3

METABOLIC PROCESSES IN HARVESTED PRODUCTS

Metabolism is the entirety of biochemical reactions occurring within cells. Many components of metabolism, especially those which are beneficial or detrimental to the quality of postharvest products, are of major interest to postharvest biologists. The acquisition and storage of energy and the utilization of stored energy are central processes in the control of the overall metabolism of a plant. The acquisition of energy through photosynthesis and its recycling *via* the respiratory pathways are compared in Table 3.1. Respiration occurs in all living products, while photosynthesis does not occur in products devoid of the green pigment chlorophyll.

The various organs of intact plants have a high degree of specialization as to carbon acquisition, allocation and storage. Leaves, for example, photosynthesize but seldom act as long-term storage sites for photosynthates. Petioles and stems transport fixed carbon, but typically have only a limited photosynthetic potential and when utilized for storage, often only act as temporary sinks (e.g., the stems of the Jerusalem artichoke). Flowers, roots, tubers, and other organs or tissues likewise have relatively specific roles with regard to the overall acquisition of carbon. While attached to the plant, these plant parts (organ or tissue) derive the energy required to carry out their specific functions from photosynthesizing leaves. There is, therefore, in intact plants an interdependence among these different parts with divergent primary functions. Severing these parts from the plant at harvest disrupts this interdependence and can, therefore, influence postharvest behavior. For example, the detaching of leaves, whose primary function is to fix carbon dioxide rather than the storage of carbon, markedly restricts or terminates photosynthesis, leaving them with extremely low reserves that can be used for maintenance. Storage organs, on the other hand, if sufficiently mature, have substantial stored carbon that can be recycled for utilization in maintenance and synthetic reactions.

In contrast to this high degree of specialization among parts in the acquisition of energy, respiration occurs in all living cells and is essential for the maintenance of life in products after harvest. The factors affecting these two general processes, energy acquisition (photosynthesis) and energy utilization (respiration), are reviewed in this chapter. These processes are affected by both internal (commodity) and external (environmental) factors that often interact. Important commodity factors include species, cultivar, type of plant part, stage of development, surface to volume ratio, surface coating, previous cultural and handling conditions, and chemical composition. Among the major external factors influencing respiratory rate are temperature, gas composition, moisture conditions, light and other factors that induce stress conditions within the harvested product.

Table 3.1. General Comparison of Photosynthesis and Respiration in Plants

	Photosynthesis	*Respiration*
Function	Energy acquisition	Energy utilization and Formation of carbon skeletons
Location	Chloroplasts	Mitochondria and cytoplasm
Role of light	Essential	Not required
Substrates	CO_2, H_2O, light	Stored carbon, O_2
End products	O_2, stored carbon	CO_2, H_2O, energy
Overall effect	Increase in weight energy	Decrease in weight
General reaction	$6CO_2 + 6H_2O \xrightarrow{\text{chloroplast}} C_6H_{12}O_6 + 6O_2$	$C_6H_{12}O_6 + 6O_2 \xrightarrow{\text{mitochondria}} 6CO_2 + 6H_2O + \text{energy}$

1. RESPIRATION

Respiration is a central process in all living cells that mediates the release of energy through the breakdown of carbon compounds and the formation of carbon skeletons necessary for maintenance and synthetic reactions after harvest. The rate of respiration is important because of these main effects but also because it gives an indication of the overall rate of metabolism of the plant or plant part. All metabolic changes occurring after harvest are important, especially those that have a direct bearing on product quality. The central position of respiration in the overall metabolism of a plant or plant part and its relative ease of measurement allow us to use respiration as a measure of metabolic rate. The relationship between respiration and metabolism, however, is very general since specific metabolic changes may occur without measurable changes in net respiration. This is illustrated by comparing changes in a number of the physical and chemical properties of pineapple fruit during development, maturation and senescence (Figure 3.1). Neither changes in the concentration of chlorophyll, reducing sugars, acidity, carotenoids, nor esters correlates well with changes in respiratory rate. Therefore, it is important to view respiration as it fits into the overall process of harvested product metabolism rather than as an end in itself.

There are two general types of respiratory processes in plants—those that occur at all times regardless of the presence or absence of light (dark respiration) and those occur only in the light (photorespiration).

1.1. Dark Respiration

The living cells of all plant products respire continuously, utilizing stored reserves and oxygen (O_2) from the surrounding environment and releasing carbon dioxide (CO_2). The ability to respire is an essential component of the metabolic processes that occur in live harvested products. The absence of respiration is the major distinction between processed plant products and living products. Respiration is the term used to represent a series of oxidation-reduction reactions where a variety of substrates found within the cells are oxidized to carbon dioxide. At the same time, oxygen absorbed from the atmosphere is reduced to form water. In its simplest form, the complete oxidation of glucose can be written as:

$$\underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{oxygen}}{6\,O_2} + \underset{\text{water}}{6\,H_2O} \rightarrow \underset{\text{carbon dioxide}}{6\,CO_2} + \underset{\text{water}}{12\,H_2O} + \text{energy}$$

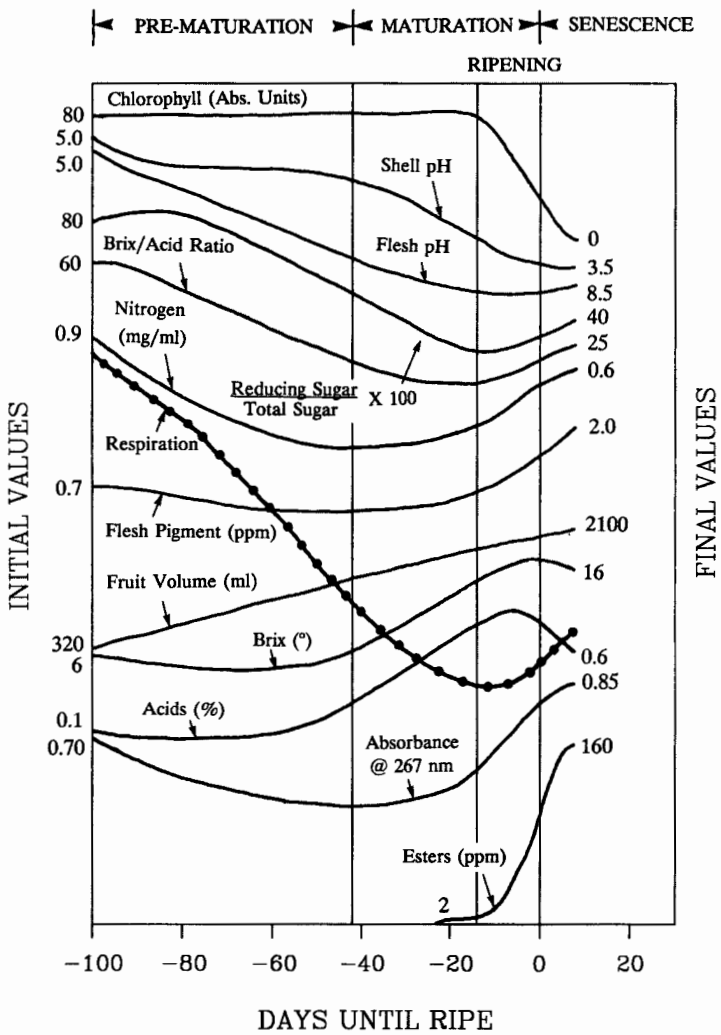


Figure 3.1. Chemical and physical changes in the pineapple fruit during prematuration, maturation, ripening and senescence (*after Gortner et al.*).[40] While respiratory rate can give a general indication of the overall rate of metabolism, it often does not correlate with specific changes occurring, e.g., changes in the % acids.

The products of this reaction are carbon dioxide, water and the energy that is required for essential cellular processes. Some of the energy generated in respiration of harvested produce is lost as heat (~46%); however, significant amounts are retained by the cells in chemical forms that may be used for these essential processes. The rate of the reaction is determined by substrate (e.g., glucose) and oxygen availability, and most importantly, temperature.

Respiration is much more complex than the generalized reaction just presented. The glycolytic, tricarboxylic acid, pentose phosphate and electron transport system pathways are involved in the breakdown of many of the common substrates utilized by the cell. Often during the oxidation of a substrate, the conversion to carbon dioxide is not complete and intermediates are utilized by the cells for synthetic reactions such as the formation of amino acids, nu-

cleotides, pigments, lipids and flavor compounds. Hence, the respiratory pathways provide precursors, often called carbon skeletons, required for the formation of a large number of plant products.

During the preharvest growth of a plant or plant product, a major portion of the carbon trapped during photosynthesis is diverted into synthetic reactions. It is through the respiratory pathways that the carbon from photosynthesis begins its transformation into the majority of the other compounds in the plant. Since the synthesis of these compounds also requires energy, derived from the respiratory pathways, a portion of the photosynthetic carbon fixed is utilized for this purpose. Therefore, a balance is reached between respiratory substrate availability and the demand for energy production and carbon skeletons. Since neither availability nor demand is static, the system is continually changing this balance during the day and over the developmental cycle of the plant or plant part.

At harvest, the relationship between carbon acquisition and utilization is radically changed when the plant product is severed from its readily replenishible supply of carbon provided by photosynthesis. Hence, a new balance must be reached; energy and carbon skeletons must now come from already existing sources within the severed product. Mature plant parts which function as carbon storage organs (e.g., seeds, roots, bulbs, tubers) have substantial stores of carbon that can be utilized *via* the respiratory pathways for an extended period. Leaves and flowers do not function as carbon storage sites and hence have very little reserves. As a consequence, the balance shifts to a situation where demand can readily deplete the supply.

After harvest, the objective is to maintain the product as close to its harvested condition (quality) as possible, thus in most products growth is considered undesirable. Postharvest conditions for these products often result in an extensive reduction to a total elimination of photosynthesis, necessitating the reliance upon existing reserves. The respiratory pathways that are operative after harvest in both intact plants and severed plant parts are the same as those prior to harvest. The major changes are the now finite supply of respiratory substrate available to the various pathways and the new equilibrium established between supply and tissue demand for it.

There are a series of steps in the respiratory oxidation of sugar or starch that involve three interacting pathways. The initial pathway is glycolysis, where sugar is broken down into pyruvic acid, a three carbon compound. The pathway occurs in the cytoplasm and can operate in the absence of oxygen. The second pathway is the tricarboxylic acid (TCA) or Krebs cycle that occurs in the mitochondria, where pyruvic acid is oxidized to carbon dioxide. Oxygen, although not reacting directly in these steps, is required for the TCA pathway to proceed, as are several organic acids. The third pathway, the electron transport system, occurs in the mitochondrial inner membrane and transfers hydrogen atoms (reducing power), removed from organic acids in the tricarboxylic acid cycle and from 3-phosphoglyceraldehyde during glycolysis, to oxygen. The electrons are moved through a series of oxidation-reduction steps that terminate upon uniting with oxygen, forming water. The energy is used to pump protons that are then allowed to flow back through a proton channel that converts the gradient to chemical energy in the form of adenosine triphosphate (ATP). ATP is then utilized to drive various energy requiring reactions within the cell. A fourth respiratory pathway, the pentose phosphate system, while not essential for the complete oxidation of sugars, functions by providing carbon skeletons, reduced NADP required for certain synthetic reactions and ribose-5-phosphate for nucleic acid synthesis. The pentose phosphate pathway appears to be operative to varying degrees in all respiring cells.

While oxygen is not required for the operation of the glycolytic pathway, it is essential for the tricarboxylic acid cycle, the pentose phosphate pathway and the electron transport system. Glycolysis can proceed therefore under anaerobic conditions, i.e., in the absence of oxygen.

The occurrence of anaerobic conditions poses a serious problem in the postharvest handling of plant products. When the oxygen concentration within the tissue falls below a threshold level (around 2%), pyruvic acid can no longer proceed through the tricarboxylic acid cycle. Pyruvic acid instead is converted to lactate and/or ethanol that can accumulate to toxic levels. Prolonged exposure to anaerobic conditions, therefore, results in cellular death and loss of the harvested product. Exposure for short periods often results in the formation of off-flavors in edible products. Depending on the tissue and length of exposure to low oxygen, the off-flavors may be eliminated upon returning to aerobic conditions.

1.1.1. Glycolysis

Glucose, derived from sucrose or starch, is broken down by the glycolytic pathway in a sequence of steps to form pyruvic acid. In the initial step, glucose has a phosphate added (i.e., is phosphorylated) (Figure 3.2). If the starting compound is free glucose, the reaction is catalyzed by the enzyme hexokinase to form glucose-6-phosphate. If, as found in many postharvest products, the glucose occurs as part of a starch molecule, phosphate is added by the enzyme starch phosphorylase, forming glucose-1-phosphate, which is subsequently converted to glucose-6-phosphate. The phosphorylation of free glucose requires energy, in the form of 1 ATP, while the phosphorylation of glucose when it is part of a starch molecule does not. The six-carbon glucose molecule progresses through fructose-1-phosphate to fructose-1,6-bisphosphate before being split by the enzyme aldolase into two 3-carbon compounds, dihydroxyacetone phosphate and 3-phosphoglyceraldehyde. The 3-phosphoglyceraldehyde molecule is the first compound to lose electrons in the respiratory pathway, forming 1,3-bisphosphoglycerate, when 2 hydrogen atoms are removed and accepted by NAD (nicotinamide adenine dinucleotide). 1,3-Bisphosphoglycerate undergoes four additional enzymatic steps, resulting in the formation of pyruvic acid. Two of the four steps from 1,3-bisphosphoglycerate to pyruvic acid yield chemical energy in the form of ATP. None of the reactions from glucose or starch to pyruvic acid require oxygen, so the glycolytic pathway can proceed normally under anaerobic conditions.

If anaerobic conditions occur in the harvested tissue due to restricted entry of oxygen or an insufficient supply in the atmosphere surrounding the commodity, pyruvic acid cannot enter the tricarboxylic acid cycle and be oxidized. The inability to enter the cycle is due to an absence of oxidized flavin adenine dinucleotide (FAD) and NAD required for the cycle to proceed. When this occurs, pyruvic acid accumulates and is usually decarboxylated to form CO_2 and acetaldehyde, which is subsequently reduced to ethanol. Pyruvate may also be reduced to form lactic acid. Alcohol (ethanol) and to a lesser extent lactic acid accumulate within the tissue. Both reactions require energy, which is provided by NADH formed during the oxidation of 3-phosphoglyceraldehyde previously in the pathway. The overall reaction in simplified form is:

$$\text{glucose} + 2\ \text{ATP} + 2\ \text{Pi} + 2\ \text{ADP} \rightarrow 2\ \text{ethanol} + 2\ CO_2 + 4\ \text{ATP}$$

When ethanol is produced from glucose, two ATP molecules are required but four are formed from each free glucose molecule giving a net yield of two ATPs. This represents one fourth of the energy yield that would be derived from the glycolytic pathway when sufficient oxygen is present and is only 1/16 that derived when glucose is fully oxidized (glycolysis and the tricarboxylic acid cycle). As cells switch their carbon flow toward lactate and alcohol formation, the production of CO_2 increases (Figure 3.2). The increase is due to the reduced energy yield under anaerobic conditions, as much more glucose must be oxidized to meet the cell's energy requirements. The complete oxidation of one glucose molecule under aerobic conditions yields

