be ascribed to differences between ovaries of the aggregate-type fruit. The capacity of the respiratory apparatus did not change during this preclimacteric peak, but the contents of rate-limiting malate and citrate increased after harvest.

It is concluded that the preclimacteric rise in CO_2 evolution reflects increased mitochondrial respiration because of enhanced supply of carboxylates as a substrate, probably induced by detachment from the tree. The second rise corresponds with the respiration during ripening of other climacteric fruits.

The climacteric nature of the ripening of Annonaceous fruit was discovered by Biale and Barcus (2). They ascribed the irregular increase in oxygen consumption after harvest to the structure of the fruit being "aggregates of many ovaries", reflecting "changes of variable tissues in several physiological stages." Later studies (3, 11, 15) confirmed the irregular shape of the respiratory curve of postharvest *Annona* fruit, and also showed that the increase in ethylene evolution lags days behind the onset of the respiratory rise. Although it is generally agreed that the climacteric respiration depends on autocatalytic ethylene production (21), and that the other ripening phenomena follow or at most coincide with the rise in ethylene (4), the early respiratory rise in *Annona* fruit appears to be ethylene independent.

In the present study, the relationship between respiration and ethylene production and the nature of the former process are analyzed in postharvest fruit of soursop, *Annona muricata* L.

MATERIALS AND METHODS

Whole and Quartered Fruits and Tissue Cylinders. Two to four mature, unripe soursop (*Annona muricata* L.) fruit, harvested every Monday morning at the Waiakea Agricultural Experiment Station on the island of Hawaii, were flown to Honolulu and used that afternoon for experimentation.

Whole fruits were weighed and placed into 3.2- or 6.4-L glass jars, sealed with modeling clay. Ethylene-free, outdoors air was passed through the jars at 200 to 250 ml min^{-1}, and the outflowing air was automatically monitored every 1 h for its content of CO_2 (IR gas analyzer, Infrared Industries Inc., Santa Barbara, CA) and ethylene (Varian 1400 gas chromatograph with Al_2O_3 column at 90°C and photoionization detector at 110°C). Fruits were temporarily removed from the jars to obtain tissue cylinders and for the preparation of discs (see below). The wound surface was treated with a 20% solution (w/v) of PEG 6000 containing 1% of the fungicide, Benomyl (50% active ingredient, E.I. DuPont de Nemours and Co. Inc.).

In some experiments, a fruit was cut longitudinally into quarters to enable different pretreatments to tissue of the same age. The wound surface was treated as above, leading to the rapid formation of a thin but firm secondary skin, underneath which the tissue remained in a healthy condition. After pretreatments, the gas exchange of the fruit quarters was monitored as described above.

In other experiments, cylinders of flesh tissue were obtained with an ethanol-sterilized cork borer, 10 mm diameter. The cylinders were weighed and about 20 g samples infiltrated with aqueous solutions of growth regulators *in vacuo*. After superficial drying, the samples were transferred to 470-ml jars lined with water-saturated filter paper. In later experiments, tissue cylinders of 22 mm diameter were cut into about 5-mm-thick segments, four of which per 470-ml jar were placed on three layers of 7 cm diameter Whatman No. 1 filter paper to which 20 ml solution was added. The gas exchange of the jars was monitored as described above using an air flow of 40 to 50 ml min^{-1}.

Fruit discs. The CO_2 and ethylene evolved were mainly produced by the fruit flesh (and skin), the contribution of the seeds being small. For the determination of RQ[2] values discs about 4 mm thick were manually sliced under semi-sterile conditions from 7-mm-diameter cylinders of fruit flesh, obtained with an ethanol-sterilized cork borer. Weighed samples of four randomized discs were immediately transferred to Warburg flasks without bathing medium to avoid different solubilities of respiratory gases, and with or without 0.2 ml 10% NaOH-solution in the central well. Gas exchange was determined with a Gilson respirometer.

In the other experiments, discs about 1 mm thick were simi-

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[2] Abbreviations: RQ, respiratory quotient; ψ, water potential; DNP, 2,4-dinitrophenol; CCCP, carbonylcyanide *m*-chlorophenylhydrazone; SHAM, salicylhydroxamic acid; NAA, 1-naphthylacetic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; AOA, (aminooxy)acetic acid; AVG, aminoethoxyvinylglycine; CHI, cycloheximide; CAP, chloramphenicol.

larly prepared, and weighed samples of randomized discs were immediately suspended in the final medium to minimize osmotic shock and membrane damage.

Water Potential. Water potential, ψ, was determined by immersing samples of 6 to 10 discs in sorbitol solutions ranging from 200 to 1200 mOsmol, and weighing the samples from time to time after blotting, until constant weight.

Respiration Studies. To measure the rate of respiration, weighed samples of usually 10 discs were suspended in 4 ml medium per Warburg flask, fitted with sidearm and inner well. Since mono- and divalent cations may affect membrane stability (23) and ripening characteristics differently (22), the cation composition as well as water potential and acidity of the media were made similar to those of the fruit tissue. The cation content varied per fruit and within the fruit being, on average, in $\mu eq \cdot g^{-1}$ fresh weight: Ca, 5.2; Mg, 9.2; K, 51; and Na, 1.8. The bathing media therefore contained 3 mM $CaCl_2$, 5 mM $MgCl_2$, and 50 mM KH_2PO_4, titrated with drops of 2 M H_3PO_4 to pH 4.0. They were made about isotonic to 630 mOsmol with sorbitol. In many experiments, the sorbitol was wholly or partly replaced by isotonic amounts of respiratory substrates. When these substrates were carboxylates, the free acids were weighed out, dissolved, and brought to pH 4.0 with drops of 10 M NaOH solution before being made to volume. If substances had to be dissolved in organic solvent, instead of ethanol to which disc respiration strongly responded, inert dimethylformamide was used (18).

