

INTRODUCTION

The process of senescence in plants has long been of interest to scientists of many disciplines. Inquiries into this process have included studies of the physiological, biochemical, and structural changes which occur preceding cell, organ or plant death (see reviews by Varner, 1961, 1965; Sacher, 1967, 1973; Butler and Simon, 1971; Beevers, 1976).

Current theories implicate the vacuole as playing a central role in senescence, either through the storage and subsequent release of hydrolytic enzymes into the cytoplasm (Dodge, 1971; Butler and Simon, 1971; Matile and Winkenbach, 1971; Matile, 1975), or by a loss of ability to function as a "recycling center" for the degradation of cytoplasmic components and return of usable substrates to cellular function (Thomson and Platt-Aloia, 1976). Either, both, or neither of these possible vacuolar functions could be important aspects of senescence, and changes in the ultrastructure of plant cell vacuoles have been described in a number of different senescing systems (Matile and Winkenbach, 1971; Villiers, 1971; Berjack, 1972; Wilson, 1973; Thomson and Platt-Aloia, 1976). This consistency of structural changes of the vacuole has been described in a variety of circumstances of senescence.

Biochemical, physiological as well as structural studies of senescence indicate a certain degree of uniformity of events irrespective of whether the cause leading to death is natural (cotyledons or attached leaves), induced (leaf discs; water or temperature stress), or due to disease. At the biochemical and physiological levels, there appears to be a general decrease in many cellular components. A decrease in protein content has been reported in several instances (Sacher, 1967; Matile and Winkenbach, 1971; Beevers, 1976; Lees and Thompson, 1979), however, there is some controversy as to whether this is due to increased proteolysis, (Anderson and Rowan, 1965, 1966; Beevers, 1968) or decreased synthesis (Osborne, 1962; Wollgiehn, 1967; Beevers, 1968). Support for the latter hypothesis might be implicated by the apparent loss of ribosomal RNA during leaf senescence (Eilam et al., 1971).

A decrease in lipid components of the cell is usually first evident in the chloroplasts. A loss of chlorophyll (Freeman et al., 1978) is manifested as a yellowing of green tissues, the most spectacular of which is the synchronous autumnal senescence of the leaves of deciduous trees. The transition from chloroplast to chromoplast also involves a degradation of the thylakoid membranes and is reflected by a loss of galactolipids and sulpholipids (Draper, 1969) early during the senescence process. This is then followed by a decline in phospholipids from the membranes, apparently not only chloroplast membranes, but from the endoplasmic reticulum, as well (McKersie et al., 1978). Freeman et al. (1978) found decreases in total lipid, galactolipid, and phospholipid throughout maturation and senescence of citrus leaves. However, the rate and degree of apparent degradation was greatly increased during senescence.

In addition to these biochemical changes, decreases in metabolic functions also occur which are similar in several, diverse systems. Photosynthetic capacity declines commensurate with chlorophyll loss (Woolhouse, 1967). Although respiration is

apparently maintained to some degree, it also drops considerably during the final stages of senescence (Beevers, 1976).

The similarity of structural changes during senescence were summarized at the ultrastructural level by Butler and Simon (1971), and these can be correlated as structural manifestations of the bio- chemical and functional changes just described. First, there appears to be a decrease in the number of ribosomes, and a characteristic breakdown of the chloroplasts. The mitochondria are fairly persistent through the final stages of senescence, although structural modifications and a reduction in numbers are common. Further senescence is indicated by a swelling and vesiculation of the endoplasmic reticulum, which eventually disappears along with the dictyosomes. Finally the tonoplast ruptures, presumably releasing hydrolytic enzymes. The nucleus and plasma membrane are the last structures to degenerate.