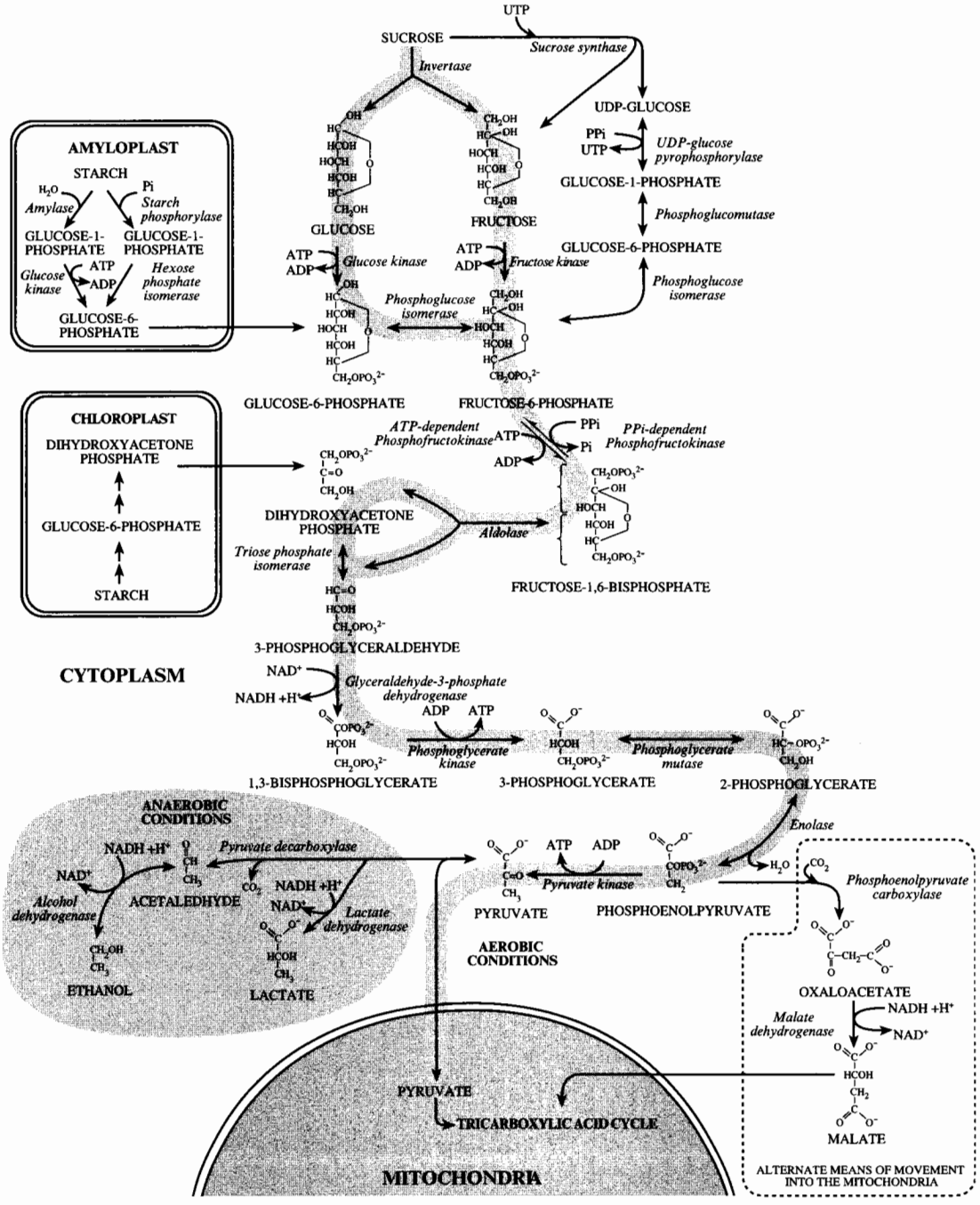


Figure 3.2. The glycolytic pathway for the aerobic oxidation of glucose or glucose-1-phosphate to pyruvate. The pathway can flow in either the gluconeogenic (forming sugars) or the glycolytic direction, the latter being the predominant direction. During ripening of fruit such as banana, the gluconeogenic direction may predominate.[7] Under anaerobic conditions the movement of pyruvate into the tricarboxylic acid cycle is inhibited, and NADH + H formed in the oxidation of 3-phosphoglyceraldehyde is utilized to reduce acetaldehyde to ethanol or pyruvate to lactate by way of the shaded portion of the pathway.

~32 ATP equivalents, while under anaerobic conditions a net of only 2 ATPs are formed from a glucose molecule. While anaerobiosis has disastrous consequences for living tissue in terms of loss of stored reserves and accumulation of undesirable compounds, it is also the basis of a very important processing technique, fermentation. Potential energy remains stored in the form of alcohol that can be recaptured if oxygen is supplied.

1.1.2. Tricarboxylic Acid Cycle

Pyruvic acid produced by the glycolytic pathway is further broken down in the tricarboxylic acid (TCA) cycle, also known as the Krebs cycle, and the citric acid cycle. Tricarboxylic acid refers to the three carboxyl groups that are present on some of the acids in the cycle, while citric acid is an important early intermediate in the sequence of reactions. The reactions of the tricarboxylic acid cycle occur in the matrix of the mitochondria and on the surface of the inner membrane. Pyruvic acid, therefore, must move from the cytoplasm, where glycolysis occurs, into the mitochondria for further oxidation to proceed.

In the initial step, pyruvic acid is decarboxylated as it combines with Coenzyme A forming the 2 carbon compound acetyl CoA (Figure 3.3). Acetyl CoA then combines with the 4 carbon molecule oxaloacetic acid, yielding citric acid that undergoes a series of oxidative and decarboxylation reactions ending with the formation of oxaloacetic acid, allowing the cycle to begin again. Energy is captured as reduced NAD (i.e., NADH) at the conversion of isocitric acid to α-ketoglutaric, α-ketoglutaric acid to succinyl CoA, and malic acid to oxaloacetic acid. A single ATP is produced on the conversion of succinyl CoA to succinic acid, and FAD is reduced at the conversion of succinic acid to fumaric acid. Carbon dioxide is liberated from pyruvic, isocitric and α-ketoglutaric acids.

Each revolution of the tricarboxylic acid cycle, a three carbon pyruvate molecule releases three carbon dioxide molecules and produces reducing power in the form of four NADH molecules and one $FADH_2$ molecule. Combined with the two NADH molecules from the glycolytic pathway, a total of 10 reduced NADs are formed with the complete oxidation of a single glucose molecule. Only 12 of the 24 protons (H) are from glucose; the remaining 12 are from water that is added at various steps in the cycle.

1.1.3. Electron Transport or Cytochrome System

NAD reduced to NADH in the TCA cycle, in glycolysis and by other reactions in the cell is recycled by the removal of the electrons. NADH cannot, however, directly reduce oxygen to form water. The electrons are removed through a series of reactions forming a positive potential gradient, from compounds of low reduction potentials to higher reduction potentials (i.e., from lower to greater tendency to accept electrons), culminating in a reaction with oxygen that has the greatest tendency to accept electrons (Figure 3.4). During the process, protons are pumped across the inner mitochondrial membrane, forming a proton gradient. The proton gradient is released through a protein complex (ATP synthase), and energy is conserved in a biologically usable form as ATP. ATP is used to drive reactions, especially synthetic, that require energy inputs. In actively metabolizing cells, the efficiency of energy trapping in the electron transport system is only about 54%. A mole of glucose has a calorie potential of approximately 686 kcal·mole^{-1}. Only a small amount of energy is lost in the initial transfer of energy as electron pairs to NAD and FAD in glycolysis and the tricarboxylic acid cycle. However, during the transfer of the energy to ATP in the electron transport system, the energy potential drops to approximately 263 kcal·mole^{-1}. The remaining energy escapes as respiratory (vital)

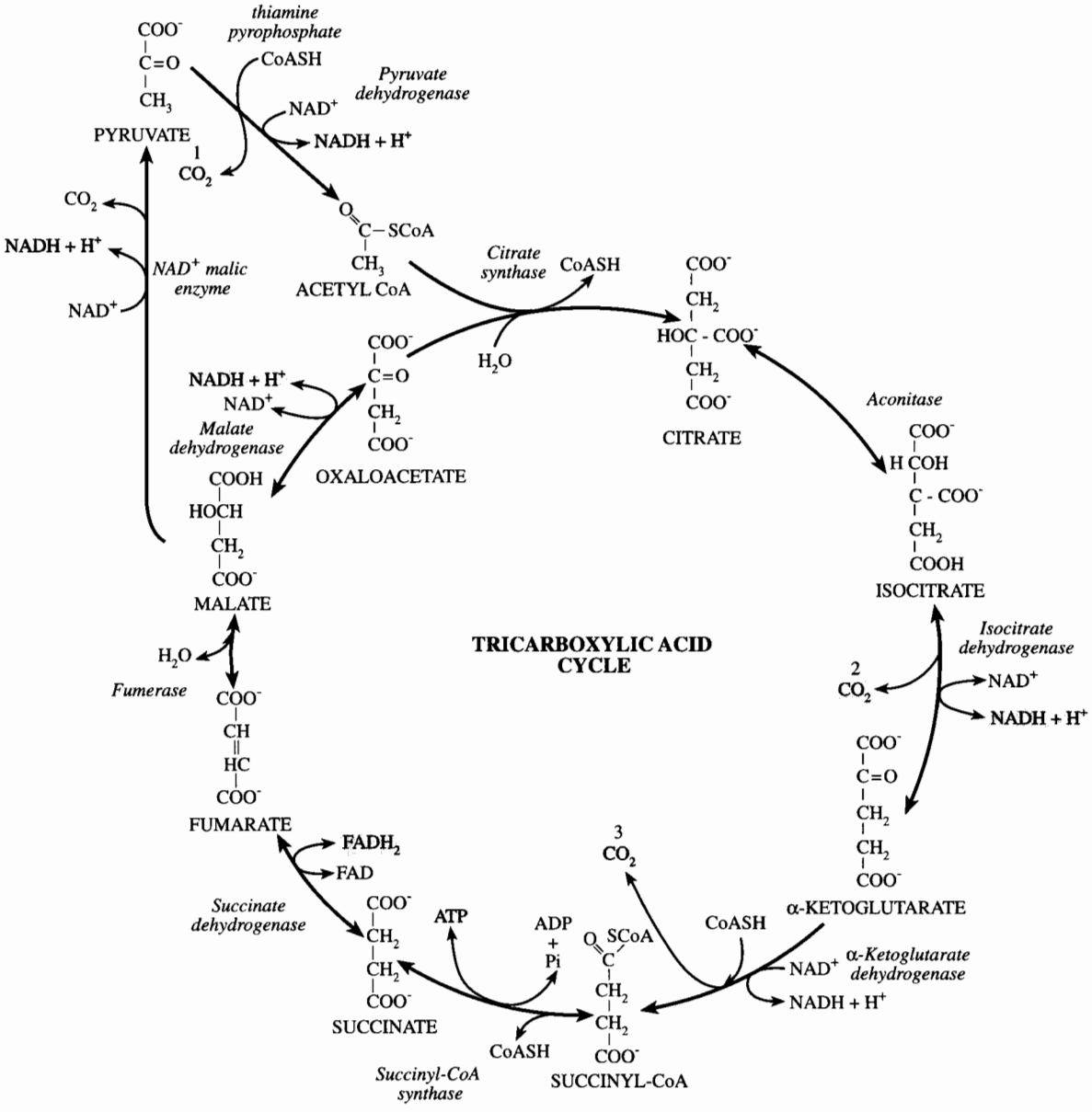


Figure 3.3. The tricarboxylic acid cycle results in the complete oxidation of 1 pyruvate molecule with each complete sequence through the cycle forming CO_2, ATP, NADH + H, and $FADH_2$. NADH + H and $FADH_2$ are then oxidized in the electron transport system (Figure 3.4).

heat, a normally detrimental factor that must be dealt with during the postharvest handling and storage period. Therefore, the overall function of the electron transport system is to trap energy in a biologically usable form (ATP) and recycle NAD and FAD required for certain reactions in the various metabolic pathways. The major components of the electron transport system have been elucidated. Figure 3.4 shows the sequence of steps involved. Each component enzyme is specific and can only accept electrons from the previous component in the

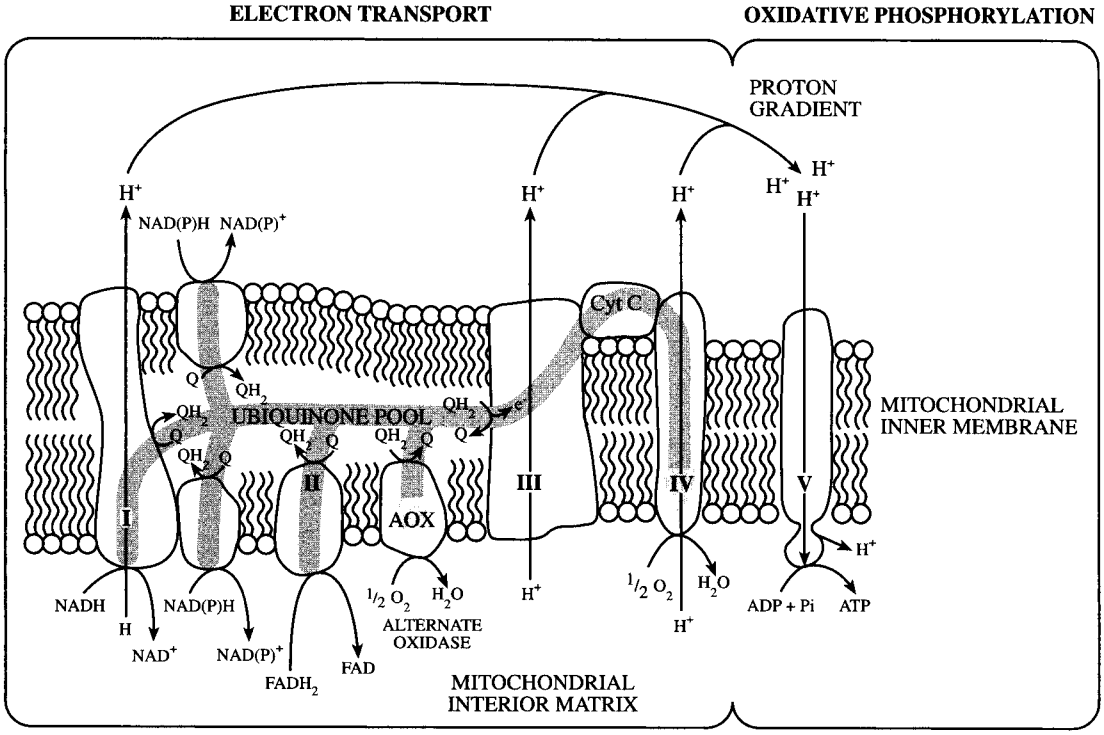


Figure 3.4. This model illustrates the organization of the electron transport system, located within the inner membrane of the mitochondria. Electrons released during oxidative steps in glycolytic pathway and the tricarboxylic acid cycle (trapped as NADH and $FADH_2$) move (shaded arrow) through a series of complexes to the terminal acceptor, oxygen. Four protein complexes participate in the process with complex I accepting energy from NADH and complex II from $FADH_2$. The free energy released during electron transfer is coupled to the translocation of protons (H^+) across the membrane, creating an electrochemical proton gradient. Protons on the exterior flow back through complex V, an ATP synthase complex that is coupled to the conversion of ADP + P_i to ATP, retrapping the free energy. When the alternative oxidase (AOX) is operative, only 1 proton is transferred from NADH. FAD reduction is associated with succinate dehydrogenase activity (i.e., succinate → fumerate), and when the AOX is operative, energy in QH_2 is transferred directly to oxygen, bypassing the formation of ATP. Thus, when the alternative pathway is operative, only 1 ATP equivalent is produced from NADH with the remaining energy being lost as heat.

chain. NADH and $FADH_2$, being different in energy potential, enter the chain at different points.

