At about the time that the climacteric was losing favor, postharvest research, as any field of scientific search, namely the respiratory climacteric, ethylene, and molecular determinants. Unanswered questions and questionable paradigms

The climacteric. Postharvest research, as any field of scientific investigation, requires a conceptual framework as a stimulant for ideas and as a model to test experimental evidence. For several decades, the respiratory climacteric served this role. However, by the late 1970s many ripening phenomena thought to be intimately (causally) associated with the respiratory climacteric were shown to be distinctly separate events [see Rhodes (24) for review]. Such evidence discredited the climacteric as a focal point for investigation, although the existence of the climacteric is surely real and the reason for its occurrence remains to be explained.

Ethylene. At about the time that the climacteric was losing favor, ethylene was acclaimed as the ripening hormone (22). However, Trewavas (35) since has reminded us that a tissue must be predisposed to respond to ethylene. In the case of fruit, Hansen (15) made the same point some 20 years ago when he coined the expression "readiness to ripen." Various lines of evidence can be cited in support of the need for this predisposition. One of the most illustrative is that of Eaks (13), who took advantage of the avocado's reluctance to ripen on the tree to demonstrate (Fig. 1) the fruits' increase in readiness to ripen with advancing maturity. Another notable facet of these experiments is that the 24 hr exposure to propylene produced quantitatively the same initial respiratory response regardless of the state of maturity. The mode of ethylene biosynthesis is now well-delineated (38), yet its role in ripening remains unresolved. Moreover, as exemplified in Table 1, ethylene has a multitude of physiological effects closely with the early increase in membrane microviscosity (Fig. 2), well-preceding the rise in ethylene production and increase in permeability, two of the more common parameters indicative of senescence.

Table 1. Changes in phospholipids (PL) and neutral lipids (NL), components of the membranes, during senescence of carnation and rose flowers.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Carnations (mg-g(^{-1}) dry wt)</th>
<th>Roses (mmol-g)</th>
<th>PL</th>
<th>NL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.30</td>
<td>0.55 (a^b)</td>
<td>100 b</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>3.25</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>3.50</td>
<td>0.49 (a)</td>
<td>138 a</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>3.60</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>2.20</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Neutral lipids content was estimated indirectly from their effect on microviscosity of membrane lipids. (From ref. 5, with permission.)

\(^{b}\)Mean separation in columns by Duncan's multiple range test, 5% level.

Table 2. The effect of transient water stress on membrane microviscosity in petals of carnation flowers.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Microviscosity (poise)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Stressed</td>
</tr>
<tr>
<td>0</td>
<td>2.18</td>
</tr>
<tr>
<td>24 (end of stress)</td>
<td>2.34</td>
</tr>
<tr>
<td>48</td>
<td>2.71</td>
</tr>
<tr>
<td>72</td>
<td>3.29</td>
</tr>
</tbody>
</table>

My aim in this brief presentation is to illustrate how the interplay of senescence and homeostasis can offer a context within which to question and, in part, explain various postharvest phenomena. I should like to begin by briefly noting the status of principal phenomena and related paradigms that have influenced postharvest research, namely the respiratory climacteric, ethylene, and molecular (genetic) determinants.

Unanswered questions and questionable paradigms

Senescence and Homeostasis in Postharvest Research

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My aim in this brief presentation is to illustrate how the interplay of senescence and homeostasis can offer a context within which to question and, in part, explain various postharvest phenomena. I should like to begin by briefly noting the status of principal phenomena and related paradigms that have influenced postharvest research, namely the respiratory climacteric, ethylene, and molecular (genetic) determinants.

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Short term effects of water stress

One question remains to be answered: Are the biophysical and biochemical changes part of the response mechanism to water stress an unavoidable occurrence in cut flowers? To evaluate this question, we imposed a transient mild water stress and then returned the flowers to water. The data (Table 2) indicate increased microviscosity of membranes at the end of the stress period, and also an increase in the hydrophobicity of the membrane (data not shown). These changes probably reflect compositional changes initiated even earlier. The biophysical changes subside when the stress is relieved; that is, the stimulus is removed (Table 2). Having demonstrated
Inhibition of flowering, Successful use of intermittent $S\text{ on the generally accepted assumption of, homeostasis in fruit:}$

Fruit senescence (33). Moreover, decrease is sensed in different ways to different ethylene concentrations (1). One can resort to the explanation that different tissues at different times respond in different ways to different ethylene concentrations. But that is hardly satisfactory and serves only to emphasize the need to identify earlier predisposing events and to question the hormonal status of homeostasis in fruit:

**Molecular determinants.** On the generally accepted assumption that ripening is part of development and hence subject to a genetic program, investigators have sought key, or at least unique, molecular event(s). At one point it was proposed that the synthesis of polygalacturonase was the key ripening event in tomatoes (34). It appears, however, that the onset of tomato ripening is accompanied by the elaboration of several new mRNAs (6, 33). Moreover, development no longer is seen as a simple linear sequence of events guided by a neat sequential transcription of DNA (20). Rather, development may be served by the interplay of many propensities—some genetically programmed, others homeostatic, and some even stochastic (32).

