# Effects of Cyclic Anaerobiosis on Pome Fruits[1]

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*Abstract.* The production of $CO_2$ by apple and pear fruit was measured during and after alternating 12-hr exposures to air and $N_2$ atmospheres. Typical stimulation of $CO_2$ evolution in the absence of $O_2$ was observed. Five or more anaerobic cycles imparted a permanent reduction on the subsequent aerobic respiration rate. The differences between control respiration and aerobic $CO_2$ evolution by fruit exposed to cyclic anaerobiosis indicated that the capacity for aerobic respiration was reduced by the early anaerobic cycles. Suppression of the anaerobic stimulation of $CO_2$ production was observed after several cycles suggesting that the capacity for fermentation was accumulatively impaired. Subsequent measurements of physical characteristics showed that flesh softening and chlorophyll degradation, processes which generally coincide during ripening, were differentially affected by cyclic anaerobiosis. Apple scald was induced by anaerobiosis. Pear fruits subjected to anaerobic cycles or continuous anaerobiosis did not ripen during the 14-day poststorage period at 21° C.

## Introduction

The patterns of aerobic and anaerobic carbohydrate metabolism of pome fruits are similar to those of other tissues. Under aerobic conditions the conventional Krebs cycle and cytochrome systems are operative (12, 13, 14), and during anaerobiosis carbohydrate catabolism is both qualitatively and quantitatively analogous to alcohol fermentation by yeast (10). Pome fruit respiration during development and maturation is primarily aerobic. After harvest and as senescence progresses, there is a progressive retardation of oxidative metabolism (10) and, specifically, in carbohydrate catabolism, which becomes increasingly less dependent on molecular oxygen for $CO_2$ production (7, 8). This paper presents the results of further experimentation with anaerobic environments upon respiratory behavior and several physical changes, usually associated with ripening of apple and pear fruit.

## Materials and Methods

*Pears.* Pears, *Pyrus communis,* L., var. 'Bosc', harvested at the preclimacteric stage and stored for 10 weeks at 0–2° C, were randomly assigned to twenty-eight 1.0 to 1.5 kg samples and placed in respirometers at 21°. One-half of the respirometers were subjected to alternate 12-hr aerobic and 12-hr anaerobic cycles for 5 days. Aerobic and anaerobic environments were obtained by flushing the respirometers with air or $N_2$ at rates sufficient to provide approximately one complete atmospheric change per hour (*ca.* 300 ml/min). The remaining 14 respirometers were continuously flushed with air at similar rates and served as controls. Carbon dioxide evolution of all samples was measured colorimetrically (5) at 3-hr intervals for the first 5 days except during periods of anaerobiosis when $CO_2$ output was recorded hourly. From day 5 through day 9, measurements were made at 12-hr intervals. Fourteen days after placement in respirometers flesh firmness was measured using a Magness-Taylor pressure tester with a 5/16″ diameter tip (15).

*Apples.* Five respirometers containing *ca.* 2 kg of 'McIntosh' apples, *Malus sylvestris* Miller, harvested near the pre-climacteric minimum and stored for 4 months at 0–2° C were provided for each treatment. Treatments consisted of 3, 6, or 9 successive 12-hr anaerobic and 12-hr aerobic cycles. Controls were maintained in air continuously. The atmospheric environments and measurements of $CO_2$ evolution were accomplished as described above for pears. Ground color (16) and flesh firmness (7/16″ diameter tip) (15) were recorded 14 days after placement in respirometers.

*Analysis of data.* The fitted regression lines for $CO_2$ evolution (Fig. 1, 2) adequately describe the data and reduce the deviation at odds greater than 19:1 (1, 6). Curves depicting aerobic respiration of fruits subjected to anaerobiosis (continuous solid lines) were obtained from the mean values of $CO_2$ evolution obtained at 24-hr intervals immediately prior to the changeover from air to $N_2$ and at equal time increments thereafter. Regression lines for anaerobic responses were fitted to the mean values obtained at hourly intervals during the periods of anaerobiosis and for 2 or 6 hr for apples and pears, respectively, into the subsequent aerobic cycle. Curves for control fruit respiration were calculated from the mean values for $CO_2$ evolution at 3-hr intervals (dashed lines).