The total energy balance from the oxidation of one molecule of glucose remains a subject of debate. When ADP:O ratios (the number of ATPs synthesized per 2 electrons transferred to oxygen) are calculated in isolated mitochondria, consensus values are 2.5 ATPs per NADH and 1.5 per $FADH_2$. In the glycolytic pathway, the energy balance for a single glucose molecule under aerobic conditions is -2 ATP + 4 ATP + 2 NADH. Since glycolytic NADH is in the cytosol and cannot diffuse into the mitochondria for conversion to ATP *via* the ETS, it must go through a shuttle where the energy from one NADH is transferred to the mitochondria. Two options are postulated: the glycerol phosphate shuttle (Figure 3.5) which yields only 1.5 ATP's per glycolytic NADH; and the malate-asparate shuttle which yields 2.5. Therefore, either 5 or 7 ATPs are derived during glycolysis, depending upon the shuttle method (Table

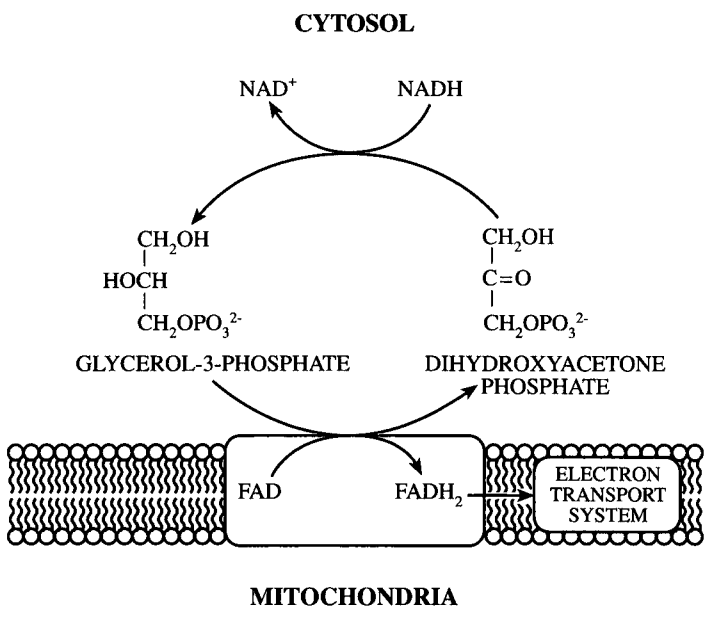


Figure 3.5. The energy from reduced NAD produced in the glycolytic pathway can be transported from the cytosol into the mitochondria (site of electron transport pathway) *via* the glycerol phosphate shuttle. NADH reduces dihydroxyacetone phosphate to glycerol phosphate, and then at the mitochondrial membrane it is oxidized back to dihydroxyacetone phosphate yielding one reduced FAD within the membrane that enters the electron transport system. Thus, the energy potential drops from 2.5 ATP equivalents to 1.5 for each reduced glycolytic NAD. An alternative malate-aspartate shuttle (not shown) is more complex, and involving the transport of glutamate and malate across the mitochondrial membrane. The malate-aspartate shuttle, however, does not result in an energy drop.

3.2). When added with the ATPs from the TCA cycle (8 NADH + 2 $FADH_2$ + 2 ATP) per molecule of glucose, the net yield is 30 or 32 ATPs.[51] The exact number (32 or 30) remains in question, each version being found in various textbooks and articles.

Carbon monoxide (CO), azide (N_3) and hydrogen cyanide (CN) are potent inhibitors of electron transport, in that they combine with the metals in the terminal cytochrome oxidase, the final enzyme in the electron transport chain. In plants, CN does inhibit the terminal cytochrome oxidase, but it also stimulates the rate of respiration. That is because plant tissues have a second terminal oxidase called the alternative oxidase which is insensitive to inhibitors of cytochrome oxidase. Electrons follow the normal electron path to ubiquinone, at which point they are transferred to oxygen to form water by the alternative oxidase. It is important to note that less than one ATP is generated per NADH in the alternative pathway; in contrast to the 2.5 ATPs generated by the normal pathway, the remainder of the energy is lost as heat. Therefore, the alternative pathway represents an inefficient energy conserving system that bypasses the normal pathway, which can substantially increase the respiratory heat load of the product.

The alternate electron transport pathway has been found in all plant tissues studied to

Table 3.2. Yield of ATPs from the Oxidation of One Glucose Molecule *via* Two Shuttle Pathways.*

Pathway	*ATP Yield per Glucose*	
	Glycerol-Phosphate Shuttle	*Malate-Asparate Shuttle*
Glycolysis (cytosol)		
Glucose phosphorylation (1 molecule)	-1	-1
Fructose-6-P phosphorylation (1 molecule)	-1	-1
1,3-bisphosphate glyceric acid dephosphorylation (2 molecules)	+2	+2
Phosphoenol pyruvic acid dephosphorylation (2 molecules)	+2	+2
Glyceraldehyde-3-phosphate oxidation (2 molecules) yields 2 NADH		
Pyruvate to acetyl-CoA (mitochondria) yields 2 NADH		
Tricarboxylic acid cycle (mitochondria)		
Succinyl-CoA → succinate	+2	+2
Succinate oxidation (2 molecules) yields 2 $FADH_2$		
Isocitrate, α-ketoglutarate and malate oxidation (2 molecules each) yields 6 NADH		
Oxidative phosphorylation (mitochondria)		
2 NADH from glycolysis (yielding 1.5 or 2.5 ATPs depending upon shuttle method)	+3	+5
2 NADH from oxidation of pyruvate at 2.5 ATPs	+5	+5
2 $FADH_2$ from succinate at 1.5 ATPs	+3	+3
6 NADH from tricarboxylic acid cycle at 2.5 ATPs	+15	+15
Net ATP Yield	+30	+32

*ATP yields are based upon consensus P/O ratios which give ATP equivalents for mitochondrial oxidation of NADH and $FADH_2$ of 2.5 and 1.5, respectively. Two shuttle pathways are given for the oxidative phosphorylation of glycolytic NADH in the mitochondria.

date. The abundance of the alternative oxidative protein increases in many plant tissues exposed to any one of several environmental and biotic stresses, as well as during the ripening and/or senescence of some fruit tissues (e.g., cold stored potato tubers, parsnip and carrot roots; ripening avocado and banana fruits). In nature, its only well documented role appears in the thermogenesis associated with the flowering of some species belonging to the families Annonaceae, Araceae, Aristolochiaceae, Cyclanthaceae, and Nymphaeaceae.[79] The elevated temperature (e.g., up to 15°C above the ambient air)[78] associated with the alternative path in certain flower parts results in the volatilization of odoriferous compounds that attract insects, thereby facilitating pollination. The alternative pathway is activated on the day of flowering and remains active for only a few hours. Although the existence of the alternative pathway has been known for over 70 years,[37] its physiological function in most tissues remains speculative. While not fully documented, several roles have been proposed, based on the principle that electron transport through the alternative path supports a high rate of respiration that is not constrained by respiratory control (i.e., when the level of ADP is low and the level of ATP is high). The nonphosphorylating alternative pathway may support higher respiration rates that would ensure a stable supply of metabolites, such as organic acids, required for biosynthetic reactions in the cells. Related to this is the "energy overflow hypothesis" by Lambers,[69] which considers the alternative path as a coarse control of carbohydrate metabolism operative when carbohydrates accumulate in greater quantities that required for growth, storage and ATP syn-

thesis. A more recent hypothesis postulates that the alternative pathway alleviates the over-reduction of the electron transport chain, which could lead to the formation of superoxide anions and other deleterious reactive oxygen species.[92] Reactive oxygen species react with phospholipids, proteins, DNA and other cellular components, ultimately resulting in cell death.

1.1.4. Pentose Phosphate Pathway

In addition to glycolysis and the tricarboxylic acid cycle, the pentose phosphate pathway can be used to oxidize sugars to carbon dioxide. The name is derived from the fact that many of the intermediates in the pathway are five carbon (penta)phosphorylated sugars. The pentose phosphate system is found in the cytoplasm, and its main function does not appear to be energy production *via* the formation of ATP in the electron transport system, but rather as a source of ribose-5-phosphate for nucleic acid production, as reduced NADP for synthetic reactions and as a means of interconversion of sugars to provide 3, 4, 5, 6, and 7 carbon skeletons for biosynthetic reactions. One example is the formation of erythrose-4-phosphate used as a backbone for shikimic acid and aromatic amino acids. In addition, NADPH is required for the synthesis of fatty acids and sterols from acetyl CoA. A major difference between the pentose phosphate pathway and the tricarboxylic acid-glycolysis systems is that NADP rather than NAD accepts electrons from the sugar molecule. NADPH is specifically required in some metabolic reactions, and it can enter into the mitochondrial electron transport system *via* an NADPH dehydrogenase.

Initial reactions in the pentose phosphate pathway include the irreversible oxidation of glucose-6-phosphate from glycolysis to 6-phosphogluconic acid, yielding a reduced NADP (Figure 3.6). Subsequently, 6-phosphogluconic acid is converted through the removal of carbon dioxide and hydrogen to a 5 carbon sugar, ribulose-5-phosphate which upon isomerization forms a ribose-5-phosphate that is essential for nucleic acid synthesis. The conversion of phosphogluconic acid to ribulose-5-phosphate is also not reversible, and reduced NADP is formed. The two initial reactions are the only oxidative (i.e., removal of electrons) steps in the pathway, and the second is the only point in the entire pathway at which carbon dioxide is removed. Subsequent steps are reversible and can recycle back to glucose-6-phosphate, the initial substrate.

Since the pentose phosphate pathway is an alternative means of oxidizing sugars, it is of interest to know which system is operative in harvested tissue. Existing evidence indicates that the glycolysis, tricarboxylic acid and pentose phosphate pathways are operative to some extent in all tissue; however, it is difficult to accurately measure the precise contribution of each pathway. In tomato fruit, the pentose phosphate pathway is thought to account for only about 16% of the total carbohydrates oxidized, a level probably common in many tissues. However, in some tissues such as storage roots, the pentose pathway appears to be responsible for 25 to 50% of the oxidation of sugars.

1.2. Photorespiration

The acquisition of carbon *via* photosynthesis and the loss of carbon through respiration can be seen as opposing processes in chlorophyll containing plant tissues. Growth is achieved when the gain in carbon exceeds losses, i.e., is above the carbon dioxide compensation point. In most species, it is known that the respiratory rate of chlorophyll containing tissue, as measured by the loss of CO_2 from the tissue, proceeds at a higher rate in the light than in the dark. This light-stimulated loss of carbon, termed photorespiration, is a process that occurs in ad-

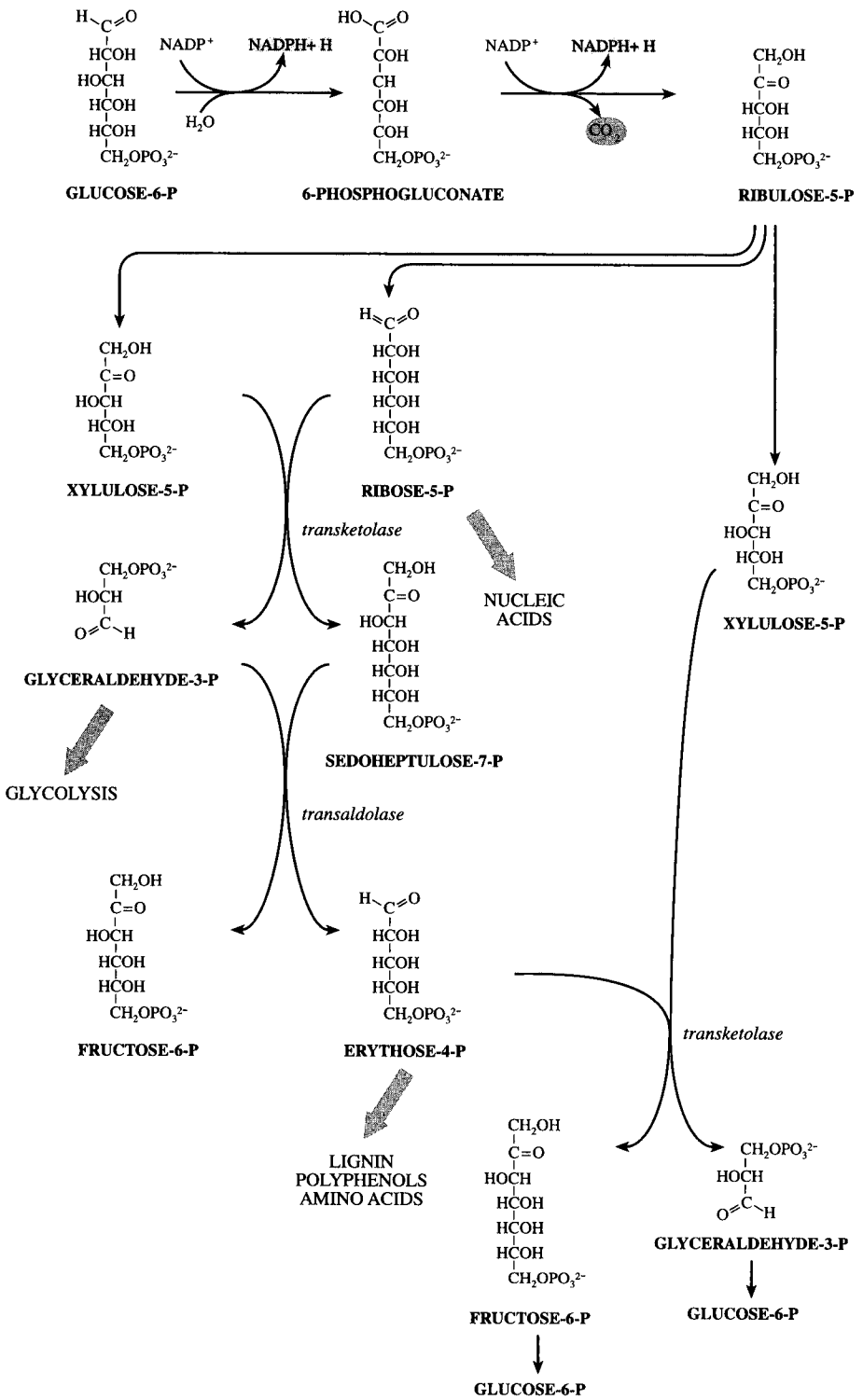


Figure 3.6. The pentose phosphate pathway, represents an alternative means for the oxidation of sugars and provides a ready mechanism for the formation of 3, 4, 5, 6 and 7 carbon skeletons for synthetic reactions. The pathway also provides NADPH + H^+ and ribose-5-phosphate, needed for nucleic acid production.

dition to or superimposed upon the normal dark respiratory processes in the plant, discussed previously.

If one uses a strict interpretation, photorespiration should not be considered a respiratory process since there is no transfer of energy between molecules, a classical requirement for respiration. Rather, it represents a form of oxidative photosynthesis. A significant portion of the carbon that is fixed into sugars in many species actually moves through this pathway. Since it has, however, generally been viewed as a respiratory process, for continuity we utilized this conventional approach.

In contrast to photorespiration, dark respiration (glycolysis, tricarboxylic acid cycle, pentose phosphate pathway and electron transport system) proceeds at essentially the same rate whether in the dark or in the light. The rate is determined by both metabolic demand and temperature. It has been estimated that 30 to 50% of the photosynthetically assimilated carbon in the leaves of some C_3 plants may be lost through photorespiration.[109]

The relative importance of photorespiration, and for that matter photobiology in general during the postharvest period, has not been studied to any appreciable extent since most products are stored in the dark or at low light levels. As a consequence, the degree to which we need to be concerned with detrimental effects of light and the potential usefulness of light during this time frame remains to be ascertained. Since photorespiration occurs in chlorophyll containing tissues that are actively photosynthesizing, it is assumed to be of greater importance in intact plants (e.g., bedding plants, woody ornamentals, transplants) than in detached plant parts. Since photorespiration decreases with both decreasing light intensity and oxygen concentration, both conditions common in postharvest handling, its rate could be readily altered.

The primary objective during the postharvest period is to maintain the product as close to the preharvest condition as possible (i.e., no significant growth in intact plants). Consequently, the balance between photosynthesis and respiratory losses may be more critical than the actual rates of each process.

Of the three primary photosynthetic carbon fixation pathways operative in higher plants, approximately 500 species possess the C_4 pathway, 250 species the CAM pathway; the remaining 300,000 are generally thought to utilize the C_3 pathway (for additional details of the pathways, see 2.2. Dark Reactions). In comparing the two primary groups, C_3 and C_4, there are a number of important characteristics that distinguish them. For example, plants having the C_3 photosynthetic pathway for carbon fixation have distinctly higher levels of photorespiration and carbon dioxide compensation points than do C_4 species (Table 3.3). The C_3 species, which comprise the majority of the woody and herbaceous ornamentals and transplants in postharvest handling and marketing, also differ in a number of other important characteristics. Photosynthesis in C_3 species is significantly inhibited by ambient oxygen levels (21%), and as a consequence, net photosynthesis is elevated and photorespiration depressed with low oxygen conditions. In addition, photosynthesis in many C_3 species also tends to saturate at lower light intensities than in C_4 species, and the optimum temperature for photosynthesis is significantly lower (Table 3.3).