This uniformity, coupled with the observation of the occurrence of temporal and spatial specificity (e.g., root cap cells and xylem vessels) of the senescent phenomenon, strongly implicated a high degree of control by the plant. The general consensus appears to be that in most instances of natural senescence there is a certain programmed series of events which are apparently under the ultimate control of the genome, and under secondary control by a complex interaction of the various plant hormones (Varner, 1965; Beevers, 1976). It might appear that the similarity of the sequence of events between naturally senescing systems and those which undergo senescence because of injury or disease is because once the process is initiated, it follows a programmed, preset pattern. However, there is a question as to whether this pattern is actually under control of the cell or plant, or whether it simply reflects a loss of control, resulting in, first the degradation of the most unstable structures and mechanisms followed by deterioration of the most stable.

Senescence is normally defined as an irreversible process which leads ultimately to the death of the cell or organism. More specifically, Beevers has pointed out that, in order to differentiate senescence from the often anabolic process of aging (development), the definition should include only "the deteriorative events which precede the death of a mature cell..." (Beevers, 1976). A precise definition of senescence may appear to be merely a problem of semantics and therefore not critical for the proper scientific investigation of this process. However, although most examples of senescence which have been studied appear to follow a similar pattern, there are examples of apparent senescence which show some divergence from this sequence.

One of the most notable "senescing" systems which does not seem to follow, precisely, the biochemical patterns outlined for senescence, is ripening fruits. Numerous studies have equated ripening with senescence (Sacher, 1973; Bain and Mercer, 1964) however those properties which characterize senescence, as outlined above, are seldom present in ripening fruit; in fact, often the opposite trend is apparent (Rhodes, 1970; Dilley, 1970). For example, proteins and nucleic acids have been shown to increase rather than decrease during ripening (Rhodes, 1970; Dilley, 1970). The decrease in respiration typical during senescence, is certainly not present in ripening climacteric fruits. Additionally, reversibility of some aspects of ripening has been shown to be possible under some circumstances (Thomson et al., 1967). In light of these observations, the question should be raised as to whether ripening is actually a

senescent phenomenon. This is important not only for semantics and a definition of each of the processes of ripening and senescence, but if the two are different, then an understanding of the initiation and control of each phenomenon can only be achieved if they are studied as separate and distinct phenomena.

Most of the current investigations on fruit ripening are based upon three important concepts and/or discoveries made 50 years ago. The first is that of Kidd and West (1930). In a study of respiratory patterns in apples, they observed a greatly increased rate of CO₂ release during ripening. Their interpretations lead to the definition of this period in the life of a fruit as the transition from the growth phase of development to the senescent phase. Because of the increased rate of respiration, they coined the term "respiratory climacteric." Research on this aspect of ripening has been voluminous and has resulted in the concept that there are two basic types of fruit based on their respiratory pattern during ripening (Biale, 1960; Rhodes, 1970). One type is that described by Kidd and West, i.e., the climacteric fruit. This category includes most tropical and sub-tropical species (such as mangos, bananas and avocados), as well as numerous temperate-zone fruits (e.g., apple and pear). The other classification is non-climacteric fruits, and includes fruits which show only a slight or no increase in respiration during ripening (e.g., Citrus) (Biale, 1960).

At the same time that Kidd and West were proposing the concept of the climacteric, Denny (1924) successfully isolated a gaseous component given off by ripening fruits, and identified it as ethylene. He applied pure ethylene to lemon fruits and was able to induce a change of color from green to yellow. This work led to the concept of ethylene as the "ripening hormone." Subsequently, ethylene has been detected to increase, concomitantly with the CO₂ production, during the climacteric (Young et al., 1952; Biale et al., 1954). It has also been shown to increase during, or just prior to senescence in several different systems (Suttle and Kende, 1978; Kende and Baumgartner, 1974; Abeles, 1973). This observation of the production of ethylene, both by ripening fruits and by senescing systems, led to an equation of ripening with senescence rather than, as proposed by Kidd and West (1930), the concept that ripening precedes senescence.

Further support of this equation of ripening and senescence was introduced by Blackman and Parija (1928). They proposed the theory that both ripening and senescence were caused by a loss of the "organizational resistance" of the cells. Their explanation followed the thesis that proper functioning of a cell required a certain amount of compartmentalization, i.e., a separation of enzymes and substrates, in order to allow control over metabolic processes. They called this compartmentation "organizational resistance," and said that once this subcellular organization was lost, hydrolytic enzymes would be released, ions and small molecules would be lost, and death would soon follow.