**Cellular senescence and homeostasis**

Although the above somewhat pessimistic interpretations are debatable, there is likely to be general agreement that a reassessment of postharvest phenomena is in order. At the same time, I believe most postharvest physiologists will agree that senescence is the biological phenomenon that best “fits” postharvest life. More than 60 years ago, Kidd and West (18) proclaimed that the climacteric is simply a manifestation of senescence. A recent committee of ASHS (37) has proposed terminology whereby “senescence” encompasses all “those processes that follow physiological maturity.” Nonetheless, senescence, like growth, is such a general phenomenon as to suggest few investigative approaches without having recourse to more specific hypotheses. As noted by Huber (16) in this symposium, several such hypotheses have been proposed, but most fit a mammalian context. Among the hypotheses, “error catastrophe” is not likely to apply to fruit senescence, where little or no cell division takes place. Variants of other hypotheses, e.g., loss of organization resistance, codon restriction, and uncoupling, have been put forward but none has proved adequate to explain observed ripening phenomena. Thus, while senescence is a fitting general concept for postharvest events, it requires further elaboration to be useful in scientific investigations. It is proposed that the juxtaposition of senescence and homeostasis may provide a useful conceptual framework and investigative leads.

The concept is not new. Samis (31), in the 1960s, proposed that loss of compensatory power (capacity to repair or remain homeostatic) characterizes the senescent cell. In retrospect, it would seem almost self-evident that cells start down the road to death when they can no longer maintain their steady state. The question arises: Are fruit cells homeostatic?

**Homeostasis and its existence in fruit**

The following may be viewed as evidence for, and characteristics of, homeostasis in fruit:

a) *Fruit cells are dynamic.* That fruit cells undergo change with time is obvious. However, the rapidity of change is not always appreciated. For instance, Brady and O’Connell (7) found that 25% to 50% of cellular proteins in the ripening banana turn over every day. Polysome levels in pear fruit cells reach new equilibria in <3 hr following a temperature shift from 20° to 40°C, or the reverse (R.J.R., T. Bos, and R. Puschmann, unpublished data). Such a dynamic state allows for rapid adjustments to or compensation for imposed stress.

b) *Fruit cells can undergo repair.* Successful use of intermittent warming to counteract low temperature injury (2, 11, 12) attests to the presence of repair. A more quantitative portrayal of repair was observed when mitochondria were isolated from fruit exposed to split doses of ionizing radiation (30). In those experiments, compensation or homeostatic repair occurred in the 24 hr between exposures so that a radiation dose (1500 krad) that would totally uncouple mitochondria resulted in only minor uncoupling if split into three equal increments with 24 hr intervening.

c) *A respiratory increase is indicative of stress and a homeostatic response.* This is surely the case for respiration following increased injury by ionizing radiation or other forms of mechanical or physical injury. It is probably also true for the response to propylene (or ethylene), as shown in Fig. 1. For the cell, stress need not be in the form of injury, but rather any perturbation from equilibrium for which the cell attempts to compensate. In this regard, it is interesting that an increase in respiration is perhaps the most universal response to ethylene. That the response is compensatory in origin is strongly implied by data such as those of Reid and Pratt (23) shown in Fig. 2. Potatoes respond to a 24-hr exposure to 10 ppm ethylene with a several-fold increase in respiration. More important, the same potatoes responded again only if there had been a sufficient interval of time between the first and second exposure, during which respiration gradually returned to normal. Reid and Pratt referred to the postethylene period as one of recovery that, in the context of our present discussion, clearly implies that ethylene produced a perturbation that needed to be compensated. Reid and Pratt also noted that oranges respond to ethylene in a similar manner, except that the effective recovery period is only about one-fourth to one-fifth as long. Perhaps it is no idle circumstance that the basal respiratory rate of oranges is four to five times that of potatoes, thus implying a proportionately faster rate of compensation or homeostasis.

d) *Homeostasis decreases with fruit age.* Such a decrease is sensed intuitively and is also clearly evident when comparing radiation treatment of preclimacteric and climacteric pear fruit (30). Following a 200-krad dose, preclimacteric pears responded with a 250% increase in respiration. A similar dose to pears at the climacteric peak elicited only one-tenth the response. The loss of capacity to restore ribosomal synthesis (19) following irradiation was also indicative of a decline in homeostasis with progressive senescence.

e) *Cellular respiration is largely mitochondrial in origin and mitochondria remain highly functional while the ripening cell is deteriorating.* The former is tacitly assumed though not entirely proved. The latter contention is supported by several studies, as summarized elsewhere (28).
Senescence and homeostasis in an integrative context

Accepting the foregoing, one can imagine alternative explanations for the climacteric and for the response to ethylene other than those commonly accepted.