During photosynthesis in C_3 species, a relatively large amount of glycolic acid is synthesized; however, the molecule cannot be metabolized in the chloroplasts. Upon movement out of the chloroplast and into peroxisomes, glycolic acid is oxidized to glyoxylic acid, which is subsequently converted to glycine (Figure 3.7). Glycine then moves into adjacent mitochondria where two molecules of glycine react to produce one molecule of serine and carbon dioxide. Since the oxidation step is not linked to ATP formation, photorespiration results in a loss of both energy and photosynthetic carbon from the plant.

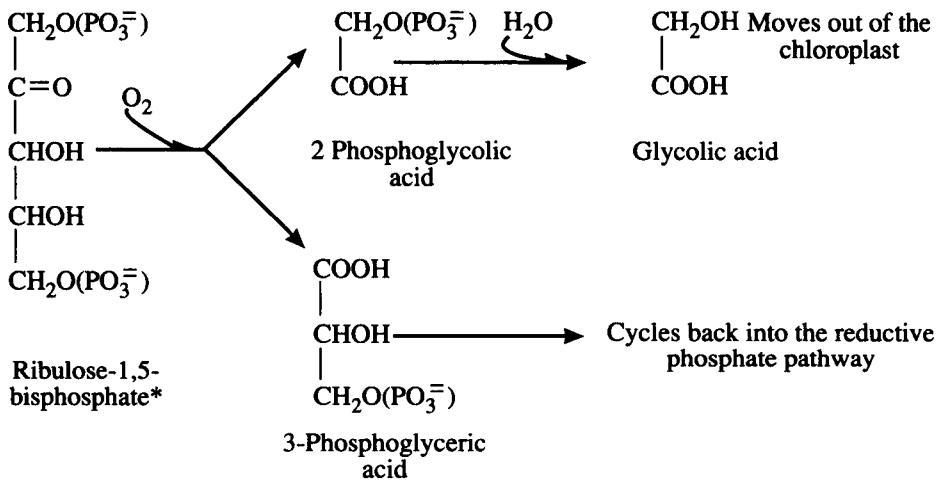
The inhibition of photosynthesis by oxygen was first observed by Otto Warburg in 1929

Table 3.3. Several Characteristics Which Distinguish C_3 and C_4 Species.*

Characteristics	*C_3 Plants*	*C_4 Plants*
Leaf anatomy	No significant differentiation between mesophyll and bundle sheath cells	Bundle sheath cells containing large numbers of chloroplasts and other organelles
Major pathway of CO_2 fixation in light	Reductive pentose-phosphate cycle (i.e., Calvin-Benson cycle)	C_4 pathway plus reductive pentose cycle
Photorespiration	High	Low
Inhibitory effect of O_2 on photosynthesis and growth	Yes	No
CO_2 compensation point in photosynthesis (ppm CO_2)	30–70	0–10
Net photosynthesis vs. light intensity	Saturation at ca. 1000–4000 foot candles	No saturation
Maximum net photosynthetic rate (mg CO_2/dm^2 leaf area/hr)	15–35	40–80
Optimum temperature for net photosynthesis (°C)	15–25	30–45
Transpiration rate (g H_2O/g dry wt)	450–950	250–350

**After:* Kanai and Black[56]

and has subsequently been known as the **Warburg effect,** in the same manner as the inhibition of sugar breakdown by oxygen was named the Pasteur effect after Louis Pasteur. The inhibition of photosynthesis by oxygen involves the competition between molecules of carbon dioxide and oxygen for the same binding site on ribulose bisphosphate carboxylase, the primary photosynthetic carboxylation enzyme. The higher the oxygen level, the more favored the oxygenation reaction and the greater the production of glycolic acid, the substrate for photorespiration.



When the oxygen concentration is lowered, the carboxylation reaction is increasingly favored.

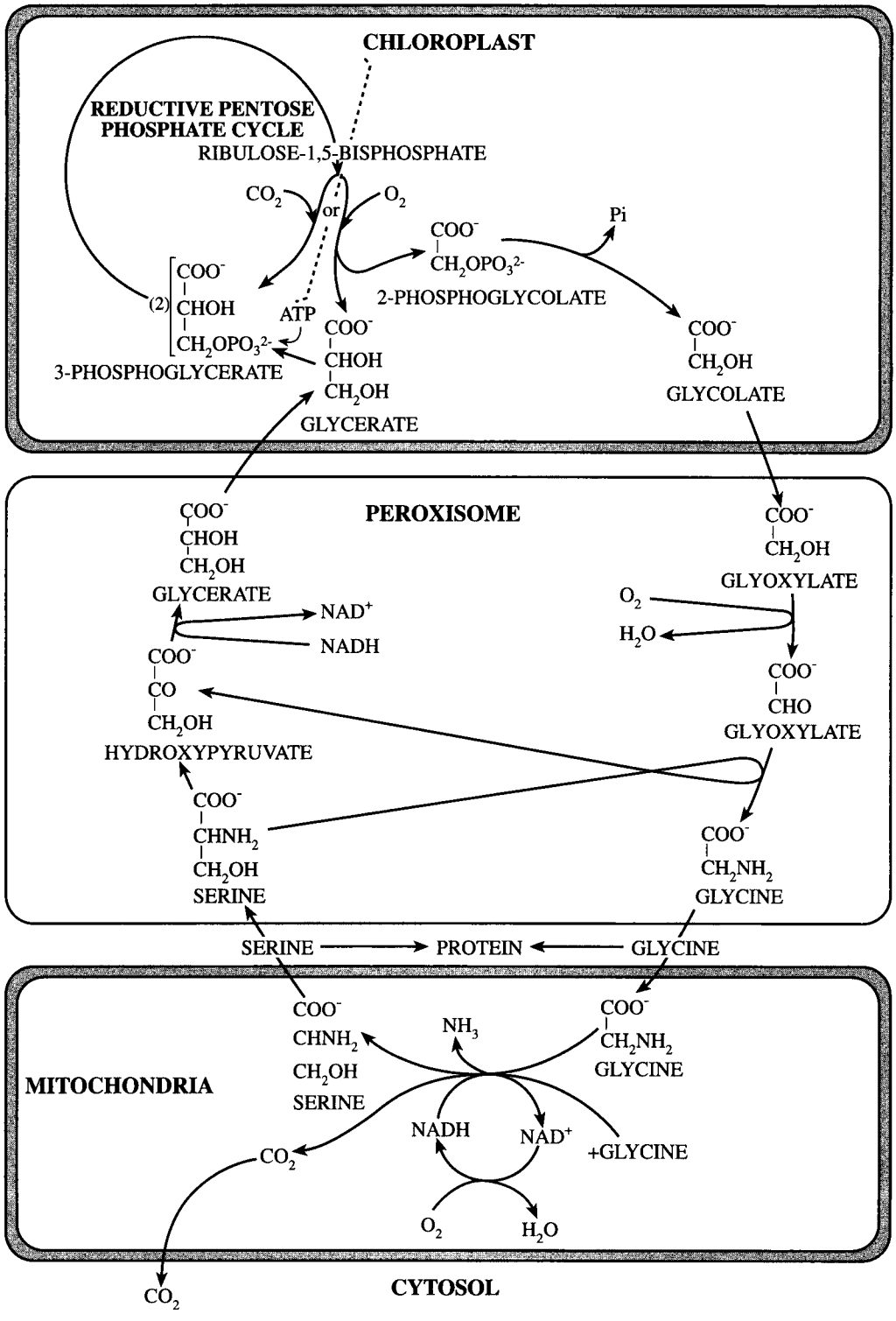
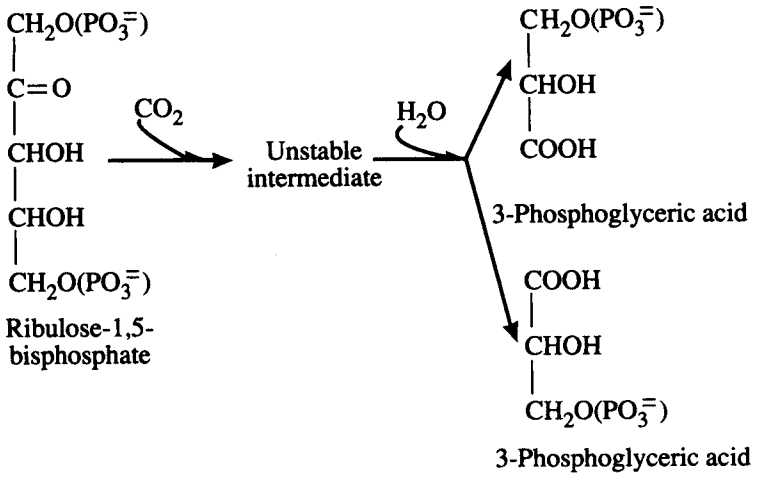


Figure 3.7. The metabolic pathway for carbon metabolism during photorespiration. Glycolate produced in the chloroplasts is transported to peroxisomes, where it is oxidized to glycine. Glycine is then converted in the mitochondria, forming serine and liberating carbon dioxide. Serine can then be cycled back through the peroxisomes and converted to glycerate, which re-enters the C_3 cycle giving a net loss of 1 molecule of carbon dioxide per molecule of glycolate formed.



The rate of photorespiration is difficult to measure precisely in an illuminated leaf since a portion of the carbon dioxide respired is photosynthetically refixed before it escapes from the leaf. The **carbon dioxide compensation point,** the concentration of carbon dioxide in the atmosphere where carbon dioxide fixed equals that respired, is often used as an index of photorespiration. Species which have high compensation points (30–70 ppm carbon dioxide) have high rates of photorespiration, and conversely, those with low compensation points (0–10 ppm carbon dioxide) have low photorespiration rates. For C_3 species, the difference in the rate of photosynthesis at 21% oxygen and 2% oxygen is also used as a measure of photorespiration, since photorespiration is almost totally blocked (the oxidation of ribulose-1,5-bisphosphate) by low oxygen.

2. PHOTOSYNTHESIS

Photosynthesis is the process by which green plants capture light energy and convert it into chemical energy that is allocated between growth and maintenance reactions.[104] Photosynthesis is not commonly considered a significant postharvest metabolic process, since many harvested products contain few chloroplasts and/or are usually stored in the dark. However, a number of products have the potential to photosynthesize, and many, although not all, may derive a benefit from this process upon removal from the production area. These products fall into two major groups: 1) intact plants such as ornamentals, leafy cuttings and tissue cultures; and 2) chlorophyll containing detached plant parts such as green apples or pepper fruits, petioles, shoots, leaves and others. Therefore, a distinct group of postharvest products are, at least theoretically, not totally severed from an external source of energy that may be used for maintenance. In some cases, even small inputs of free energy after harvest may substantially reduce or eliminate the products' dependence upon stored reserves.[68]

With intact plants, there are two general options for handling the product. Conditions can be created or selected that will maintain the plants' photosynthetic environment. This requires light, an appropriate carbon dioxide concentration and temperature, and sufficient water to maintain an adequate moisture balance within the plant. In contrast to the site of production, the postharvest environment is maintained at a lower level of these parameters, a level that will ensure maintenance of the product rather than enhanced growth and development. In many postharvest environments for intact plants, appropriate plant moisture status is the parameter that is most commonly handled improperly.

A postharvest environment may also be selected for intact plants that will minimize the

metabolic rate of the product. Therefore, in contrast to an environment conducive for photosynthesis, an environment can be selected to minimize the utilization of stored energy reserves. This is the primary option selected for the handling of both intact plants and detached plant parts and is accomplished largely by product temperature management.

Products that were photosynthetic organs prior to being severed from the plant at harvest (e.g., lettuce, amaranths, spinach) are logical candidates to derive a benefit from light during storage. This, however, is rarely the case. One reason is that the light energy trapping efficiency of plants, even under optimum conditions, is low (usually under 5%), the remaining energy being dissipated primarily as heat. This elevates the leaf temperature and leads to counterproductive increases in the use of stored energy reserves *via* the respiratory pathways. In intact plants, leaf temperature is decreased through the cooling effect of evapotranspiration. One gram of water removes 540 calories of heat upon being transformed from a liquid to a gas. Severed plant parts, however, do not have a readily replenishable source of water that can be used for cooling *via* evapotranspiration. As a consequence, product temperature increases.

An additional problem with utilizing photosynthesis to help maintain harvested chlorophyll containing plant parts is that the temperatures at which the products are normally stored are substantially below those required for optimum photosynthesis. The lower temperatures are essential, however, for successful storage since they decrease the metabolic rate of the product and the utilization of stored energy reserves.

In products that benefit from photosynthesis after harvest, the amount of external energy needed prior to harvest differs from that required after harvest. This difference is based on a distinction between the primary goals of the product before and after harvest. Prior to harvest, growth is a primary goal; therefore, carbon and energy acquisition must be greater than respiratory utilization. After harvest, during the postharvest handling period, growth is seldom desirable. Rather, the objective is to maintain the product as close to its harvested condition as possible (i.e., minimize change). Therefore, photosynthesis after harvest is seen as way of maintaining the energy balance within the plant, rather than as a means of providing excess energy for the purpose of carbon accumulation.

Photosynthesis occurs within specialized plastids, the chloroplasts, found primarily in leaves. The most important pigment in these plastids is chlorophyll, but other pigments such as carotenoids and phycobilins also participate in photosynthesis. The simplified overall reaction occurring in photosynthesis can be written as:

$$6\,CO_2 + 6\,H_2O + \text{light } (h\nu) \xrightarrow[\text{green plant}]{} C_6H_{12}O_6 + 6\,O_2$$

where carbon dioxide is fixed and oxygen from water is released. Photosynthesis can be divided into two interconnected processes: the light reactions that trap energy from light and release oxygen from water, and the dark reactions that use the energy to fix carbon dioxide.

2.1. Light Reactions

The light reactions involve the splitting of water with the release of oxygen:

$$\text{light } (h\nu) + H_2O + NADP \rightarrow \tfrac{1}{2}O_2 + NADPH + H$$

and the light driven formation of ATP from ADP and Pi (photophosphorylation). The reactions trap light energy (photons) and transport it in the form of electrons from water through a series of intermediates to NADP, where it can be stored as NADPH (Figure 3.8). Two separate light reactions act cooperatively in elevating the electrons to the energy level required for

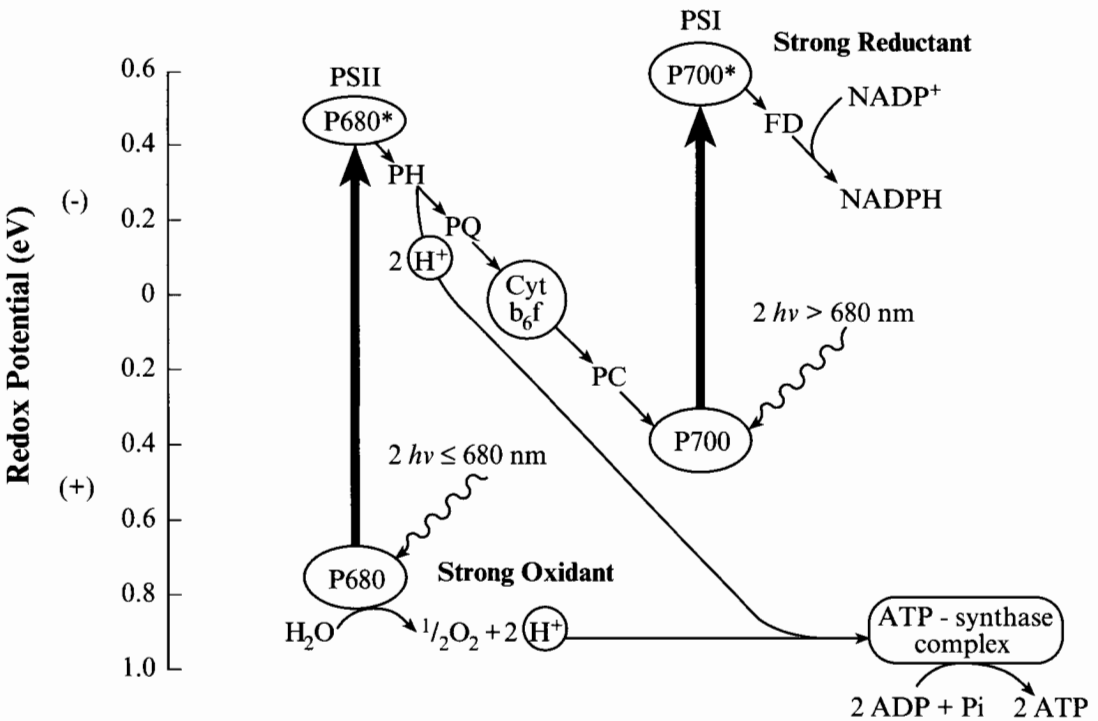


Figure 3.8. The two light reactions in photosynthesis, photosystems II (PSII) and I (PSI), trap light energy and convert it to ATP and NADPH + H^+; oxygen is also liberated in the process. Energy is absorbed by photosystem II which results in the splitting of water (photolysis), releasing O_2 and H^+ and the excitation of electrons to a high energy level where they can be accepted by the first carrier in a series which transfers the electrons to the chlorophyll of photosystem I. Additional light energy absorbed by the chlorophyll molecule increases the energy level of the electrons which are trapped by an electron acceptor and subsequently transferred to ferredoxin (FD). NADP is reduced, utilizing the H^+ formed in the photolysis of water, yielding NADPH + H^+.

their transfer to NADP. In this process, the electrons are transported *via* an electron transport chain that operates on the same alternating oxidation-reduction principle as the respiratory electron transport system, though it is distinctly different.