Analysis of Organic Acids and Soluble Carbohydrates. HPLC analyses were performed as described by Paull *et al.* (16), except that an automatic injector and integrator calculating the amounts from peak area were used.

The statistical measure used in the tables and figures is the standard deviation, ρ_{n-1}.

RESULTS

Intact and Quartered Fruits and Tissue Cylinders. The typical gas exchange of an intact fruit in the flow-through system is depicted in Figure 1. On the 1st d after the day of harvest, CO_2 evolution increased and went through a first peak on the 2nd d. At that time, ethylene production was not yet detectable with this system, but sampling from closed jars revealed production rates of, *e.g.* 6 $nl \cdot kg^{-1} \cdot h^{-1}$, only 6×10^{-5} of the maximum rate in Figure 1. The steady production rate varied from fruit to fruit, the highest rate determined being 3 $\mu l \cdot kg^{-1} \cdot h^{-1}$.

After a steady decrease, the CO_2 output rose again from the 3rd d on. This second rise invariably coincided with the increase in ethylene evolution. Plotting the CO_2 and ethylene values during the start of their increase against each other indicates that the ethylene level had to surpass a threshold value before the respiratory rise could occur (Fig. 2). Since the dose-response curve of ethylene action is semilogarithmic, the ethylene data were plotted on a log scale. During this stage of autocatalytic ethylene production, closely resembling the ripening of other climacteric fruit, other ripening changes also took place: flavor development, skin browning, and softening. The water potential, ψ, of the tissue cells dropped rapidly (Fig. 1), partly because of loss of wall pressure. In the course of day 5, a rapidly developing membrane leakage prevented further determination of ψ.

Beyond that stage, the ethylene production dropped very rapidly, but respiration rose again owing to catabolic processes accompanying cell death and fungal development.

We tried to influence the occurrence of the first and second respiratory rises and of ethylene evolution by pretreatments with 2% to 4% O_2, 5 to 10 $\mu l \cdot l^{-1}$ ethylene, or 400 $\mu l \cdot l^{-1}$ HCN. Because of the different maturity of different fruits, a single fruit was longitudinally divided into four quarters, one for each treatment and an air control. The treatments were given during 15 or 6 h on the night after harvest or the following morning, respectively.

The first hour(s) after cutting, 1 to 5 $\mu l \cdot kg^{-1} \cdot h^{-1}$ wound ethylene was formed, except at the low oxygen treatment. The quarters remained in a healthy condition throughout the duration of the experiments. Although in the experiments, of necessity without replications, differences in CO_2 and ethylene productions were observed, these differences were too variable and irreproducible to allow reliable conclusions.

Another approach was the treatment of tissue cylinders by infiltration *in vacuo* with solutions of growth regulators affecting the biosynthesis or action of ethylene. Although such cylinders absorbed 19% to 21% of their fresh weight during infiltration, solutions at usual concentrations had no effect at all, probably owing to apparent lack of oxygen. Only with extreme concentrations could reproducible effects be obtained. For instance, at 1 mM NAA or 1 mM $AgNO_3$, concentrations preventing any ethylene production, the cylinders remained in their original, firm and juicy state, whereas 20 mM ACC led to tissue softening. Also because cylinders given the two control treatments (no infiltration and water infiltration) dried out without becoming soft, it was concluded that the results of these treatments could not be compared with normal ripening. Instead, cylinder segments were placed on filter paper drenched with the solutions to prevent lack of moisture and of oxygen. Two mM ACC induced softening, but even 1 mM AOA or AVG plus 0.05 mM $CoCl_2$ failed to prevent ethylene evolution, so that apparently solute penetration was inadequate under these circumstances. The points of time of the two respiratory rises could never be changed and (wound) ethylene often occurred from the beginning. Attempts to affect the relationship between CO_2 and ethylene evolution were thereupon discontinued.

Respiration of Tissue Discs. The course of the RQ during postharvest development was determined for two reasons. First, some of the irregularities in CO_2 evolution (Fig. 1) might result from (de)carboxylations not connected with respiration. Second, respiration data from whole and quartered fruits, measured as CO_2 evolution, had to be compared with those from tissue discs in Warburg flasks, determined as O_2 uptake. For these reasons, at different stages of postharvest development, particularly at the early ones, the gas exchange of dry tissue discs was determined in the absence and presence of NaOH solutions. In nine experiments with four fruits, the RQ was slightly below unity (RQ = 0.937 ± 0.067). The variation mainly occurred between individual fruits, the RQ remained constant throughout postharvest development.

The rate of basal respiration of tissue discs in a suitable medium was always higher, at the same temperature, than that of the fruit from which they were freshly prepared. Figure 3 shows that disc respiration varied with that of the fruit, being temporarily enhanced at the preclimacteric peak.