## Results and Discussion

*Respiration.* The data described by Fig. 1 and 2 clearly show that the Pasteur effect (conservation of carbon in the presence of oxygen) was operative throughout the experiment. The ratio of $CO_2$ produced by fruits subjected to an anaerobic atmosphere to $CO_2$ produced aerobically was greater than unity for both apple and pear fruit. Since the source of $CO_2$ in these fruits is mainly carbohydrate, with some contribution from malic acid, no assumptions about fermentation products are necessary to demonstrate qualitatively that the Pasteur effect was operative (2). It is generally agreed that as the oxygen concentration is increased from zero, accumu-

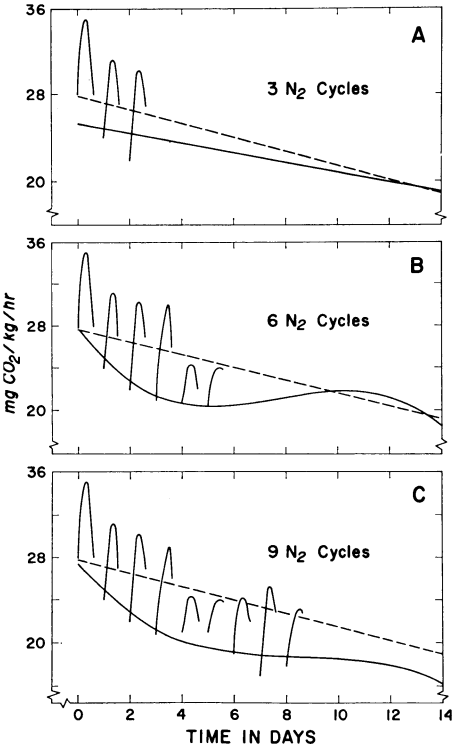


*Fig. 1.* Respiration rates of 'McIntosh' apples receiving three, six, or nine 12-hour anaerobic exposures (A, B, and C, respectively). Continuous curve depicts aerobic respiration of fruits subjected to anaerobiosis; dashed curve is control respiration; arched curves show the $CO_2$ production rates during the anaerobic cycles.

[1]Received for publication November 2, 1968. Journal Article No. 4538 from the Michigan Agricultural Experiment Station.

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lation of fermentation products decreases, and oxidative pyruvate catabolism increases. Furthermore, the rate of carbohydrate dissimilation is progressively reduced resulting in a net conservation of carbon. This is thought to be a consequence of the dependency of adenosine triphosphate (ATP) turnover rates on molecular oxygen (10).

In Blackman's classical work (3) apples, after exposure to a 48-hr "nitrogen experience", resumed the normal aerobic respiration rate within several days. He concluded that the period of anaerobiosis left no permanent effect on respiratory metabolism. In the present studies, cyclic periods of anaerobiosis resulted in a subsequent permanent reducion of $CO_2$ evolution in air (Fig. 1, 2). The aerobic respiration of apples receiving 3 anaerobic cycles (36 hr $N_2$) was, after 14 days, about the same as for control apples, but exposure to 9 anaerobic cycles for a total of 108 hr $N_2$ resulted in a permanent reduction in the aerobic respiration rate. Six cycles (72 hr $N_2$) had an intermediate effect on subsequent aerobic respiration (Fig. 1).

In pears (Fig. 2), the reduction in aerobic $CO_2$ evolution by fruit subjected to anaerobiosis increased during the first 4 anaerobic cycles and remained relatively constant thereafter. The marked increase in $CO_2$ production following a change from aerobic to anaerobic conditions tended to diminish as the number of anaerobic exposures increased (Fig. 1, 2). Beginning with the fifth $N_2$ cycle for apples and the fourth cycle for pears, the magnitude of the $N_2$-stimulated $CO_2$ evolution was decreased such that the peak response approximated the same level as control fruit respiration at the same point in time. These data suggest that the capacity for aerobic respiration was reduced during the early $N_2$ cycles and the capacity for fermentation was diminished during later cycles.

*Physical characteristics.* Pears given five 12-hr anaerobic exposures failed to soften during the 14-day test period at 21° C. The inhibition of the mechanism for cell wall softening was not overcome when fruits were returned to an aerobic environment. The average flesh firmness (pressure) of these fruits was 8.2 lb., whereas the control fruits were too soft to be measured (less than 3 lb.). Although these pears were maintained in an oxygen-free environment for a total of 60 hr (five 12-hr cycles) fermentation products were not discernible by taste or odor at the end of the 14-day period. In