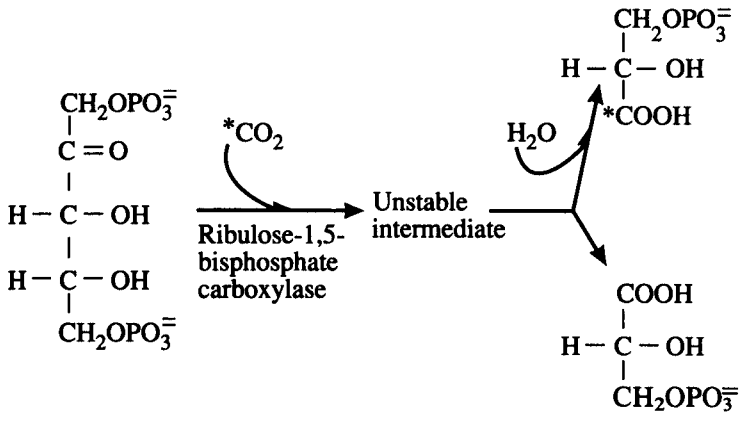
2.2. Dark Reactions

The energy trapped in the light reactions as NADPH and ATP can be used in a number of reactions within the plant; however, its primary role is in the fixation of carbon from atmospheric CO_2 (dark reactions). In plants commonly encountered after harvest, there are three primary means of fixation of CO_2: the C_3, C_4, and CAM pathways.

2.2.1. C_3–Reductive Pentose Phosphate Pathway

The C_3 or reductive pentose phosphate pathway (PPP) is operative within the majority of plant species. The name reductive PPP is to distinguish it from the oxidative PPP which shares some of the same enzymes. The pathway is also referred to as the Calvin cycle after Melvin Calvin,

who with his colleagues elucidated the cyclic pathway in the 1950's. In the pathway, CO_2 from the air is fixed by reacting with ribulose-1,5-bisphosphate (5 carbon sugar) to form two 3-carbon phosphoglycerate (PGA) molecules.



Ribulose-1,5-bisphosphate (2) Phosphoglyceraldehydes

The energy captured in the light reactions is used to convert PGA back to ribulose-1,5-bisphosphate (Figure 3.9) for the continuation of the process. Each cycle fixes a single carbon

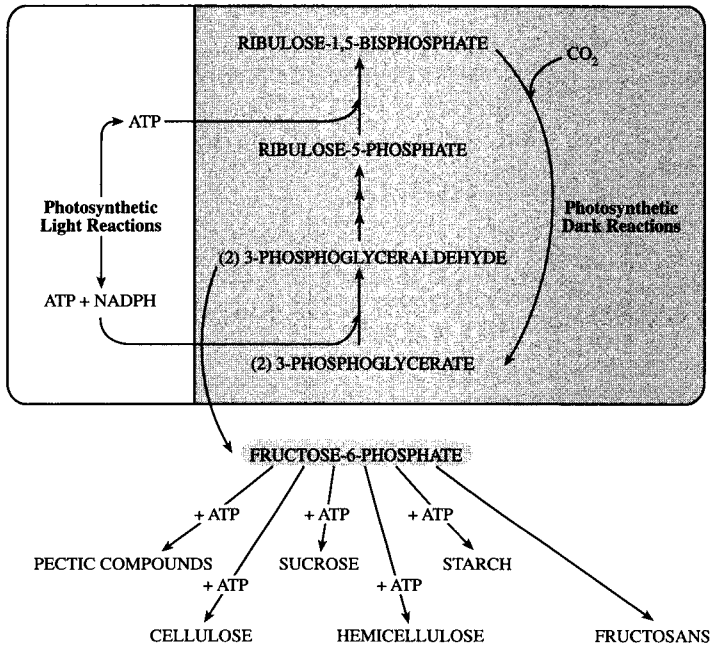


Figure 3.9. An overview of the reductive pentose phosphate or Calvin-Benson photosynthetic cycle. NADPH and ATP formed in the light reactions (Figure 3.8) are used to convert 3-phosphoglyceraldehyde back to ribulose-1,5-diphosphate to complete the cycle. As the number of 3-phosphoglyceraldehyde molecules increases, they are converted to hexose sugars and subsequently into the diverse array of carbon compounds found within the plant.

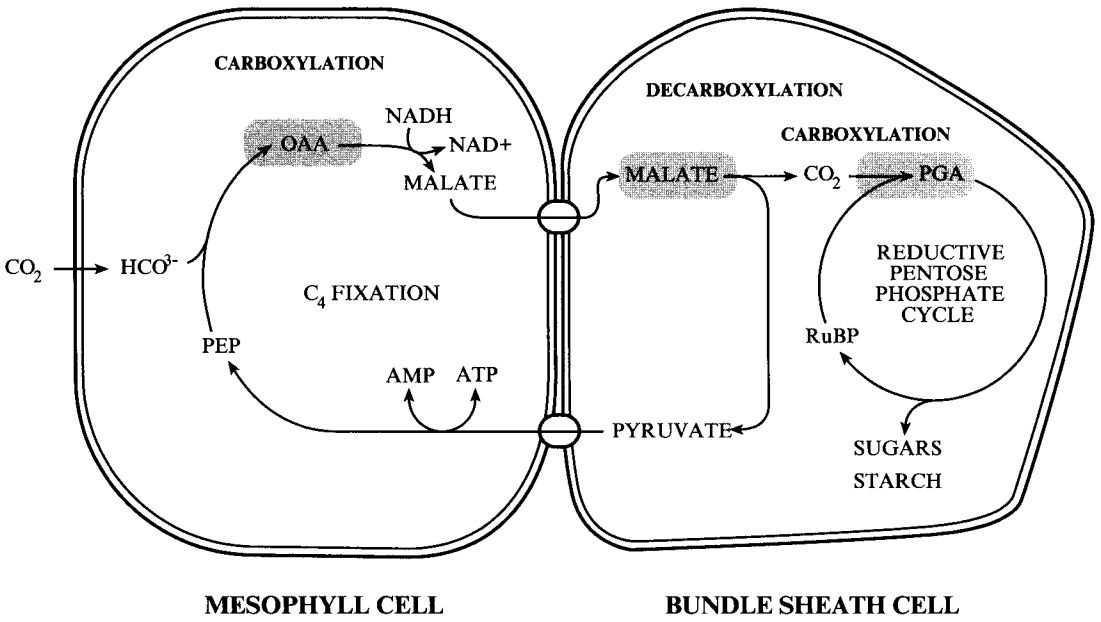


Figure 3.10. In C_4 plants, carbon dioxide is initially fixed (carboxylated) in the mesophyll cells of the leaf by reacting with phosphoenolpyruvate, forming oxaloacetate and subsequently malate. Malate is transported into bundle sheath cells, where it is decarboxylated, liberating CO_2 that is refixed *via* the reductive pentose phosphate pathway. Decarboxylation in the bundle sheath cells greatly increases the CO_2 concentration, increasing the efficiency of the C_3 pathway.

dioxide molecule. The chemical energy captured (nine ATP equivalents) is required for the fixation of one molecule of carbon dioxide, three as ATP and six equivalents in the reducing power of two NADPH molecules.

2.2.2. *C_4 Pathway*

In some species of plants, carbon dioxide reacts with phosphoenolpyruvic acid in the mesophyll cells, forming the four carbon compound oxaloacetate (hence the name C_4 pathway). Oxaloacetate is then converted to malate (Figure 3.10) that diffuses into the bundle sheath cells of the vascular bundles, where a carbon dioxide molecule is removed (decarboxylated) from the malate, yielding pyruvate that subsequently recycles back to phosphoenolpyruvate. The carbon dioxide removed is not lost but is refixed *via* the reductive pentose phosphate pathway in the bundle sheath cells. Here the oxygen concentration is low, and because of the release of carbon dioxide, its concentration is higher, greatly increasing the efficiency of the carboxylation reaction of the C_3 pathway (i.e., very low photorespiration).[19] Two variations of the pathway have also been found: 1) oxaloacetate → asparate → oxalacetate → malate → pyruvate; and 2) oxaloacetate → asparate → oxalacetate → phosphoenolpyruvate. In each case, the product formed in the bundle sheath cells with the removal of carbon dioxide (e.g., pyruvate or phosphoenolpyruvate) cycles through a series of reactions back to phosphoenolpyruvate in the mesophyll cells. Therefore in C_4 plants, the enzymes required for both the C_4 and C_3 pathways are present but in different cells. The C_4 pathway, however, is a more efficient means of carbon fixation than the reductive pentose phosphate pathway. Many species with the C_4 pathway (e.g., corn) evolved in geographical regions with hot, dry climates, enhancing their resistance to high temperatures and water use efficiency.

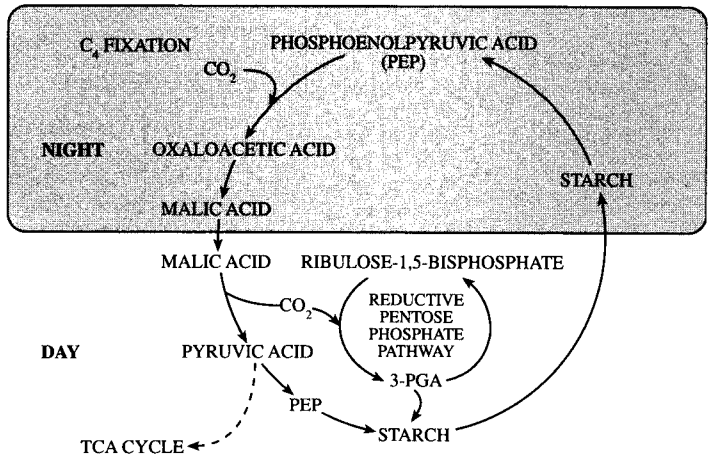


Figure 3.11. The CAM or crassulacean acid cycle found in some plants fixes CO_2 at night *via* the C_4 pathway when the stomata are open, with the formation of malic acid that is stored in the vacuole. During the day (stomata closed), malic acid moves out of the vacuole and is decarboxylated. The CO_2 is refixed in the chloroplasts, using the reductive pentose phosphate pathway. Some of the carbon is converted to starch that is recycled in a series of reactions, eventually forming phosphoenolpyruvic acid, starting the CAM cycle over again.

2.2.3. Crassulacean Acid Metabolism

A third means of fixing carbon is found in crassulacean acid metabolism (CAM) plants (e.g., pineapple). These plants trap carbon dioxide at night when their stomates are open rather than during the day as in C_3 and C_4 species whose stomates open in the light. Carbon dioxide is fixed through the action of the enzyme phosphoenolpyruvate carboxylase, forming oxaloacetate from phosphoenolpyruvate (Figure 3.11). During the day, when the stomates are closed, malate formed from oxaloacetate has carbon dioxide removed (decarboxylated) and refixed *via* the reductive pentose phosphate cycle. In CAM plants, both the C_3 and C_4 cycles are operative and found within the same cells as the CAM cycle. The pathway has evolved in plants that grow in very hot, arid regions where opening their stomates at night, rather than during the day, minimizes water loss.

3. METABOLIC CONSIDERATIONS IN HARVESTED PRODUCTS

3.1. Dark Respiration

3.1.1. Effects of Respiration

Photosynthesis provides the carbohydrates that plants use for growth and storage, while respiration is a mechanism by which the energy stored in the form of carbon compounds is released. In the general equation for the oxidation of a hexose sugar, the substrate and oxygen are converted into carbon dioxide, water and energy. The rate of conversion is modulated by temperature and the concentration of oxygen and carbon dioxide. The conversion is therefore

significant for both the stored product and the environment surrounding the product. The two major functions of dark respiration are the release of energy stored in chemical form as starch, sugars, lipids, and other substrates, and the formation of carbon skeletons to be used in various synthetic and maintenance reactions. The effects of respiration substantially alter the methods employed in handling and storing many products and, hence, are of considerable commercial importance.

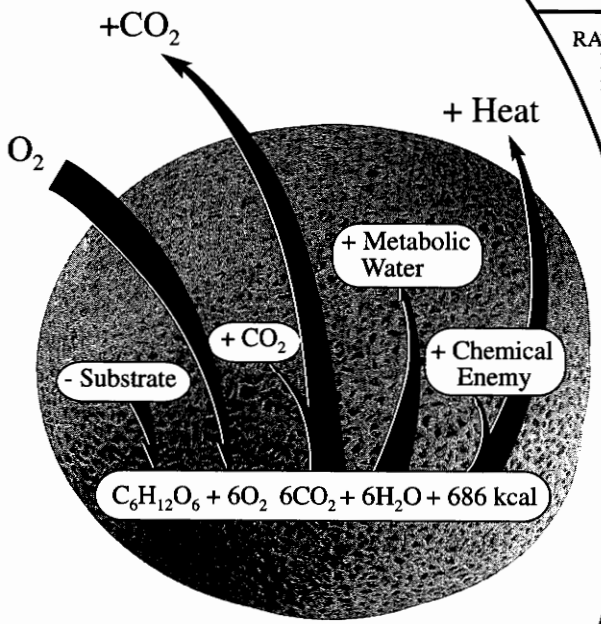
The loss of substrate from stored plant products results in a decrease in energy reserves within the tissue. This loss decreases the length of time the product can effectively maintain its condition. Loss of energy reserves eventually results in tissue starvation and accelerated senescence, and is especially critical in products such as leaves, flowers and other structures that are not carbon storage sites. Likewise, in a marketing system based on weight, respiratory losses of carbon represent weight losses in the product, hence a decreased value. The rate of respiration can in fact be used to predict the loss of dry weight from stored products (Figure 3.12). Respiratory losses also decrease the total food value (i.e., energy content) of edible products.

Respiration removes oxygen from the storage environment. If the oxygen concentration in the environment is severely depleted, anaerobic conditions occur that can rapidly spoil most plant products. As a consequence, the rate of respiration is important for determining the amount of ventilation required in the storage area. It is also critical in determining the type and design of packaging materials to be used, as well as the use of artificial surface coating on the product (e.g., waxes on citrus or cucumbers). The respiratory reduction in oxygen concentration in the storage environment can also be used as a tool to extend the storage life of a product. Since oxygen concentration has a pronounced effect on the rate at which respiration proceeds, a respiration mediated decrease in the ambient oxygen concentration can create a modified environment that may be used to slow respiration. This principle, used since Roman times, represents the basis for present day storage practices for several highly perishable products.

Elevated ambient levels of carbon dioxide generated by respiration can also be used to decrease respiration since its accumulation impedes the rate at which the process proceeds. The degree of inhibition of respiration by carbon dioxide and the sensitivity of the tissue to high carbon dioxide concentration varies widely among products. Carbon dioxide produced during the respiratory process, if allowed to accumulate, can be harmful to many stored products. For example, lettuce,[72] mature green tomatoes, bell peppers[82] and other products are damaged by high carbon dioxide. So it is essential that the carbon dioxide concentration be maintained at a safe level through adequate ventilation or absorption.