The basal respiration of discs could be enhanced by the addition of substrates. According to Paull *et al.* (16), sucrose, glucose, fructose, and organic acids, particularly malate, accumulate in postharvest soursop fruit. Disc respiration increased only slightly when the sorbitol was replaced by one of the soluble sugars, but malate strongly promoted O_2 uptake (Table I). After removal of the constraint of oxidative phosphorylation by addition of the uncoupling agent DNP, malate was the only substrate further accelerating electron transport. The stimulating effect of malate was not specific, being also exerted by other carboxylates, mostly members of the tricarboxylic acid cycle (Table II). Tricarboxylic acid cycle intermediates often promoted respiration to the same extent. Sometimes higher concentrations of, *e.g.*, succinate became inhibitory during the 4 h of experimentation. On the contrary, saturating concentrations could not be obtained for malate and citrate (Fig. 4).

The most potent stimulator of electron transport was ascorbate, iso-ascorbate being also a suitable electron donor (Table

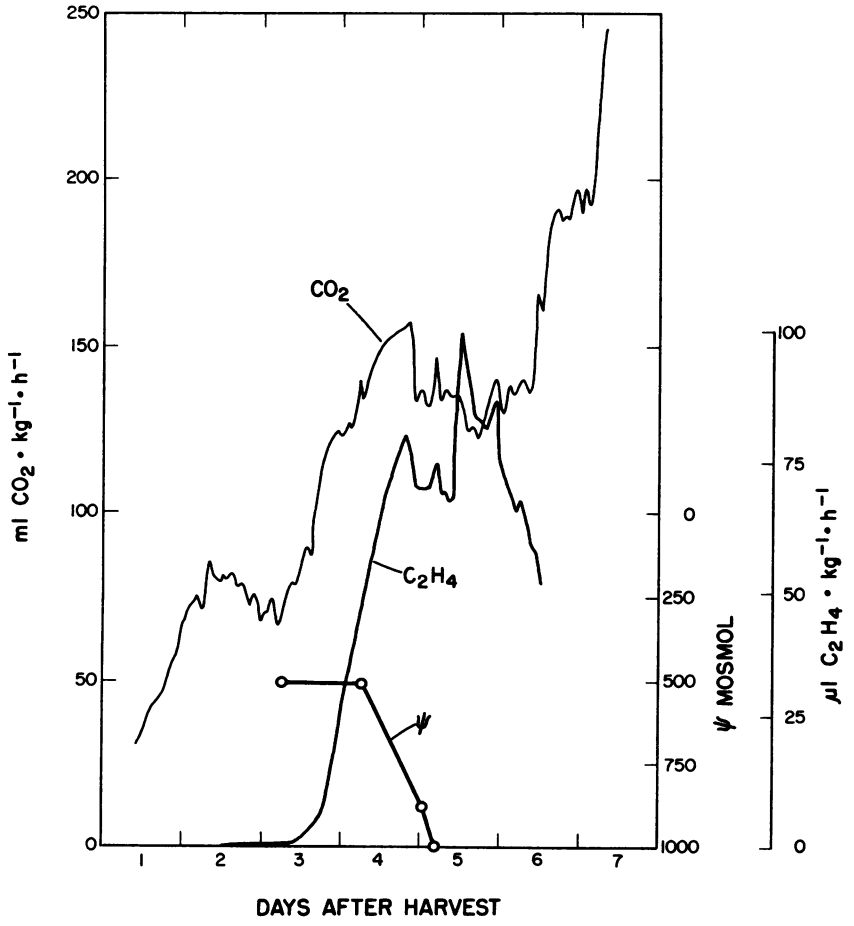

FIG. 1. Typical course of postharvest evolution of CO_2 and ethylene from intact fruit, and of the water potential, ψ, of tissue discs.