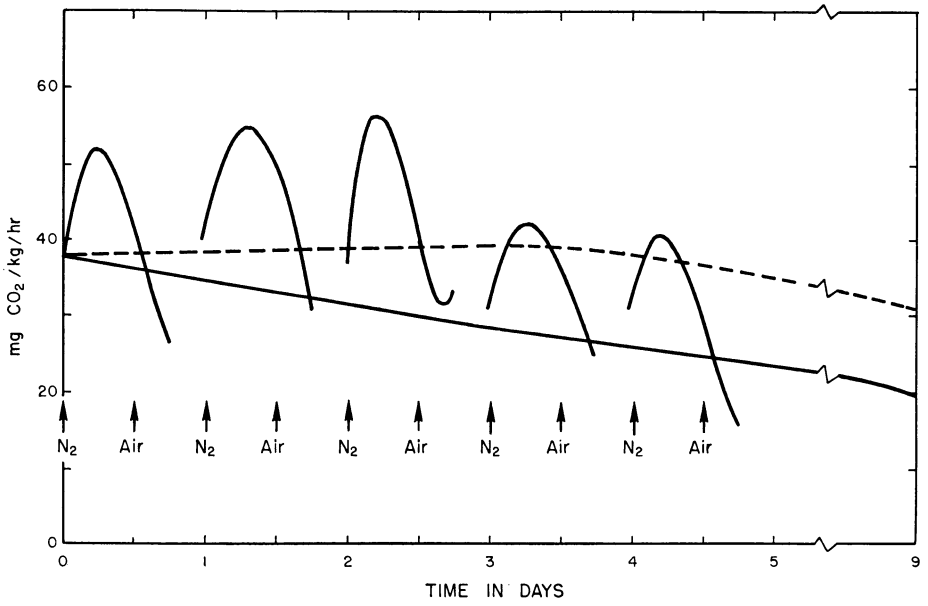


*Fig. 2.* Respiration rates of 'Bosc' pears given five 12-hr anaerobic exposures. Continuous curve depicts aerobic respiration of fruits subjected to anaerobiosis; dashed curve is control respiration; and short arched curves show the $CO_2$ production rates during the anaerobic cycles.

apples, 3 or 6 anaerobic exposures did not significantly hasten flesh softening with respect to the controls; fruits receiving nine 12-hr anaerobic cycles showed significantly greater softening (Table 1). These differing responses to anaerobiosis suggest that apple fruits responded to oxygen more rapidly than pear fruits.

Apple fruits exposed to anaerobic cycles retained a greener ground color than control fruits (Table 1). This may be explained on the basis of suppressed ethylene production, since the production of ethylene in apple (4) and pear (11) fruits is oxygen dependent. During periods of anaerobiosis ethylene production was probably suppressed resulting in a concomitant decrease in the rate of chlorophyll degradation. Apple fruits maintained in air throughout the experiment were continually exposed to ethylene, whereas those held in an oxygen-free atmosphere for 3 or more 12-hr periods were exposed to ethylene for a shorter period of time and chlorophyll degradation was retarded. Although ethylene production was probably accelerated after the fruits were returned to air (4), the duration of ethylene exposure was shorter than for the post-climacteric control fruits which produced sufficient quantities for de-greening.

Ground color changes from green to yellow and decreasing flesh firmness occur with apple fruit aging. Under the conditions of these experiments, however, softer fruits were found to have a greener ground color (Table 1) suggesting that cyclic anaerobiosis uncoupled these 2 separate, but associated, parameters of senescence.

Subjecting apple fruit to cyclic anaerobiosis resulted in an increase in the incidence and severity of storage scald symptoms (Table 1). Dilley et al. (7) showed that apple scald could be induced anaerobically and develops aerobically. Further, scald development is directly proportional to the duration of anaerobiosis. The data in Table 1 suggest that the duration of the aerobic period following anaerobiosis was also important in determining the severity of scald development. Fruit given three $N_2$ cycles followed by more than 11 days in air were more severely scalded than fruit receiving 6 or 9 $N_2$ cycles followed by shorter periods in air. Scald observed on fruit maintained in air throughout the experiment was relatively minor.

Water loss from apple fruit during

*Table 1.* Influence of the number of 12-hr anaerobic cycles on flesh firmness and ground color of 'McIntosh' apples 14 days after initial treatment.[x]

| | Number of anaerobic cycles | | | |
|---|---|---|---|---|
| | 0 | 3 | 6 | 9 |
| Mean pressure (lb.) | 10.25 ab | 10.40 a | 9.98 b | 9.34 c |
| Mean ground color[y] | 1.74 a | 3.12 b | 2.98 b | 3.82 b |
| Weighted scald (%)[z] | 4.1 a | 24.0 c | 16.3 b | 14.0 b |
| Water loss (%) | 2.5 a | 2.6 a | 2.4 a | 2.6 a |

[x]Means within a row not followed by the same letter differ at odds greater than 99:1 (9). Pretreatment pressure and ground color values were 12.24 lb. and 3.25, respectively.