This concept of ripening, senescence, and organizational resistance was largely ignored for over thirty years. Even with the advent of the electron microscope, which demonstrated structural evidence for the existence and importance of subcellular compartmentation, early studies nevertheless drew similar conclusions that ripening is a senescent process. Sacher (1962) found increased permeability of membranes coincident with the climacteric. Then Bain and Mercer (1964) examined ripening pears

with the electron microscope. They found some evidence of swelling and an apparent incipient degradation even in the mature, hard fruit recently harvested from the tree. As ripening progressed, indicated by color change and increasing respiration (climacteric), they found increasing vesiculation of the plastids and other organelles (except the mitochondria). At the climacteric peak, the protoplasm was almost completely disorganized. The apparent stability of the mitochondria was used as evidence of sufficient preservation such that fixation artifact was not considered to be a factor in the determination. Concomitant with the ultrastructural study, Bain and Mercer measured changes in impedance as evidence of increasing leakiness of the membranes of the pear tissue during ripening. Their conclusion was that ripening does involve a loss of the organizational resistance of the tissue as evidenced by the structural breakdown of the organelles, and by the increasing loss of selective permeability of the cell membranes as evidenced by leakage.

Since 1964 there have been numerous studies of degradative changes in the ultrastructure, and on increases in permeability or leakiness of cells of ripening fruits. The majority of ultrastructural studies have focused on changes in the plastids (Knee et al., 1977; Thomson, 1967; Mohr and Stein, 1969; Spurr and Harris, 1968; Rosso, 1968). Almost all structural studies have interpreted the changes observed to be degenerative.

There are, however, three notable exceptions to this consensus. One is that of Spurr and Harris (1968) who, although describing rather extensive changes in the ultrastructure of plastids of pepper fruits during ripening, interpreted these changes to be the result of a dynamic synthesis, rather than degradation. Thomson (1969), came to a similar conclusion from his study of ripening oranges, i.e., the only noticeable change in the ultrastructure of ripening oranges was a transition in structure of the plastids and *that* the other organelles did not deteriorate or change significantly. These interpretations are supported by the finding of Thomson et al. (1967), that chromoplasts of ripe oranges can be induced by treatment, or can, by natural means, return to green chloroplasts. This latter observation is important by its demonstration of the apparent retention of synthetic capabilities (chlorophyll and membranes) by the chromoplasts.

Additionally, recent studies indicate that the apparent loss of selective permeability of fruit cell membranes during ripening might be due to the increasing delicacy of the cells (Simon, 1977). This fragility could be caused by breakdown of the cell walls and would result in bursting of the cells when placed in hypotonic media used for many studies of leakage. Although this question is not yet entirely resolved (Ben-Yehoshua, 1964; Brady et al., 1970; Solomos and Laties, 1973; Wade and Bishop, 1978), these studies focus attention on the possible importance of the increasing fragility of the cells of ripening fruits.

Cell rupture would be manifested as apparent increased leakage and loss of compartmentalization. Damage to the delicate cells would also appear, in the electron microscope, as increased deterioration and degradation of the organelles, cells and tissues. Both types of damage might be interpreted as senescence phenomena.

The question remains therefore, whether ripening should be considered a senescence phenomenon, or whether it is a developmental process which precedes senescence. This is the primary question addressed by this dissertation. At the beginning of this

study it was assumed that an ultrastructural investigation of a ripening fruit by several different modes of preparation (i.e., thin section, freeze fracture- and scanning electron microscopy) would allow a thorough investigation and examination of the structural changes occurring during this process. Furthermore, it would provide a more accurate assessment of the truly degradative, versus artifactual changes.