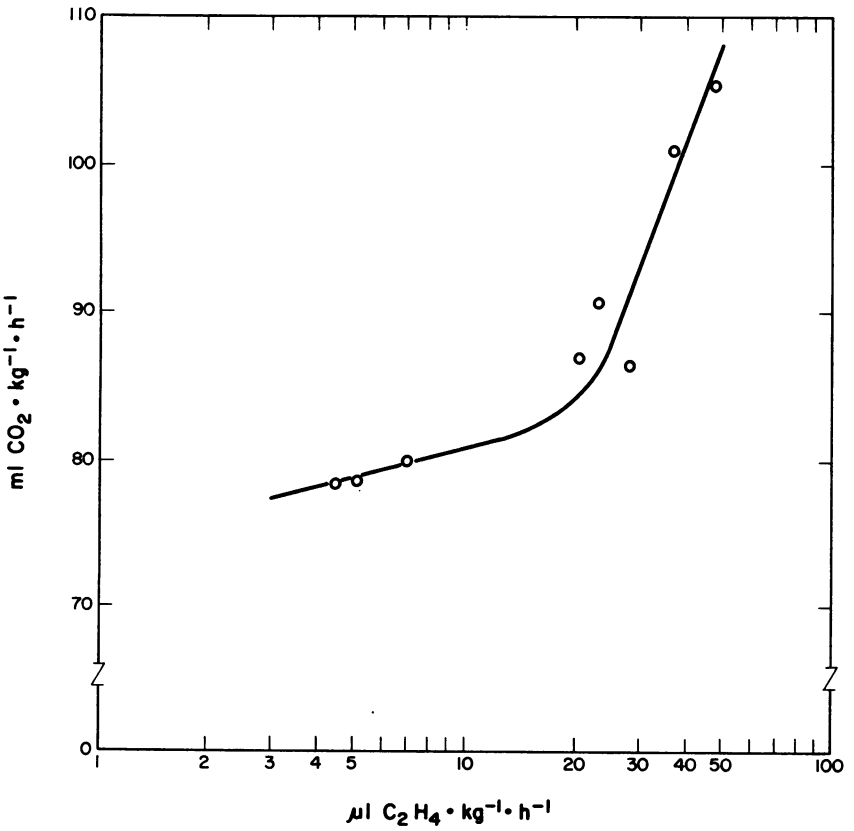

FIG. 2. Relationship between evolution of CO_2 and ethylene at the onset of the autocatalytic ethylene production and the second respiratory rise with the fruit of Figure 1 on day 3.

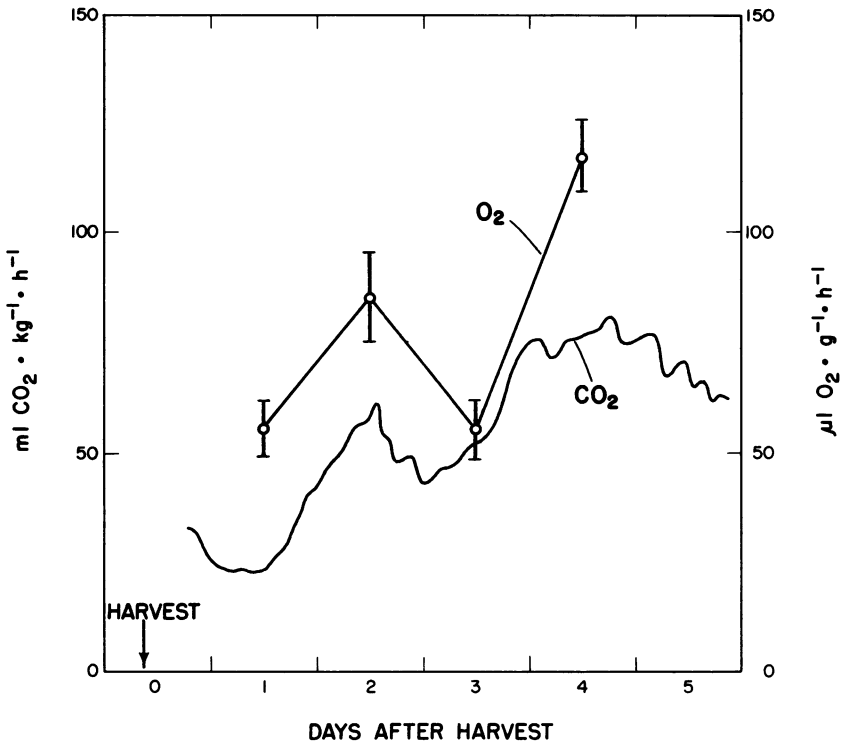

FIG. 3. Carbon dioxide production of a whole fruit and O_2 uptake of freshly prepared discs from that fruit, both at 24.5°C. Fruit respiration was monitored hourly, the respiration of 10 discs per flask was determined during 180 min in triplicate in a Gilson respirometer, the medium containing inorganic salts and 0.5 M sorbitol, at pH 4.0.

Table I. *Effects of Substrates and an Uncoupler on Disc Respiration*

Triplicate experiment at 24.5°C with 10 discs per flask in media in which sorbitol was replaced by isotonic amounts of sugars or malate, at pH 4.0. After 90 min, DNP was added to a final concentration of 20 μM, and respiration was determined again after 30 min adaptation.

	Rate of O_2 Uptake	
	− DNP	+ DNP
	$\mu l \cdot g^{-1} \cdot h^{-1}$	
Sorbitol—control	87 ± 6	142 ± 10
Sucrose	96 ± 2	135 ± 6
Glucose	104 ± 11	148 ± 9
Fructose	96 ± 1	145 ± 4
Malate	139 ± 11	179 ± 16

Table II. *Effects of Carboxylates on Disc Respiration*

Duplicate experiments at 24.5°C with 10 discs per flask in media containing inorganic ions and isotonic amounts of carboxylates instead of sorbitol, at pH 4.0.

	Rate of O_2 Uptake		
	Exp. 1	Exp. 2	Exp. 3
	$\mu l \cdot g^{-1} \cdot h^{-1}$		
Sorbitol—control	63 ± 3	71 ± 4	70 ± 1
Malate	94 ± 1	107 ± 4	
Succinate	91 ± 6		
Citrate	91 ± 6		
cis-Aconitate		70 ± 15	
trans-Aconitate		40 ± 3	
Oxoglutarate		98 ± 7	
Glutarate		0	
L-Ascorbate			165 ± 9
D-Ascorbate			105 ± 5
Acetate	4 ± 3		

II). Glutarate and acetate completely blocked oxygen uptake at substrate level, at 0.15 and 0.25 M, respectively. Lower concentrations of glutarate and acetate also proved inhibitory. Because these acids might inhibit enzymes of the tricarboxylic acid cycle competitively, their inhibition was followed at different levels of the supposed enzyme substrate. However, higher substrate levels were unable to substantially alleviate the measure of inhibition (Table III).