Water is produced during the respiratory process (termed metabolic water) and becomes part of the water present within the tissue. Metabolic water, however, represents only a very minor addition to the total volume of water within the tissue and hence is of minimal significance (Figure 3.12).

Energy, the final product in the respiratory equation, has a significant influence both upon the maintenance of the product and the preferred storage environment. The complete oxidation of one mole of a six carbon sugar such as glucose results in the formation of 686 kcal (2,868 kJ · mol^{-1}) of energy. In actively growing tissues, a significant portion of this energy is utilized in chemical forms by the cell for synthetic and maintenance reactions. A substantial amount of energy is lost, however, as heat, also referred to as "vital" heat, since the energy conservation during the transfer among molecules is not 100% efficient. In actively metabolizing tissues, around 46% of the total respiratory energy is lost as heat. This amount, however, varies among different types of plants and organs, and the general condition of the tissue. The amount of heat produced by the product can be calculated (i.e., within ~10%)[41] directly from the respiratory rate of the product (Figure 3.12). Knowledge of the amount of heat produced is important in determining the cooling requirements for a product and therefore the size of



	GLUCOSE		OXYGEN		CARBON DIOXIDE		WATER	ENERGY
moles	1		6		6		6	
weight	180 g	+	192 g	=	264 g	+	108 g	

Muskmelons (100 kg) which are 90% moisture are stored at 5°C and have a respiratory rate of 9 mg CO_2/kg • hr and a fresh rate loss of 3%/day.

RATE OF DRY WEIGHT LOSS

For every 180 g of sugar oxidized, 264 g of CO_2 is produced by the product. Therefore, the rate of dry matter loss in grams of glucose/kg fwt of fruit/day is equal to: $\left[\frac{\text{respiration (mg } CO_2\text{/ kg • hr)}}{1000 \text{ (mg/g)}}\right]\left[\frac{180}{264}\right]\left[\frac{24 \text{ hr}}{\text{day}}\right]$

The muskmelons lose: $\left[\frac{9 \text{ mg } CO_2\text{/ kg • hr}}{1000}\right]$ [.68] [24] = 0.147 g/kg fwt/day or with 100 kg of fruit = 14.7 g/load/day.

RESPIRATORY OR VITAL HEAT PRODUCED

One mole of glucose yields 686 kcal, therefore, for every 6 moles of CO_2 given off, 686/6 kcal has been produced. Then one mole of CO_2 represents 114 kcal or 114,000 cal/44g (weight of 1 mole of CO_2) = 2,591cal/mg CO_2. One Btu = 252 cal. Then the number of Btu's produced by 1 ton when 1 mg of CO_2/kg • hr is given off can be calculated by:

$\left[\frac{1 \text{ mg}}{\text{kg • hr}}\right]\left[\frac{2{,}591 \text{ cal}}{252 \text{ cal/Btu}}\right]\left[\frac{1000 \text{ kg}}{\text{metric ton}}\right]\left[\frac{24 \text{ hr}}{\text{day}}\right]$ = 247 Btu / metric ton • day or 224 Btu/British ton • day.

Therefore, the 100 kg of melons will produce the following number of Btu's/day:

$\left[\frac{9 \text{ mg } CO_2\text{/ kg • hr}}{\text{kg • hr}}\right]\left[\frac{.247 \text{ Btu}}{\text{kg • day}}\right]$ [100 kg fruit weight] = 222 Btu/day

METABOLIC WATER PRODUCED

The radio of the weight of CO_2 to water produced = 264/108. Therefore, the melons produce the following metabolic water: $\frac{264 \text{ g } CO_2}{108 \text{ g } H_2O} \times \frac{9 \text{ mg } CO_2\text{/kg • hr}}{x \text{ mg } H_2O\text{/kg • hr}}$ = 3.68 mg H_2O/kg • hr or .00000368 mg H_2O/kg • hr.

The total amount of water produced by all of the melons per week

$= \left[\frac{.00000368 \text{ kg } H_2O}{\text{kg • hr}}\right]\left[\frac{24 \text{ hr}}{\text{day}}\right]\left[\frac{7 \text{ days}}{\text{week}}\right]$ [100 kg fruit weight] = .0618 kg H_2O/week. The percent of the total water.

that is metabolic water in one week = $\frac{.0618 \text{ kg } H_2O \text{ / 100 kg • week}}{90 \text{ kg } H_2O \text{ in 100 kg fruit}}$ = .0687% of the total water in the fruit.

Therefore, the 3% weight loss/week represents: 3 kg fresh weight - .0103 kg CO_2/100 kg • week + .0618 kg metabolic water/100 kg • week = 2.96 kg fresh weight lost due to evapotranspiration.

Figure 3.12. Respiration results in the utilization of substrate (e.g., glucose) and oxygen and the formation of carbon dioxide, water and energy. Knowing the rate of respiration (mg CO_2 produced per kg of product each hour), we can calculate the rate of dry weight loss and the amount of heat and metabolic water produced. Examples of these calculations for muskmelons are illustrated in the figure.

the refrigeration system needed to maintain the desired temperature of the storage room. The amount of heat produced also influences the size of the fans required to move air around the product in storage, package design and stacking method.

3.1.2. *Respiratory Substrates*

Respiration depends on the presence of a substrate. In many tissues, this substrate is a storage form of carbohydrate such as starch in the sweetpotato root or inulin in Jerusalem artichoke tubers. These more complex molecules are broken down into simple sugars that enter the respiratory pathways to provide energy for the plant. In some species, carbon may be stored as lipids. The avocado fruit contains approximately 25% lipid on a fresh weight basis and pecan kernels approximately 74%. Organic acids, proteins and other molecules may also be utilized as respiratory substrates in plants, although in most cases these secondary substrates are not produced for this purpose. Under conditions where the tissue is depleted or "starved" of carbohydrate or lipid reserves, these secondary respiratory substrates are utilized. This situation is more likely to occur in postharvest products such as leaves or flowers that do not represent storage organs and therefore have relatively little reserve substrate. Proteins may also be hydrolyzed into their component amino acids and catabolized in the glycolytic pathway and tricarboxylic acid cycle.

When these various substrates are utilized and completely oxidized, different amounts of oxygen are consumed in relation to the amount of carbon dioxide evolved. The ratio of the two is called the **respiratory quotient** (RQ). The RQ provides a general indication of the particular substrate being used as the primary source of respiratory energy. For example, the oxidation of a common carbohydrate, lipid and organic acid give the following respiratory quotients.

Type of Substrate	*Substrate*	*Reaction*	*Respiratory Quotient (CO_2/O_2)*
Carbohydrate	glucose	$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$	1.00
Lipid fatty acid	palmitic acid	$C_{16}H_{32}O_2 + 11O_2 \rightarrow C_{12}H_{22}O_{11} + 4\,CO_2 + 5\,H_2O$	0.36
Organic acid	malic acid	$C_4H_6O_5 + 3\,O_2 \rightarrow 4\,CO_2 + 3\,H_2O$	1.33

The RQ was of greater interest early last century when analytical techniques were limited. There is still a diversity of opinions as to the actual value of the RQ. Along with tissue type, temperature and tissue age, a number of other factors significantly alter the RQ. In addition, substrates are not always completely oxidized, and several types of substrates may be used simultaneously by the cells, each greatly complicating interpretation of the RQ obtained. Other factors that affect the apparent RQ are the differential permeabilities of the tissues to oxygen and carbon dioxide, as well as respiration at oxygen levels approaching anaerobic conditions.

3.1.3. *Control Points in the Respiratory Pathway*

Changes in the cell's internal environment by external (e.g., temperature) or internal (e.g., substrate availability) factors often result in significant alterations in the respiration rate. The alteration may be due to shifts in the activity at the regulatory sites in the pathways or due to changing priorities among different pathways. Control of respiration rate in plant cells can be regulated at various points in the respiratory pathways and by a number of means. Substrate supply can control the rate of respiration by regulating substrate available for a particular

reaction. For example, if glucose-6-phosphate levels are high, the reaction catalyzed by phosphoglucoisomerase shifts toward the formation of fructose-6-phosphate in order to maintain an equilibrium. Substrate control is probably more important when demand for intermediates generated by the tricarboxylic acid cycle is high. Control can occur through the activity of an enzyme and to a lesser extent the enzymes concentration. Enzyme activities are modulated by substrate and product concentration, cofactors such as metal ions, compounds that activate or inhibit the enzyme, and the rate of enzyme synthesis and degradation. For rate limiting reactions, the concentration of an enzyme is thought to represent a coarse control. In contrast, enzyme activation is considered a means of fine control. The availability of phosphate acceptors (ADP) represents an extremely important means of respiratory control. Restricting the rate of flow of electrons through the electron transport chain and, hence, the rate of oxidation of NADH limits the rate of a number of reactions. However, if NADH is reoxidized by an alternative reaction, oxidative phosphorylation is diminished in its regulatory role. High levels of ATP also directly inhibit certain enzyme reactions (e.g., phosphofructokinase and pyruvate kinase). Therefore, the levels of ADP, NAD and NADP and their reduced products represent important modulators of respiration.

The tricarboxylic acid cycle appears to be largely regulated by mitochondrial energy status (ADP, ATP). However, low oxygen and high carbon dioxide are also known to have a pronounced effect on the rates of specific enzymes in the cycle. High carbon dioxide inhibits the conversion of succinate to malate, and malate to pyruvate, in apple fruit tissue.[63] Key enzymes controlling the rate of the glycolytic pathway are phosphofructokinase and pyruvate kinase, while in the pentose phosphate pathway the activity of glucose-6-phosphate dehydrogenase is controlled by the NADPH/NADP ratio.

3.1.4. Factors Affecting the Rate of Dark Respiration

The control of postharvest respiratory responses is strongly influenced by a number of commodity and environmental factors. For many products, high respiratory rates are closely correlated with reduced storage life. Proper management of these factors is imperative for maintaining quality and maximizing storage life.

a. Temperature

Temperature has a pronounced effect on the respiratory rate of harvested products. As product temperature increases, reaction rates increase;[90] however, the degree of increase is not the same for all the reactions within a tissue (e.g., the optimum temperature for photosynthesis is usually lower than the optimum temperature for respiration). The rate of change in reactions due to temperature is commonly characterized using a measure called the Q_{10}, which is the ratio of the rate of a reaction at one temperature (T_1) versus the rate at that temperature plus 10°C [(rate at $T_{1+10°C}$)/rate at T_1]. The Q_{10} is often quoted for respiration, in that it gives a very general estimate of the effect of temperature on the overall tissue metabolic rate. There are, however, many exceptions in different metabolic pathways; for example the respiratory rate of potato tubers decreases with decreasing temperature while the formation of sugars from starch increases below 10°C (Figure 3.13). For many products the Q_{10} for respiration is between 2.0 and 2.5 for every 10°C increase in temperature within the 5°C to 25°C range. If we are interested in maintaining a product as close to its condition at harvest, the use of low temperatures to reduce changes due to metabolism is essential. By decreasing the temperature from 25°C to 15°C when the Q_{10} is 2, the respiration rate will be half that at 25°C and halved again if the temperature is reduced from 15°C to 5°C. As the temperature increases from 25°C

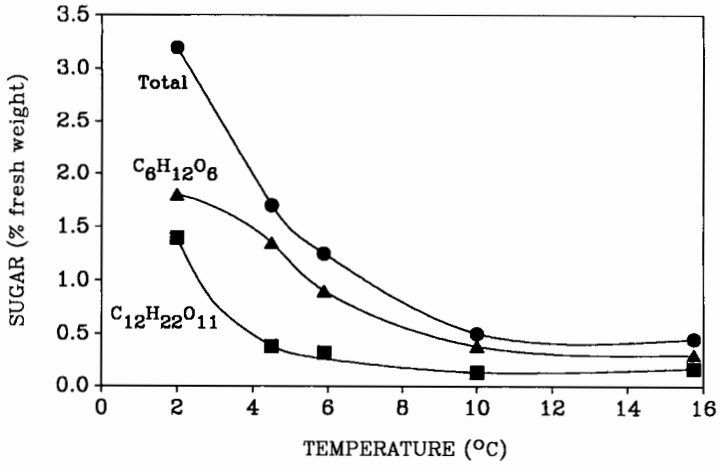


Figure 3.13. The effect of temperature on the formation of sugars from starch in potato tubers cv. Majestic (*redrawn from Burton*).[24]

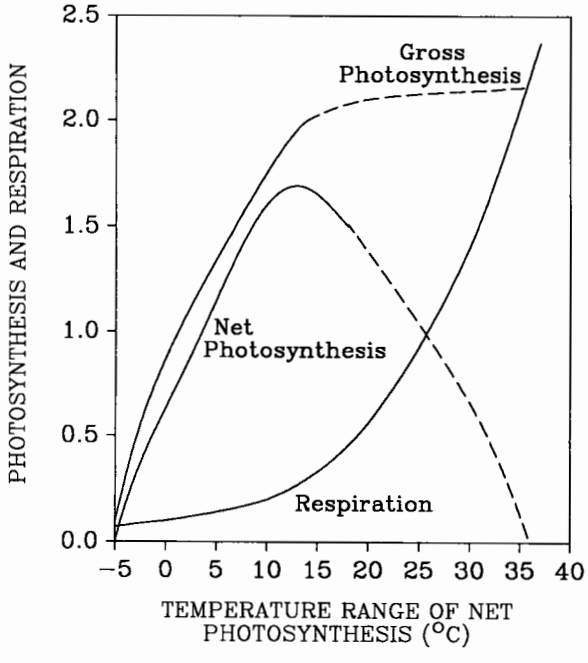


Figure 3.14. The effect of temperature on photosynthesis, respiration and net or apparent photosynthesis of Swiss stone pine seedlings (broken lines represent estimates, *redrawn from Tranquillini*).[101]

into the 30°C to 35°C range, the Q_{10} declines for most products, and at very high temperatures reaction rates are actually depressed, probably due to the loss of enzyme activity. The actual temperature range over which there is a linear increase in Q_{10} and the maximum and minimum temperature for a particular metabolic process vary widely among species and the type of tissue monitored (Figure 3.14). For example, respiration in *Populus tremuloides* stems can be measured at -11°C,[35] a temperature at which an apple fruit would be frozen solid, terminating respiration.

It is important to note that while the ambient temperature at which the product is stored is of critical importance in determining the metabolic rate, the product temperature is typically

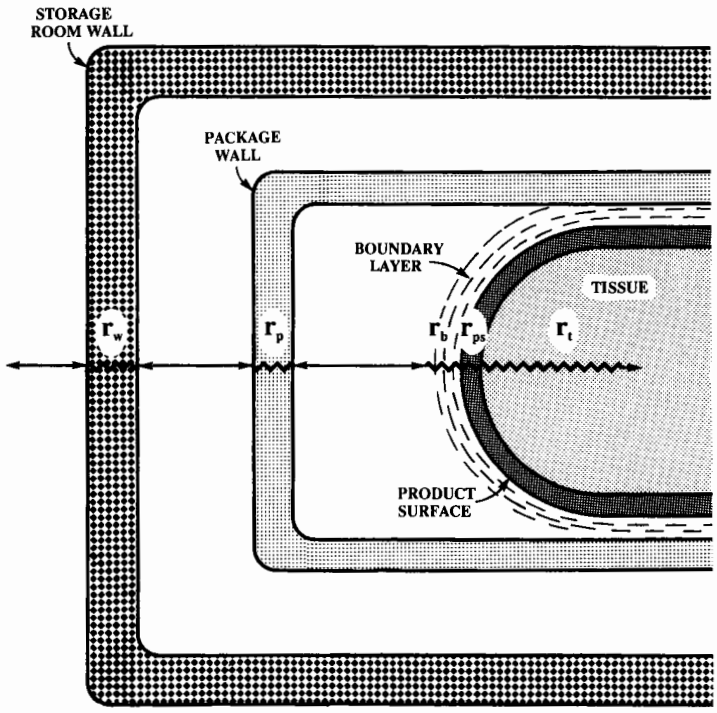


Figure 3.15. Diagrammatic presentation of the resistances to gas exchange in a harvested product. Total resistance is r_w (storage walls) + r_p (package wall) + r_b (boundry layer) + r_{ps} (product surface) + r_t (tissue). The resistances for r_w, r_p and r_{ps} are often manipulated for certain postharvest products to extend storage life.

slightly higher than the ambient temperature due to the heat liberated from the respiratory process. This often slight difference in temperature is quite important due to its effect on maintaining the moisture balance of the harvested product (see chapter 9).

b. Gas Composition

The gas composition of the atmosphere that surrounds the product can influence both its respiratory and general metabolic rate. Oxygen, carbon dioxide and ethylene are the most important gases influencing respiration. Pollutant gases such as sulfur dioxide, ozone, propylene, and others can also have a significant effect if their concentration becomes sufficiently high.