The respiratory climacteric can be seen as a homeostatic response to the incipient injury resulting from ripening (as driven by a combination of genetic proclivities and environmental conditions) and the cells’ decline toward death (28). If, as generally accepted (37), ripening and postripening are part of senescence, then ripening may be sensed by homeostatic tendencies within the cell as something to be averted or compensated. One can argue that ripening and death serve an evolutionary purpose, and, while true, that is not to say that individual cells (or beings) welcome senescence.

There is ample evidence, as reviewed elsewhere (28), that the mitochondria in fruit cells remain functional and capable of conserving energy well into the post-climacteric phase, when other cellular components are exhibiting various signs of degradation. In recent experiments Bennett et al. (3) have confirmed earlier evidence that ATP accumulates in the ripening cell. It can be argued, therefore, that the climacteric is essentially a mitochondrial respiratory phenomenon (26), whereby the organelles provide energy for cellular homeostasis and, in so far as they are autonomous, their own self-preservation.

The senescence–homeostasis juxtaposition can be visualized as shown in Fig. 3. The two counter-propensities give rise to the climacteric. Ultimately, senescence prevails and fruit enter the postclimacteric phase. However, as highlighted by plotting the differential of the climacteric, the transition point is at the climacteric inflection, not the peak (27). It is at the inflection point that homeostasis first begins to give way to senescence. Such an interpretation implies that practical technologies to exploit homeostasis, such as intermittent warming, are more likely to be successful if applied before the climacteric inflection.

If the postulate of senescence–homeostasis juxtaposition has any validity, it should apply also to nonclimacteric fruit. It is plausible that in such tissues, with their absence of autocatalytic ethylene production, homeostasis is simply an integral of the basal respiratory rate. Hence, the observation that oranges with a basal respiratory rate about four-fold greater than potatoes exhibit a roughly four-fold faster compensation after exposure to ethylene (23).

Ethylene must be considered in any hypothesis regarding the climacteric and ripening. It is generally agreed that what distinguishes a climacteric from a nonclimacteric fruit is autocatalytic ethylene production by the former (4). But what is ethylene’s role? In a recent and interesting philosophical exchange, Canny (8) has questioned whether such a simple molecule as ethylene can possibly carry the ‘‘information’’ necessary to bring about its many and diverse effects in plants. Fim (14) countered with the argument that ethylene need carry only one bit (‘‘on’’ or ‘‘off’’) of information and that the necessary diverse predispositions exist among the different receptor cells. Canny (9) did not object to this interpretation, merely arguing that diverse responses require the existence of diverse information, either on the part of the promotor or the receptor.

These arguments reflect the essential point that fruit cells must be ready to ripen before they can respond to ethylene. Otherwise, ethylene will simply ‘‘turn on’’ a metabolic response as it does in immature avocados (Fig. 1), potatoes (Fig. 2), carrots (10), oranges, and most other plant tissues. Can one distinguish between the respiratory response to ethylene of nonclimacteric or ‘‘non-
[Text from the image]

That there is a need to question some of the existing paradigms in the interpretation of the respiratory climacteric and the role of ethylene. A juxtaposition of senescence and homeostasis allows for new connections between ethylene, polyamines, and methylation. Likely to be most telling of ethylene’s mode of action is its promotion of a respiratory rise. Increased respiration is the most widely observed response of plant cells to ethylene. It is not at all clear why it occurs. Membrane perturbations have been proposed, but thus far not sufficiently proved. Stress is often mentioned, but stress, much like senescence, is a fitting phenomenon that offers few if any clues to causative events.

In a speculative view it is interesting to note S-adenosyl methionine’s central position in ethylene production, polyamine biosynthesis, and methylation (Fig. 4). Effects of ethylene at this multiple metabolic juncture should not be ruled out. Roberts et al. (25), among others, have pursued an ethylene-polyamine interrelationship. Although little evidence has been forthcoming, an involvement of ethylene in methylation has been discussed at least since Lieberman’s (21) discovery more than 20 years ago that methionine was the ethylene precursor. The diverse ways in which the interconnections between ethylene, polyamines, and methylation could affect development and metabolism seems only to be limited by the imagination—and the need for sound information.

Caveats and conclusions

The inclusion in this brief commentary of relevant published information has been deliberately incomplete and its selection likely biased in support of the position taken. Nonetheless, I believe that a juxtaposition of senescence and homeostasis allows for new interpretations of the respiratory climacteric and the role of ethylene. That there is a need to question some of the existing paradigms regarding ripening, the climacteric, and ethylene is probably acceptable to almost everyone. This commentary is merely a part of that questioning process.

Literature Cited