The inhibitory effect of acetate could not be prevented by the simultaneous addition of DNP. On the contrary, the partial inhibition by 25 mM acetate was reinforced by the uncoupler (Fig. 5). Since DNP exerted a similar inhibitory effect at excess concentrations (data not shown), this may indicate a reduction of the electron transport capacity by acetate.

CCCP stimulated electron flow, but to a considerably smaller extent than did DNP (Table IV). The effect of CCCP was initially diminished because this substance inhibited O_2 uptake before becoming stimulatory.

Effects of inhibitors of electron transport on disc respiration are summarized in Table V. The Cyt pathway inhibitor, cyanide, did not inhibit at first; rather, it promoted O_2 uptake. After 24 h, no respiratory activity was left. Together with the inhibitor of the alternate path, SHAM, which had little activity of its own, KCN strongly repressed respiration. Azide inhibited respiration considerably, and at moderate concentrations its action was enhanced by SHAM. The other inhibitor of the Cyt electron transport chain, antimycin A, the inhibitor of the alternate path, disulfiram, and the inhibitor of ATPase, oligomycin B, all were completely inactive, probably because of failure to penetrate the tissue discs.

Since the basal respiration of the discs varied like the respiration of the whole fruit in the course of postharvest development (Fig. 3), the capacity of disc respiration might be expected to change during the preclimacteric rise. This could not be studied by keeping discs *in vitro* for prolonged durations because isolated discs age as shown by their invariably increased respiration rates (Table VI). Inhibitors of cytoplasmic (CHI) and mitochondrial (CAP) protein synthesis reduced aging, respiration of discs stored

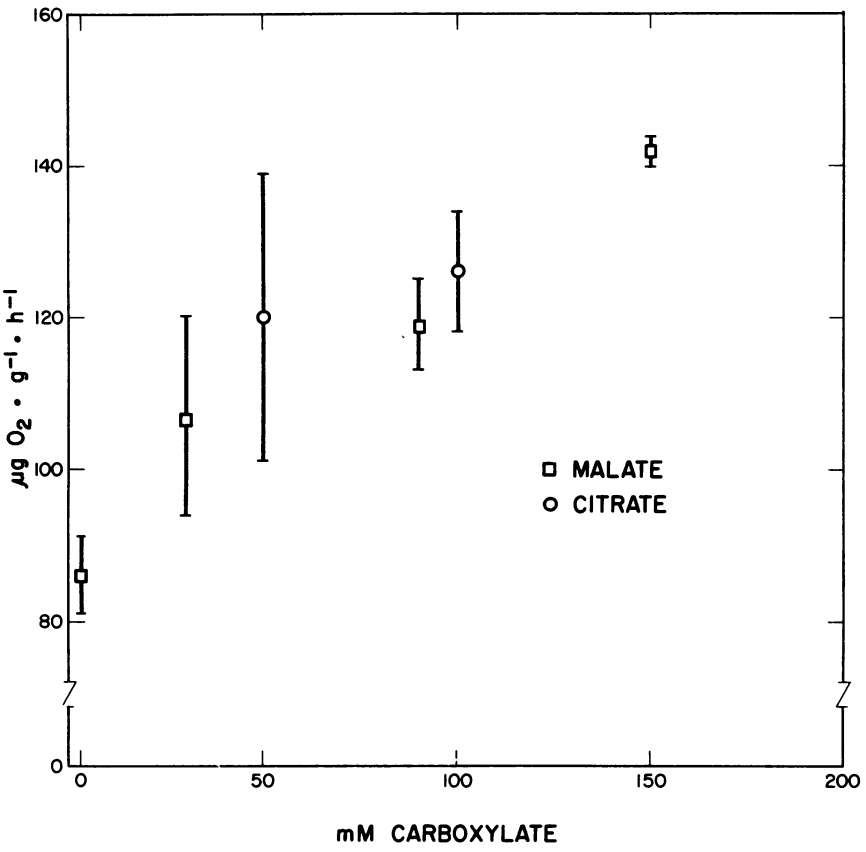

FIG. 4. Stimulation of disc respiration by malate and citrate. Triplicate experiment at 24.5°C with 10 discs per flask in which sorbitol was partly or wholly replaced by isotonic amounts of malate or citrate, pH 4.0.

Table III. *Inhibition of Oxoglutarate-Stimulated Disc Respiration by Glutarate*

Experiment at 24.5°C and pH 4.0 with 10 discs per flask in media in which sorbitol was partly or wholly replaced by oxoglutarate. After 90 min, glutarate was added. The data are from single determinations 150 to 240 min after start of the experiment.

	Rate of O_2 Uptake at Following Oxoglutarate Concn. (mM)			
	20	40	80	160
	$\mu l \cdot g^{-1} \cdot h^{-1}$			
No addition	80	92	111	121
25 mM Glutarate	71	49	58	103
50 mM Glutarate	26	44	32	31
100 mM Glutarate	27	13	16	12

in vitro being more similar to that of freshly prepared discs.

Fresh discs were used for determination of respiratory capacity during the development of the preclimacteric rise, by stimulating their rate of oxygen uptake as much as possible by the addition of substrates and uncouplers (Table VII).