The fruit tissue studied was the avocado (*Persea americana*). As a climacteric fruit, the avocado exhibits typical physiological changes during ripening (CO₂ production and ethylene release). Measurement of these parameters allows a means of determining the precise stage of ripening of an individual fruit at the time of sampling with a fair degree of accuracy. Additionally, Young and his colleagues (Awad and Young, 1979) have correlated the activity of several wall hydrolytic enzymes (pectinmethylesterase, polygalacturonase and cellulase) with the progress of the climacteric of avocado fruits. This provides a means for correlation of these enzymatic activities with the changes in the wall ultra- structure during ripening. It also permits a correlation of ultra- structural transitions which might indicate synthesis and release of these enzymes.

Another advantage of the avocado as a tissue for the study of fruit ripening is the unusual characteristic that it will not begin to ripen until it is harvested from the tree. This property not only allows control over the initiation of ripening, it also provides the opportunity for the separation of the process of maturation from that of ripening. Additionally, avocado fruit can be "stored" on the tree for up to six or more months at a mature stage of development, thus, with the late fall-maturing variety, Fuerte, and the early spring-maturing Hass variety, fruit are available for study almost all year long.

Finally, unlike most other fruits, the avocado mesocarp does not undergo a color change during ripening. This allows a separation of plastid structural changes due to color change from those changes due to deterioration.

The primary question addressed by this study is, therefore: Is ripening in avocados a senescence phenomenon? Does ripening involve a deterioration of subcellular ultrastructure, or is the structural integrity of the organelles maintained throughout ripening and in the post climacteric, soft fruit?

REFERENCES

- Abeles, F. B. 1973. Ethylene in Plant Biology. Acad. Press, New York, 302 p.
- Anderson, J. W. and Rowen, K. S. 1965. Activity of peptidase in tobacco-leaf tissue in relation to senescence. Biochem. J. 97:741-746.
- Anderson, J. W. and Rowen, K. S. 1966. The effect of 6-Furfurylamino-purine on senescence in tobacco-leaf tissue after harvest. Biochem. J. 98:401-404.
- Awad, M. and Young, R. E. 1979. Postharvest variation in cellulose, polygalacturonase, and pectinmethylesterase in avocado (*Persea americana* Mill, c.v. Fuerte) fruits in relation to respiration and ethylene production. Plant Physiol. 64:306-308.
- Bain, J. M. and Mercer, F. V. 1964. Organization resistance and the respiration climacteric. Aust. J. Biol. Sci. 17:78-85.
- Beevers, L. 1968. In; Biochemistry of Plant Growth Substances. F. Wrightman and G. Letterfield, eds. pp. 1417-1435. Runge Press, Ottawa.