During normal plant growth and development in the field, there are seldom large or long term alterations in gas atmosphere composition. After harvest, however, plant products are normally bulked tightly together and placed in containers and storage areas that have restricted air flow (Figure 3.15). Restricted air flow creates additional resistances for gas movement into and out of the product and hence alters the concentration of gases within the tissue. Typically, reduced gas exchange leads to a decrease in the internal oxygen and an increase in carbon dioxide. However in some crops, such as submerged aquatics (e.g., Chinese water chestnut, lotus root) and some root and tuber crops that grow normally under conditions of high gas diffusion resistance, the opposite may be true. Therefore, postharvest conditions commonly result in significant alterations in the gas environment to which the product experienced prior to harvest, and these changes can influence metabolic activity.

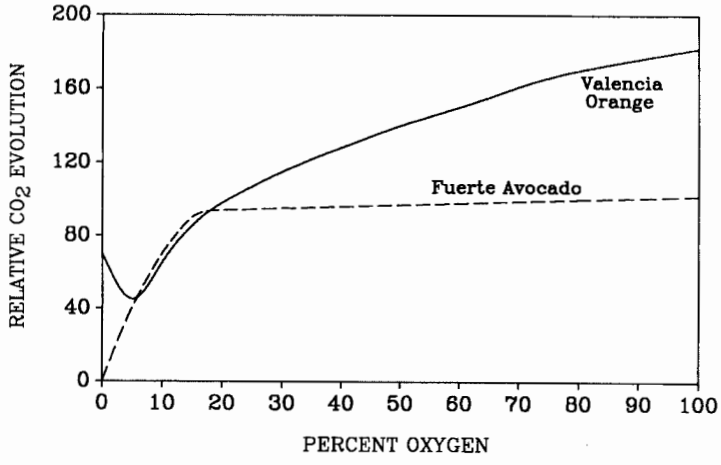


Figure 3.16. The effect of oxygen concentration on the respiration of Fuerte avocadoes and Valencia oranges. Note the "Pasteur effect" (stimulated respiration) on oranges at very low oxygen levels (*redrawn from Baile*).[10,12]

The effect of oxygen concentration on harvested plant products has been known since around the beginning of the 19th century.[50] Berard in 1821 noted that fruits held in an environment devoid of oxygen did not ripen, and if not kept too long under the low oxygen conditions, ripened normally upon return to air.[9] The rate of respiration is closely tied to the oxygen concentration in harvested products. As the internal oxygen concentration decreases, respiration decreases (Figure 3.16) until the oxygen concentration reaches the **extinction point** or critical concentration at about 1 to 3% oxygen. This concentration represents the point at which aerobic respiration *via* the tricarboxylic acid cycle is blocked and anaerobic fermentation begins. Below the extinction point, the rate of respiration increases. The increase in respiration at very low oxygen concentrations is known as the **Pasteur effect,** after Louis Pasteur, who first studied the phenomenon in microorganisms. Although the Pasteur effect is found widely in plants, it does not occur in all harvested products. For example, there is no increase in respiration of the avocado fruit even at 0% oxygen (Figure 3.16). The Pasteur effect is due to a cessation of the tricarboxylic acid cycle, as NAD and NADP are not available, having been converted to the reduced forms (NADH and NADPH). The reduced forms cannot transfer their energy to the electron transport chain in the mitochondria to produce ATP as the final step in the chain requires oxygen. For the cell to maintain itself, ATP is required; hence the rate of glycolysis has to be increased, since it does not directly require oxygen. This change also leads to the production of ethanol and lactic acid that requires the NADH produced in the glycolytic pathway.

The precise reason why the respiration rate is reduced at oxygen concentrations above the critical concentration in most products is not known. A number of possible explanations have been suggested. The enzyme phosphofructokinase, which catalyzes the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate in the glycolytic pathway, is inhibited by ATP and citric acid, both of which are formed in the oxygen dependent tricarboxylic acid cycle and may represent the modulating factors. It is also possible that oxygen concentration modulates one or more of the glycolytic enzymes. Respiration rate could also be decreased through an effect on several of the enzymes in the tricarboxylic acid cycle. In green banana fruit, low oxygen limits the rate of the enzymatic steps between either oxaloacetate or pyruvate and citrate and between 2-oxoglutarate and succinate.[78]

Table 3.4. Effect of Temperature and Oxygen Concentration on the Respiratory Rate of Various Commodities.*

	Carbon Dioxide Production ($mg \cdot kg^{-1} \cdot hr^{-1}$)					
	In Air (°C)			*In 3% O_2 (°C)*		
	0	*10*	*20*	*0*	*10*	*20*
Asparagus	28	63	127	25	45	75
Beans, broad	35	87	145	40	55	80
Beans, runner	21	36	90	15	25	46
Beetroot, storing	4	11	19	6	7	10
Beetroot, bunching with leaves	11	22	40	7	14	32
Blackberries 'Bedford Giant'	22	62	155	15	50	125
Blackcurrants 'Baldwin'	16	39	130	12	30	74
Brussels sprouts	17	50	90	14	35	70
Cabbage 'Primo'	11	30	40	8	15	30
Cabbage 'January King'	6	26	57	6	18	28
Cabbage 'Deccma'	3	8	20	2	6	12
Carrots, storing	13	19	33	7	11	25
Carrots, bunching with leaves	35	74	121	28	54	85
Calabrese	42	105	240	—	70	120
Cauliflower 'April Glory'	20	45	126	14	45	60
Celery, white	7	12	33	5	9	22
Cucumber	6	13	15	5	8	10
Gooseberries 'Leveller'	10	23	58	7	16	26
Leeks 'Musseburgh'	20	50	110	10	30	57
Lettuce 'Unrivalled'	18	26	85	15	20	55
Lettuce 'Kordaat'	9	17	37	7	12	25
Lettuce 'Klock'	16	31	80	15	25	45
Onion 'Bedfordshire Champion'	3	7	8	2	4	4
Parsnip 'Hollow Crown'	7	26	49	6	12	30
Potato, main crop 'King Edward'	6	4	6	5	3	4
Potato, new (immature)	10	20	40	10	18	30
Peas (in pod) early, 'Kelvedon Wonder'	40	130	255	29	84	160
Peas main crop, 'Dark Green Perfection'	47	120	250	45	60	160
Peppers, green	8	20	35	9	14	17
Raspberries 'Malling Jewel'	24	92	200	22	56	130
Rhubarb (forced)	14	35	54	11	20	42
Spinach 'Prickly True'	50	80	150	51	87	137
Sprouting broccoli	77	170	425	65	115	215
Strawberries 'Cambridge Favorite'	15	52	127	12	45	86
Sweetcorn	31	90	210	27	60	120
Tomato 'Eurocross BB'	6	15	30	4	6	12
Turnip, bunching with leaves	15	30	52	10	19	39
Watercress	18	80	207	19	72	168

**Source:* Robinson et al.[94]

The respiratory rate of most stored products can be decreased by lowering the oxygen to a concentration that is not below the extinction point for that product (Table 3.4). The actual critical concentration of oxygen appears to vary among products. In addition, the external concentration of oxygen that gives the appropriate internal concentration for minimizing respiration varies with the rate of oxygen utilization by the tissue, the tissue's diffusive resistance, and the differential in oxygen partial pressures between the interior and the exterior. There-

fore, at higher temperatures, the external concentration of oxygen must be increased to maintain a given oxygen level within the tissue due to the increased rate of utilization of the oxygen by the tissue.

In general, a significant decrease in the respiratory rate for most stored products does not occur until the external oxygen concentration is below 10%. The optimum external concentration for a number of products held in cold storage is in the 1 to 3% range; however, there are exceptions. For example, sweetpotatoes shift to anaerobic metabolism at external oxygen concentrations below 5 to 7 percent.[25] Much of the variation in optimum external oxygen concentration among types of products and even among cultivars can be accounted for by factors other than the external oxygen concentration.

The use of low oxygen in the storage of plant products has the potential to decrease the overall metabolic rate and a diverse array of specific biochemical changes. At the product level, the net effect may be seen as delayed ripening, aging or the development of certain storage disorders. Low oxygen environments, however, are not commercially used for many commodities for several reasons. The very short time span between harvest and the retail sale of many products and the availability of the product year round from different production locations often makes its use unnecessary. Likewise, for most products the costs are substantially greater than the benefits obtained, though there are notable exceptions. Approximately a half a million metric tons of apples are stored each year, in the United States alone, utilizing low oxygen conditions. This storage method extends the availability of the crop for 4 to 10 months over conventional storage practices, greatly increasing the net worth of the industry.

Carbon dioxide impedes respiration, resulting in a net and often quite significant decrease in respiration in some products. The effect of carbon dioxide, although not universal, has been shown in seedlings, intact plants and detached plant parts, and occurs both under aerobic and anaerobic respiratory conditions. The degree to which respiration is impeded increases in relation to the concentration of carbon dioxide in the atmosphere. For example, in pea seedlings, the inhibitory action of carbon dioxide at concentrations up to 50% increases approximately with the square root of the concentration.[58] Carbon dioxide, therefore, appears to retard the rate of respiration but does not totally block it.[75]

Under aerobic conditions respiration is impeded by high carbon dioxide when sufficient respiratory reserves are present, a common condition with most postharvest products. However, under conditions where the tissue is depleted of a ready source of stored reserves, respiration is no longer decreased by high carbon dioxide. The precise mechanisms of action of high carbon dioxide that results in a decrease in respiration are not known. The inhibitory effect is not due to permanent injury to the tissue, since upon removal of the carbon dioxide, respiration returns to normal. High carbon dioxide concentrations under aerobic conditions affect the tricarboxylic acid cycle at the conversion of succinate to malate and malate to pyruvate in apple fruit.[63] Succinate dehydrogenase, which converts succinate to malate, is the enzyme most significantly impeded. The level of reduction in the presence of 15% carbon dioxide results in toxic levels of succinate accumulating in apples, causing damage to the tissue.[53] The influence of carbon dioxide on other tricarboxylic acid cycle enzymes appears negligible.

A high carbon dioxide concentration during storage does not depress respiration in all tissues. In some cases, respiration may be unaffected or even significantly increased by elevated carbon dioxide. The respiratory rate of potato tubers, onion and tulip bulbs, and beetroot has been shown to be substantially increased, in some instances up to 200%, upon exposure to extremely high levels of carbon dioxide (30 to 70%)[100] and in lemon fruits by 10% carbon dioxide. Carrot roots are not affected.

The mechanisms that lead to the stimulation of respiration by high carbon dioxide may be attributable in part to the fixation of carbon dioxide by malic enzyme and phosphoenolpyruvate carboxylase (Figure 3.2). The initial products formed in lemon fruit after brief

Table 3.5. Effect of Ethylene and Cyanide on the Respiratory Rate as Measured by Oxygen Uptake of Various Types of Plant Tissue.*

	$\mu L\ O_2 \cdot g^{-1}$ *fresh wt* $\cdot$ *hour*$^{-1}$		
Tissue	*Control*	*Ethylene*	*Cyanide*
Fruit			
Apple	6	16	18
Avocado	36	150	150
Cherimoya	35	160	152
Lemon	7	16	21
Grapefruit	11	30	40
Stem			
Irish Potato	3	14	14
Rutabaga	9	18	23
Root			
Beet	11	22	24
Carrot	12	20	30
Sweetpotato	18	22	24

**Source:* Solomos and Biale.[98]

exposure to $^{14}CO_2$ are malic, citric and aspartic acids.[108] High concentrations of carbon dioxide may, therefore, facilitate the formation of tricarboxylic acid cycle intermediates and thereby stimulate respiration. The stimulation may also be related to secondary effects of the carbon dioxide molecule on the pH of the cytoplasm. The effect of elevated carbon dioxide on cellular pH is complex and varies with tissue. Carbon dioxide is readily soluble in the cytoplasm and the vacuole, existing as bicarbonate and hydrogen ions with the dissociation of carbonic acid. When lettuce was exposed to 15% carbon dioxide[96] and avocado to 20%,[71] the pH of the cytoplasm declined 0.4 units while the lettuce vacuole pH declined 0.1 units. When the lettuce and avocado were moved back into air, the pH returned to near the pretreatment level. The change in pH of freshly harvested green peas exposed to elevated carbon dioxide was compensated for by a decrease in malic acid concentration giving essentially no net change.[105] Short transitory changes in pH caused by returning the tissue to ambient carbon dioxide conditions may activate the carboxylation of phosphoenolpyruvate to oxaloacetate and subsequently malate.[106] At present the effect of even small changes in cellular pH on respiration is not known. It is known that along with a change in cellular pH in avocado, there is a decline in ATP levels and respiratory enzymes, which are also transitory.[70,71]

Ethylene is a phytohormone that can significantly stimulate the respiratory rate of a number of harvested products. This was first illustrated by the work of Denny[30,31] on citrus fruit and later with bananas.[48] The effect of ethylene is of considerable interest to postharvest biologists in that harvested products synthesize ethylene. In most cases, however, an increase in respiratory rate *per se* represents only a minor concern in relation to major biochemical changes in quality that may also be induced by ethylene (e.g., accelerated floral senescence, loss of chlorophyll, abscission).

A relatively wide range of vegetative and reproductive tissues respond to ethylene by increasing their respiratory rate (Table 3.5), and the increase is dependent upon the continued presence of the gas.[93,98] Respiration is not stimulated by ethylene in all tissue. For example, the respiration of strawberry fruit, pea[39] and wheat seedlings,[73] and peanut leaves[57] is not stimulated. Flower respiration typically declines after harvest, followed by an increase as they begin to senesce. Enhanced respiration induced by ethylene in flowers may be an indirect effect, through an acceleration of the senescence process. Many of the tissues in which respiration is

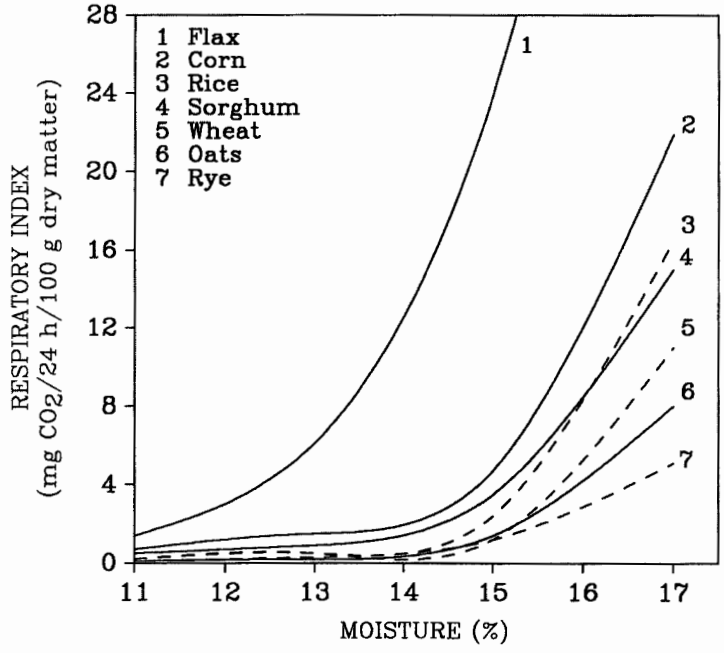


Figure 3.17. Effect of seed moisture level on respiration rates (*redrawn from Bailey*).[4]

accelerated by ethylene have significant storage energy reserves. It has been suggested that the ability of a tissue to respond to ethylene with an increase in respiration is closely correlated with the presence of the alternative electron transport pathway (Table 3.5). In these tissues, both cyanide and ethylene stimulate respiration, although through different mechanisms. The respiratory increase in either case does not necessarily involve the induction of ripening or stimulated ethylene synthesis, since it can be found in potato tuber tissue that neither ripens nor has autocatalytic ethylene synthesis.

c. Moisture content of the tissue

In general, respiration and metabolic processes decrease with decreasing tissue moisture content. There are, however, many interacting factors such as species, tissue type and physiological condition that significantly alter the plant or plant part's response to a particular moisture status. The change in respiratory rate can be influenced by moisture changes after harvest or preharvest differences in moisture content. For example, the respiratory rate of the storage roots of sweetpotato cultivars is closely related to the cultivar's percent moisture.