Apart from a slightly different reaction to ascorbate with or without DNP, the respiration on the 2 subsequent days was remarkably similar in its response to substrates and uncouplers. Even the originally different levels of basal respiration, characteristic for the discs at the two stages of ripening, approached each other in the course of the experiments.

Since the respiratory apparatus apparently did not change during the preclimacteric rise, the substrate levels were determined in samples taken simultaneously from the same fruit at the 2 subsequent days at which the discs used for Table VII were prepared. The results (Table VIII) are in agreement with those obtained earlier by Paull *et al.* (16). They show that, whereas the level of soluble carbohydrates did not vary greatly, the two main carboxylates, particularly malate, increased considerably during the preclimacteric rise.

The chromatogram of the organic acids showed a third peak at nearly the same retention time as glutaric acid, from which it could not be separated at co-chromatography (Fig. 6). However, since this small peak only slightly changed and increased, the higher respiration at the preclimacteric peak cannot be ascribed to decrease in concentration of an inhibitory organic acid.

DISCUSSION

Experiments with mature whole fruits, as in Figure 1, demonstrate that the production of CO_2 first increases about 2 d after harvest and several days ahead of the rise in ethylene evolution. Data obtained from tissue disc respiration, showing a constant RQ value slightly below unity throughout, indicate that all postharvest CO_2 evolution arises from respiration without participation of any other major decarboxylation reaction.

To the current view first expressed by Biale and Barcus (2), that the irregular shape of the respiratory curve of postharvest soursop and other Annonaceous fruit is caused by different developmental stages of its constituent ovaries, the alternative can be proposed that a respiratory rise, as normally encountered with climacteric fruit, is preceded by a preclimacteric peak. Two arguments underlie the latter proposal. First, the second respiratory rise coincides with autocatalytic ethylene evolution that, according to Figure 2, has first to surpass a threshold value. This was also found in, *e.g.*, tomato (20) and is characteristic of the climacteric type of fruit ripening (21). Also, the other processes typical of soursop ripening occur simultaneously. Second, the observation that the respiration of isolated tissue discs follows the same pattern as whole fruit respiration (Fig. 3) proves that the preclimacteric peak is a characteristic of the respiration of any part of the fruit flesh and not brought about by a few

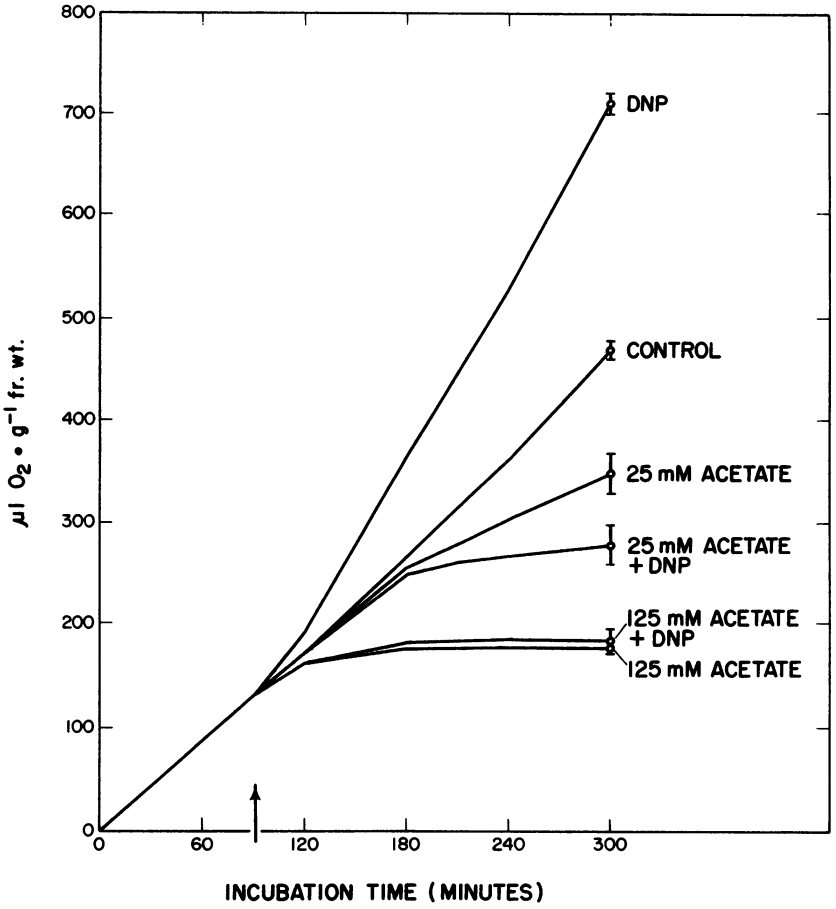

FIG. 5. Effects of acetate and DNP on disc respiration. Duplicate experiment at 24.5°C with 10 discs per flask in media in which sorbitol was not or partly replaced by isotonic amounts of acetate, at pH 4.0, added together with 25 μM DNP (final concentration) after 90 min.

Table IV. *Effect of Uncoupling Agents on Disc Respiration*

Triplicate experiments at 24.5°C with 10 disks per flask in media containing, with the inorganic ions, 0.5 M sorbitol or 0.15 M malate, at pH 4.0. Uncouplers added after 90 min, total duration 240 min.