[y]Green = 4; yellow = 1 (13).

[z](Slight X1 + moderate X2 + severe X3) × (No. fruit in sample)$^{-1}$ × 100.

the 14-day period was not significantly affected by anaerobiosis.

Thus, the development of senescence in pome fruits was retarded by reducing the capacity for fermentation and by the additive suppressing influence of increasing anaerobic cycles on subsequent $CO_2$ production in air.


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# Vascular Blockage, Water Absorption, Stomatal Opening, and Respiration of Cut 'Better Times' Roses Treated with 8-Hydroxyquinoline Citrate and Sucrose[1]


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*Abstract.* The efficacy of 8-hydroxyquinoline citrate in prolonging the life of cut roses was due to decreased vascular blockage in stems, increased water absorption, and stomatal closure. Sucrose increased the respiratory rate of rose petals but 8-hydroxyquinoline citrate did not influence respiration. Sucrose also prolonged life of cut roses by reducing stomatal opening but water absorption was reduced. A solution of 200 ppm 8-hydroxyquinoline citrate and 3% sucrose improved longevity of cut roses by reducing moisture stress through increased water absorption and retention. Roses held in this solution lasted twice as long as roses held in water.


## INTRODUCTION

MANY workers have reported extension of vase-life and improved quality of cut flowers using mixtures of quinoline salts and sucrose (3, 8, 9, 14). Larsen and Cromarty (7) suggested that the extended life of flowers in 8-hydroxyquinoline citrate (8-HQC) was due principally to the reduction of microbial growth. Quinoline salts are bactericidal (18) and also control stomatal opening (15, 17). Larsen and Scholes (8) recognized 8-HQC as a stomatal closing agent but concluded that prolonged cut-flower life was due to the bactericidal properties of quinoline compounds. The influence of quinoline salts on stomatal opening cannot be overlooked since Odom (11) has shown that cut flowers treated with 8-hydroxyquinoline sulfate (8-HQS) did not wilt. Scholes (14) has shown that in 8-HQS cut roses gained more weight and absorbed more solution than in water. This increased water absorption and gain in fresh weight was reflected in improved keeping quality. Coorts et al. (3) found that in an 8-HQS-sucrose solution cut roses transpired at a 10–15% higher rate than in tap water; whereas, in sucrose solution roses transpired at a 30% lower rate than in tap water. They postulated that 8-HQS may have a beneficial effect on water uptake by reducing physiological plugging. Aarts (1) reported that 8-HQS had no effect on vascular blockage of *Alnus glutinosa*. Durkin and Kuc (5) showed that the life of cut rose flowers was curtailed by water deficiency induced by vascular blockage or physiological plugging. They proposed that senescence in cut roses might be retarded by using materials which reduce vascular blockage and an "anti-desiccant" which might reduce water stress within the plant. Durkin (4) showed that plugging of the rose vascular system is caused by oxidation of the naturally occurring stem tannins by the enzyme, peroxidase.

Aarts (1, 2) concluded that the main prerequisite for long life of cut flowers is an undisturbed water uptake. He showed that flower stems could become partially plugged even when held in sterile solutions. Aarts demonstrated that sucrose limited transpiration and water uptake and closed stomates of *Mathiola incana* leaves. He attributed the reduced water uptake to the high osmotic potential of the sucrose solution.

Scholes (14) indicated that 200 ppm 8-HQS reduced the respiration rate in cut roses compared to controls. Roses treated with 8-HQS had a slight increase in the respiratory rate just prior to the end of keeping life. Coorts et al. (3) demonstrated that 200 ppm 8-HQS did not influence respiration of cut roses, but combined with 4% sucrose, increased respiration 50% over control roses on the 6th day after harvest. The respiratory rate of roses held in sucrose or in sucrose-8-HQS mixture did not differ on the 6th day after harvest. Coorts et al. concluded that the keeping quality of roses was enhanced by preservatives which increase respiration, rather than inhibit it. Scholes concluded there was no relationship between the effectiveness of a chemical in increasing keeping quality and its effect on the rate of respiration.

The purpose of this paper is to report the effects of 8-HQC and sucrose on vascular stem blockage, water


[1]Received for publication November 4, 1968. Work was conducted in cooperation with Gulf Coast Experiment Station, University of Florida, Bradenton.


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