- Beevers, L. 1976. Senescence. In; Plant Biochemistry. J. Bonner and J. E. Varner, eds. pp. 771-794. Acad. Press. New York.
- Ben-Yehoshua, S. 1964. Respiration and ripening of discs of the avocado fruit. *Physiol. Plant.* 17:71-80.
- Berjak, P. 1972. Lysosomal compartmentation: Ultrastructural aspects of the origin, development and function of vacuoles in Lepidium sativum. *Ann. Bot.* 36:73-81.
- Biale, J. B. 1960. The postharvest biochemistry of tropical and subtropical fruits. *Adv. Food Res.* 10:293-354.
- Biale, J. B., Young, R. E. and Olmstead, A. J. 1954. Fruit respiration and ethylene production. *Plant Physiol.* 29:168-174.
- Blackman, F. F. and Parija, P. 1928. Analytic studies in plant respiration. I. The respiration of a population of senescent ripening apples. *Proc. Roy. Soc. London.* B103:412-445.
- Brady, C. J., O'Connell, P. B. H., Smydzuk, J. and Wade, N. L. 1970. Permeability, sugar accumulation, and respiration rate in ripening banana fruits. *Aust. J. Biol. Sci.* 23:1143-1152.
- Butler, R. D. and Simon, E. W. 1971. Ultrastructural aspects of senescence in plants. *Adv. Gerontol. Res.* 3:73-129.
- Denny, F. E. 1924. Hastening the coloration of lemons. *J. Agricul. Res.* 27:757-769.
- Dilley, D. R. 1970. Enzymes. In: *The Biochemistry of Fruits and their Products*. A. C. Hulme, ed. pp. 179-205. Acad. Press, London and New York.
- Dodge, A. D. 1971. The mode of action of the bipyridylum herbicides, paraquat and diquat. *Endeavor.* 30:130-135.
- Draper, S. R. 1969. Lipid changes in senescing cucumber cotyledons. *Phytochemistry* 8:1641-1647.
- Eilam, Y., Butler, R. D. and Simon, E. W. 1971. Ribosomes and polysomes in cucumber leaves during growth and senescence. *Plant Physiol.* 47:317-323.
- Freeman, B. A., Platt-Aloia, K., Mudd, J. B., and Thomson, W. W. 1978. Ultrastructural and lipid changes associated with the aging of Citrus leaves. *Protoplasma.* 94:221-233.
- Kende, H. and Baumgartner, B. 1974. Regulation of aging in flowers of *Ipomea tricolor* by ethylene. *Planta* 116:279-289.
- Kidd, F. and West, C. 1930. Physiology of fruit. I. Changes in the respiratory activity of apples during their senescence at different temperatures. *Proc. Roy. Soc. London.* B106:93-109.
- Knee, M., Sargent, J. A. and Osborne, D. J. 1977. Cell wall metabolism in developing strawberry fruits. *J. Exp. Bot.* 28:377-396.
- Lees, G. L. and Thompson, J. E. 1979. An evaluation of the role of membrane self-digestion in cotyledon senescence. *Z. Pflanzenphysiol.* 95:199-211.
- Matile, Ph. 1975. *The Lytic Compartments of Plant Cells*. Springer-Verlag. Wein and New York. 183 pp.
- Matile, Ph. and Winkenbach. 1971. Function of lysosomes and lysosomal enzymes in the senescing corolla of the morning glory (*Ipomoea purpurea*). *J. Exp. Bot.* 22:759-771.
- McKersie, B. D., Lepock, J. R., Kruuv, J. and Thompson, J. E. 1978. The effect of cotyledon senescence on the composition and physical properties of membrane lipid.

- Biochim. Biophys. Acta 508:197-212.
- Mohr, W. P. and Stein, M. 1969. Fine structure of fruit development in tomato. *Can. J. Plant Sci.* 49:549-553.
- Osborne, D. J. 1962. Effect of kinetin on protein and nucleic acid metabolism in Xanthium leaves during senescence. *Plant Physiol* 37:595-602.
- Rhodes, M. J. C. 1970. The climacteric and ripening of fruits. In: *The Biochemistry of Fruits and Their Products*. A. C. Hulme, ed. pp. 521-533. Acad. Press, London and New York.
- Rosso, S. W. 1968. The ultrastructure of chromoplast development in red tomatoes. *J. Ultrastruct. Res.* 25:307-322.
- Sacher, J. A. 1962. Relations between changes in membrane permeability and the climacteric in banana and avocado. *Nature* 195:577-578.
- Sacher, J. A. 1967. Studies of permeability, RNA and protein turnover during aging of fruit and leaf tissues. *Symp. Soc. Exp. Biol.* 21:269-304.
- Sacher, J. A. 1973. Senescence and postharvest physiology. *Ann. Rev. Plant Physiol.* 24:197-224.
- Simon, E. W. 1977. Leakage from fruit cells in water. *J. Exp. Bot.* 28:1147-1152.
- Solomos, T. and Laties, G. C. 1973. Cellular organization and fruit ripening. *Nature* 245:390-392.
- Spurr, A. R., and Harris, W. M. 1968. Ultrastructure of chloroplasts and chromoplasts in Capsicum annum. I. Thylakoid membrane changes during fruit ripening. *Amer. J. Bot.* 55:1210-1224.
- Suttle, J. C. and Kende, H. 1978. Ethylene and senescence in petals of Tradescantia. *Plant Physiol.* 62:267-271.
- Thomson, W. W. 1969. Ultrastructural studies on the epicarp of ripening oranges. *Proc. First Int. Citrus Symp.* 3:1163-1169.
- Thomson, W. W., Lewis, L. N. and Coggins, C. W. 1967. The reversion of chromoplasts to chloroplasts in Valencia oranges. *Cytologia*.