Addition	Rate of O_2 Uptake		
	None	10 μM DNP	10 μM CCCP
	$\mu l \cdot g^{-1} \cdot h^{-1}$		
Sorbitol	44 ± 2	103 ± 6	63 ± 1
Malate	81 ± 6	126 ± 8	96 ± 5

Table V. *Effects of Inhibitors on Discs Respiration*

Two triplicate experiments at 24.5°C with 10 discs per flask in medium containing 0.15 M malate, pH 4.0. Inhibitors were added after 90 min. The data are respiration rates from 150 until 240 min after start of the experiment.

Addition	Rate of O_2 Uptake	
	Exp. 1	Exp. 2
	$\mu l \cdot g^{-1} \cdot h^{-1}$	
None	116 ± 6	114 ± 2
0.4 mM KCN	132 ± 4	
4 mM SHAM	91 ± 7	
0.4 mM KCN + 4 mM SHAM	6.0 ± 1.5	
10 mM NaN_3		7.1 ± 2.2
1 mM NaN_3		28 ± 8
1 mM NaN_3 + 4 mM SHAM		4.2 ± 0.7

Table VI. *Basal Respiration Rates of Fresh Discs and Discs Aged in the Absence or Presence of Inhibitors of Protein Synthesis*

Two duplicate experiments on subsequent days at 24.5°C with 10 discs per flask in sorbitol medium, pH 4.0. Aging during 16 h overnight in the same medium, with or without 0.1 mM CHI or 0.1 mM CAP.

	Rate of O_2 Uptake	
	Exp. 1	Exp. 2
	$\mu l \cdot g^{-1} \cdot h^{-1}$	
Previous day (fresh)	54 ± 2	68 ± 7
Aged	102 ± 4	96 ± 4
Aged in CHI	74 ± 1	78 ± 7
Aged in CAP	75 ± 9	88 ± 4
Fresh discs	68 ± 7	79 ± 4

advanced ovaries. Also, it is a common observation that the fruit ripens and softens as a whole.

Attempts to experimentally modify the gas exchange in postharvest fruit parts and tissue cylinders met with little success. Inhibitors of ethylene synthesis and action failed to change the times at which the respiratory rises occurred. This may well be because, apart from other experimental difficulties, all samples had of necessity to be subjected to the perhaps overriding pretreatment of the harvest. The effects of cutting off the supplies from the vegetative parts will be further discussed below. Experimentation with fruits still attached to the trees, difficult as this may already be in the case of soursop, was not feasible under our circumstances where the fruit was grown on another island.

The other way to further analyze the nature of respiration during postharvest fruit development was a study of the respiratory apparatus in tissue discs, especially before and during the

Table VII. *Respiratory Capacity of Discs from a Fruit before and during the Preclimacteric Peak*

From a fruit of which the development of the preclimacteric peak was monitored by hourly determination of CO_2 evolution, discs were prepared on two subsequent days for duplicate experiments at 24.5°C with 10 discs per flask in media containing 0.5 M sorbitol, or 0.25 M sorbitol plus 0.075 M succinate ('Succinate'), or 0.25 M ascorbate, pH 4.0. Twenty μM DNP or 10 μM CCCP were added after 90 min. Total duration of the experiments was 240 min.

	Rate of O_2 Uptake			
	Before		During preclimacteric peak	
	First 90 min	Last 90 min	First 90 min	Last 90 min
Sorbitol	52 ± 7	63 ± 1	75 ± 6	69 ± 1
+DNP		127 ± 6		129 ± 10
+CCCP		73 ± 3		75 ± 3
Succinate	129 ± 7	77 ± 9	120 ± 11	69 ± 11
+DNP		7 ± 0		6 ± 1
+CCCP		74 ± 18		88 ± 14
Ascorbate	145 ± 6	106 ± 1	152 ± 6	126 ± 3
+DNP		153 ± 2		125 ± 4
+CCCP		117 ± 3		126 ± 6

Table VIII. *Contents of Carboxylates and of Soluble Carbohydrates in Samples Comparable to Those Used in Table VII*

Duplicate 2.0-g samples at 2 subsequent days were analyzed by HPLC.

	Carboxylates and Soluble Carbohydrates Content	
	Before	During preclimacteric peak
	mmol·g⁻¹ fresh wt	
Citric acid	8.4 ± 1.0	12.3 ± 1.4
Malic acid	14.0 ± 1.6	38.0 ± 5.5
Sucrose	52.2 ± 4.6	65.7 ± 4.3
Glucose	116 ± 5	120 ± 5
Fructose	116 ± 4	121 ± 4

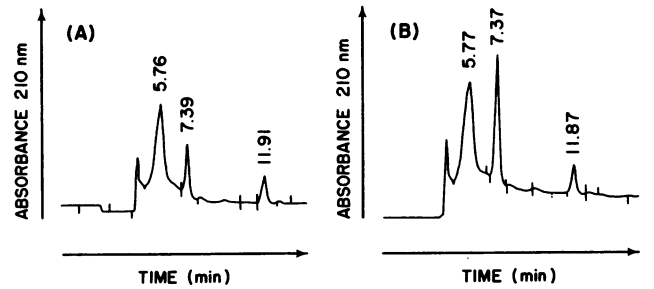

FIG. 6. Typical chromatograms of organic acids in samples before (A) and during (B) the preclimacteric peak. Retention times (min): 4.45, inorganic ions; 5.76, citrate; 7.38, malate; 11.91, unknown (glutarate: 11.66 min).

occurrence of the preclimacteric peak. The preparation of the tissue discs was aimed at obtaining cells with a minimum of damage. Therefore, such conditions as low temperature and nonisotonic solutions, as commonly used in disc preparations (13), were avoided. Experiments usually started about 30 min after disc preparation. The size of the soursop fruit allows for repetitive sampling on subsequent days from the same fruit. The respiration of the slices reflected that of the whole fruit, although at an increased level (Fig. 3). This is generally found and described as a wound effect, also involving activation of the alternate path (23).

The characteristics of the respiration of soursop fruit discs before and during the preclimacteric rise resemble in several respects those of slices of postharvest avocado and banana fruits as described by Theologis and Laties (23). These authors, too, found cyanide to stimulate O_2 uptake, hydroxamate to be little effective, and their combination strongly inhibitory. This points to the concomitant occurrence of the Cyt and alternate paths, with an easy shift of electron flow between them, as has recently been demonstrated in senescing oat leaves (19). However, whereas in avocado and banana discs upon blocking of the Cyt and alternate paths a considerable residual respiration is left, in soursop discs the O_2 uptake is reduced to only a few per cent of the original respiration and eventually comes to a complete halt. A similar effect is exerted by NaN_3, high doses of which inhibit both pathways (9). These data indicate that O_2 consumption in the soursop fruit is confined to the mitochondria with no measurable cytoplasmic oxidation. The same was found by Lambers *et al.* (12) in roots and leaves of all 10 plant species investigated.

In ripening avocado and banana, the maximal capacity of the respiratory apparatus does not change and is large enough to account for the climacteric rise (23). Also, during the preclimacteric rise in respiration of soursop, the capacity and further characteristics of disc respiration remain unchanged, suitable substrates and uncoupling agents increasing the O_2 uptake before and during the preclimacteric rise in a similar manner (Table VII). This indicates that the rise is not caused by some intrinsic change in the mitochondrial apparatus.

The subcellular localization of the substrates and their availability to the mitochondria are not known. However, the moderate change in soluble sugars does not indicate an active participation in metabolic turnover, whereas the carboxylate level changes considerably during the preclimacteric rise (Table VIII). Insofar as acid metabolism occurs within the mitochondria, it is immediately connected with the electron transport chains. The much larger increase in malate than in citrate, however, might indicate their occurrence in the cytoplasm, where citrate can inhibit pyruvate kinase activity (24) to the benefit of phosphoenolpyruvate carboxylase-mediated malate synthesis. Cytosolic acids should be readily available to the mitochondria.

Our data on the reaction of disc respiration to exogenous substrates and uncouplers confirm the general observation that the activity of nonisolated mitochondria is determined not only by the level of ADP but also by that of suitable substrate (21). Disc respiration is much more stimulated by carboxylates than by carbohydrates and the total level of the two main endogenous acids, malate and citrate, is well below saturation (*cf.* Table VIII and Fig. 4). Also, because no inhibiting influence of endogenous factors could be detected, the more than 2-fold increase in level of endogenous carboxylates during the preclimacteric rise may well explain this rise. This conclusion shifts the question from what causes the preclimacteric respiratory rise to what regulates the increase in carboxylate level.

Since the RQ is close to unity throughout postharvest development, the carboxylates probably originate from storage carbohydrate(s), *e.g.* by phosphoenolpyruvate carboxylase activity. The predominance of malic acid supports this view. Paull *et al.* (16) observed early postharvest breakdown of starch, the modulation of which may well occur at the glycolytic level (5, 17, 21). It is difficult to evaluate the state of maturity of soursop fruit at harvest. That nevertheless the preclimacteric rise usually occurs 2 d after harvest strongly indicates that the detachment from the vegetative apparatus itself induces this rise. This view is supported by observations on mature green tomatoes, the ripening of which was prevented by their storage in low oxygen and high carbon

dioxide partial pressures immediately after harvest. In these tomatoes, starch was broken down and changes in the metabolism of sugars and organic acids occurred without control by ethylene, probably as a result of removal from the vine. Only after transfer to ambient atmosphere did polygalacturonase activity and pigment concentrations change concomitant with autocatalytic ethylene evolution (7, 10). Apparently, a similar separation between postharvest phenomena and proper ripening features occurs naturally in *Annona* fruit.

Induction of the preclimacteric rise by the harvest can be effectuated in two ways. On the one hand, the shift from exogenously supplied assimilate(s) as a respiratory substrate towards internally accumulated starch may lead to a temporary overshooting of the pathway of starch degradation (14). On the other hand, the supply from the vegetative parts to the fruits of a labile inhibitor of ethylene action has been established for a number of plant species (6, 20). Reduction after harvest in the level of such an inhibitor may allow ethylene to trigger, for example, a respiratory increase at the prevailing, low rate of its production (1, 8). The two ways, overshooting shift and ethylene activation, may be causally connected. Future research can be directed, on the one hand, as to how the traumatic effect of harvest influences the breakdown of storage carbohydrates in the soursop fruit and, on the other hand, on whether a similar response can be detected in other fruit, particularly in other evolutionary ancient plant families.

It can be concluded that respiration of postharvest soursop fruit consists of a climacteric rise as normally encountered in fruits with autocatalytic ethylene production, preceded by a probably harvest-induced, transient respiratory rise.

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