Seeds show the most dramatic effect of postharvest changes in the product's moisture status on respiration (Figure 3.17). The respiratory rate closely parallels the seed's moisture content as the seed imbibes or loses water. The respiration rate of leaves on an intact plant also declines with decreasing leaf water content; however, under severe dehydration it may temporarily increase.[21] Leaf water status is determined by soil moisture deficits or excesses.[26] In fleshy fruit, postharvest respiratory changes normally occur only after a substantial change in the internal water concentration. Hence, with most fleshy or succulent postharvest products, decreasing the internal moisture content is not a viable method for controlling the rate of respiration of the tissue. In these tissues, moisture content is often closely tied to product quality,[90] and a decrease in moisture is counterproductive. In many other products, however, longevity can be greatly ex-

tended through the repression of respiratory and metabolic activity with reduced product moisture. For example, in the storage of grains, seeds, dates, and most nuts, moisture status alteration represents an excellent means of extending a product's useful life.

d. Wounding

Wounding of plant tissue stimulates the respiratory rate of the affected cells, a response that has been known for nearly a century. Boehn[18] demonstrated that cutting potato tubers resulted in an abnormal rate of carbon dioxide production. Cutting the roots of trees[46,47] and the handling of leaves[3,38] also results in a stimulation of respiration.

Respiratory increases from wounds to plant tissue are often grouped into two general classes: a) those caused by mechanical damage—**wound respiration,** and b) those caused by infection by another organism such as fungi or viruses—**infection-induced respiration.**[103] This classification is not, however, definitive in that wounds induced by other means, although normally less frequent, are found (e.g., chemical sprays, light, pollutants). In addition, several types of wounds may occur simultaneously in the same tissue.

Mechanically induced wounds include those caused by harvesting, handling, wind, rain, hail, insects and animals. The wounds can be separated into subclasses based upon the presence or absence of surface punctures, cuts or lesions. Injuries which facilitate the diffusion of gasses to or from the underlying tissue often result in a substantial, but transient, increase in apparent respiration due to the escape of carbon dioxide that has accumulated in the intercellular spaces of the tissue. As a consequence, it is often difficult to make a clear distinction between altered gas exchange and wound effects on respiration when carbon dioxide production is used as sole means of measuring respiration.

Uritani and Asahi[103] characterized the differences in respiratory response between mechanically wounded and infected tissue (Figure 3.18), illustrating two distinct patterns. In both cases, increases in respiration coincided with increases in storage carbohydrate catabolism and an increase in soluble sugars in some tissues. Both the glycolytic[55] and pentose phosphate pathways are stimulated in response to increased demand for both primary and secondary plant products needed for wound healing. Healing includes the formation of lignin, suberin and in some cases, callus. Wound respiration, therefore, facilitates the supply of precursors and cofactors required for the biosynthesis of these wound healing layers (see Bloch[16,17]).

Infection-induced-respiratory increases are related to primary and secondary defense reactions by the cells. Plants have evolved multiple and varied techniques to combat the invasion of microorganisms. For example, rapid cell death resulting in necrotic areas confines the mycelia, limiting the number of cells infected (hypersensitive response). In addition, secondary products such as phytoalexins may be formed to minimize invasion. These infection induced processes, like those of mechanical wounding, require respiratory derived energy and secondary metabolic products, resulting in the observed increases in tissue respiration.

e. Species and plant part

Extremely large differences in respiratory rates can be found among different plant parts. The respiration rate of harvested vegetables, representing a range of plant parts, could be ranked on a dry weight basis in the following order: asparagus, lettuce, green bean, okra, green onion, carrot, tomato, beet root, green mango, red pimento[8] and other crops.[88] Differences include variation due to species and cultivar. Likewise, the respiratory rate of different plant parts, even within the same species and cultivar, can vary significantly, and there is often a wide range among parts of the same organ (e.g., the respiratory activity of the wheat seed embryo was twenty times that of the adjoining endosperm).[23]

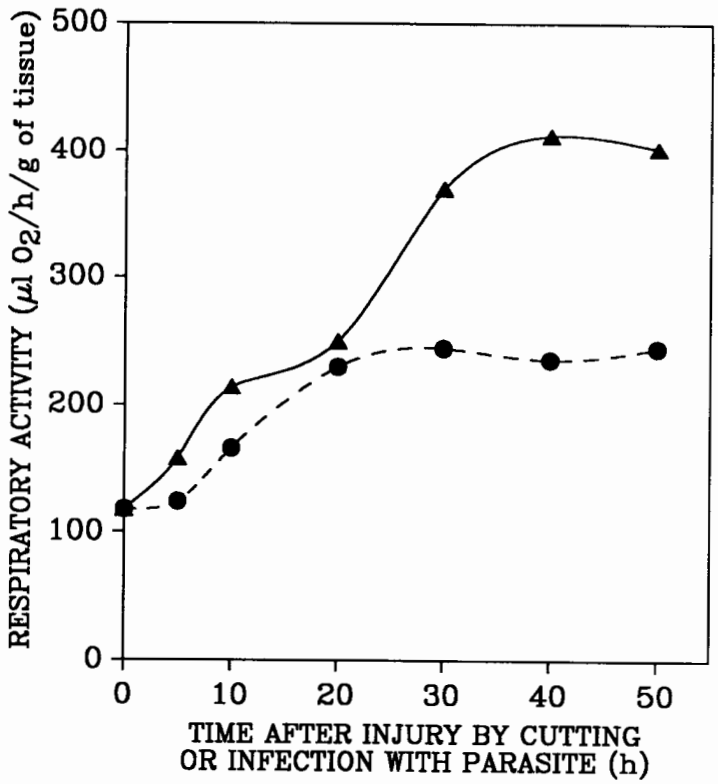


Figure 3.18. The effect of mechanical wounding (●) or infection (▲) by the fungus *Ceratocystis fimbriata* on the respiratory rate of sweet-potato storage root tissue (*redrawn from Uritani and Asahi*).[103]

The intensity of respiration of harvested products can vary widely among similar plant parts from different species. For example, flax seed have a respiratory rate that is 14 times that of barley seed at the same temperature and moisture content (11%).[4] Avocado fruit have a maximum rate of respiration at their climacteric peak nearly 8 times that of apple.[11]

f. Cultivar

While one could anticipate a significant range in respiratory rates among species, differences at the cultivar level may also be substantial. For example, cut flowers of the chrysanthemum cultivar Indianapolis White had a respiratory rate of 1.6 times that of the cultivar Indianapolis Pink, based on flower fresh weight, and 4.3 times greater expressed on a per flower basis.[67] Likewise 'McIntosh' apple fruit have been shown to have preharvest respiratory rates double that of 'Delicious'.[42,43] While not all cultivars exhibit this degree of variation, it is common and, depending on the postharvest conditions, may pose a factor for consideration during storage.

g. Stage of development

The stage of development of a plant or plant part can have a pronounced effect on the respiratory and metabolic rate of the tissue after harvest. In general, young actively growing cells tend to have higher respiratory rates than older, more mature cells. A number of factors, how-

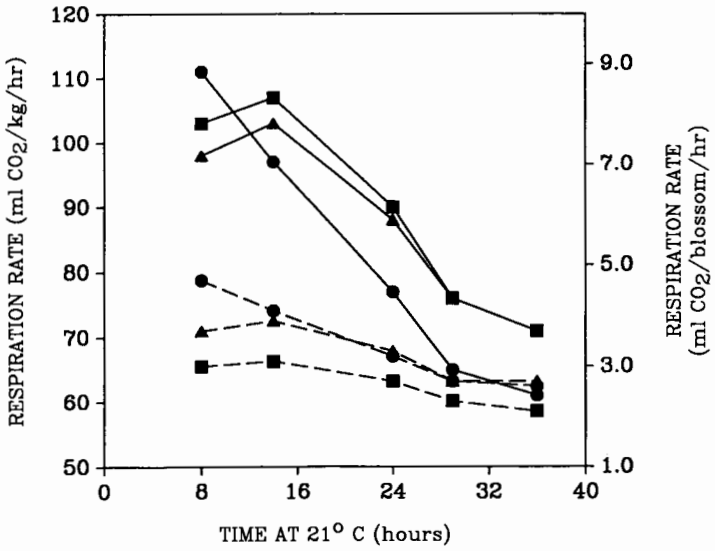


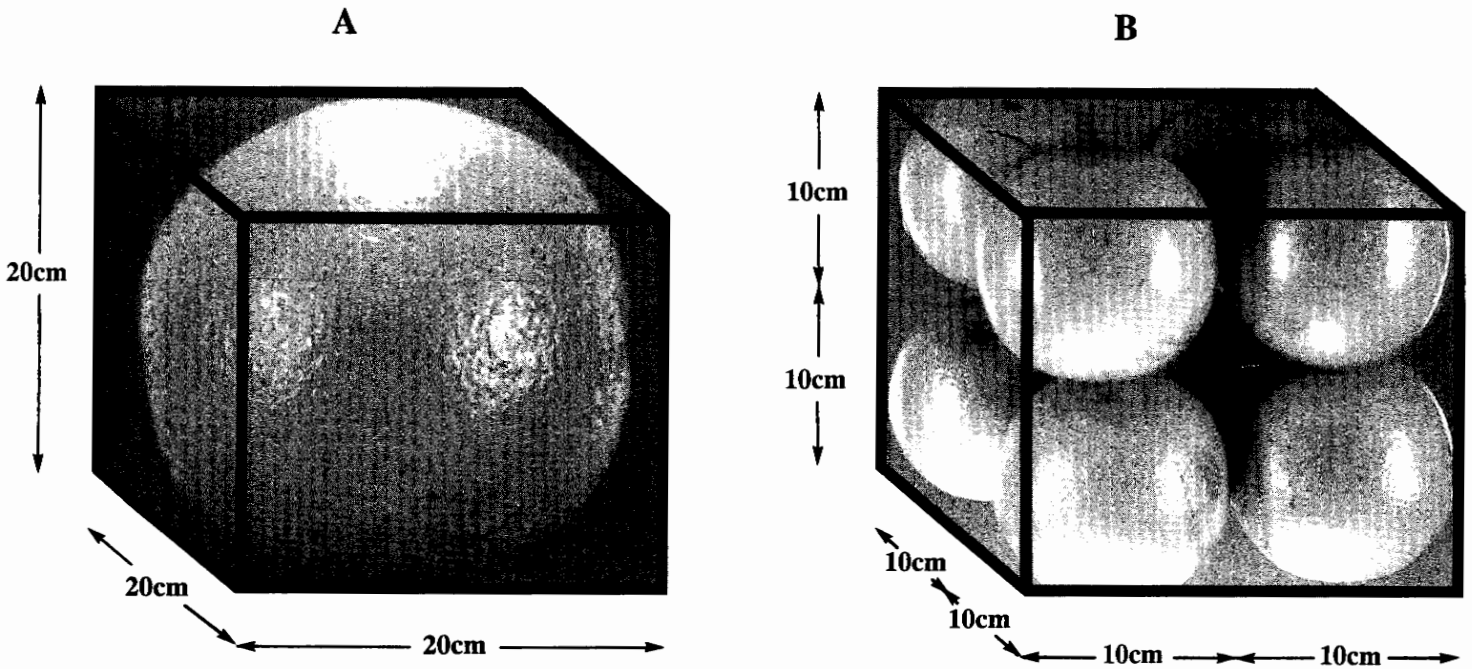
Figure 3.19. The effect of chrysanthemum (*Chrysanthemum morifolium* Ramat. cv. Indianapolis White) flower maturity (■—intermediate; ▲—mature; ●—fully mature) on flower respiration (--- = ml CO_2/kg/hr; — = ml CO_2/blossom/hr) at harvest (0 time) and during storage (*redrawn from Kuc and Workman*).[67]

ever, affect this relationship between maturity and respiratory rate, for example, species, plant part and stage of maturity. Therefore, generalizations on the effect of maturity on respiratory rate should be kept within fairly strict commodity-species bounds, and in some cases exceptions become more numerous than the rule.

The effect of maturity within a general commodity type is well illustrated in cut flowers. Carnations ('White Sims') harvested at varying stages of maturity between the tight bud stage and fully opened displayed significant differences in their respiratory rate expressed on either a weight or per blossom basis.[67] When flowers were harvested at a more mature stage of development (Figure 3.19), the respiratory rate rose. In addition, these differences tended to be maintained during the postharvest period. In contrast, however, chrysanthemum (cv. Indianapolis White) displayed the opposite trend, with the respiratory rate decreasing with maturity. Even more pronounced effects of maturation on respiratory rate can be seen in climacteric fruits when the range in maturities tested include preclimacteric, climacteric and postclimacteric stages.[33] Young apple leaves and stems have from three to seven times the rate of respiration of corresponding fully developed organs from the same plant.[85]

h. Surface area to volume ratio

The surface to volume ratio may influence the respiratory rate of some products due to its effect on gas exchange. As an object increases in size, assuming the shape is not altered, there is a progressive decrease in surface area relative to its volume. This is because volume increases as the cube of length (length × length × length) while surface area increases only as the square (length × length), as illustrated in Figure 3.20 with the comparison of two sizes of spherical fruit placed in a cubic box (20 × 20 × 20 cm). One sphere 20 cm in diameter will fit into the container or eight 10 cm diameter spheres. The composite surface area of the smaller spheres is double that of the larger sphere while the total volumes are equal. This difference provides in a



	A	B
FRUIT SURFACE AREA :	$20cm^2$ x 3.1416 x 1 fruit = $1256cm^2$	$10cm^2$ x 3.1416 x 8 fruit = $2513cm^2$
FRUIT VOLUME :	$20cm^3$ x 0.5236 x 1 fruit = $4189cm^3$	$10cm^3$ x 0.5236 x 8 fruit = $4189cm^3$

VOLUME B / VOLUME A = 1

SURFACE AREA B / SURFACE AREA A ≈ 2

Figure 3.20. The relationship between product size and the surface area available for the diffusion of gases into and out of tissue.

small diameter product a larger surface area for gas exchange for the underlying cells, shifting the uptake-utilization (oxygen) and production-emanation (carbon dioxide) equilibrium.

The shape of the majority of harvested products (leaves, flowers, nuts, etc.) deviates substantially from spherical and in some cases, they also have rough or uneven surfaces. This increases the surface area to volume ratio, facilitating diffusion. In products where the surface represents a significant barrier to diffusion due to the presence of the cuticle or periderm, increased surface area may be important. Although the actual surface to volume ratio can be substantially altered by environmental conditions during growth (e.g., the effect of thinning on fruit size), little control can be exerted over it during the postharvest period. The postharvest environment, however, can be adjusted to compensate for surface/volume conditions of a specific product to prevent undesirable internal conditions from developing.

i. Nature of the harvested product's surface

The composition of the gas atmosphere within most harvested products has an effect on their respiratory rate. Both high carbon dioxide and low oxygen concentrations have been shown to decrease the respiratory rate of cells. The internal gas composition is controlled by the rate of oxygen use and carbon dioxide production by the tissue, differences in the partial pressures of these gases between the interior and exterior environment, and the gas permeability of the tissue and any applied surface coatings. The nature of the surface of the harvested product, therefore, can have an impact upon gas diffusion resistance. High diffusion resistances result in a greater difference between the internal and external gas atmosphere. If the differential between internal and external oxygen and carbon dioxide concentration is sufficiently large, the respiratory rate of the internal cells can be altered.

Surface resistances are generally much larger than internal diffusion resistances, since there is a significant volume of intercellular air space. Surface cells are arranged much more tightly (little intercellular space), and compounds that resist gas movement (e.g., cutin, waxes) are present on the surface. Therefore, the nature of the surface of harvested products and postharvest practices that alter these surface characteristics (e.g., application of waxes) can exert a considerable influence over respiratory and metabolic rates. Lenticels, stomates, surface cuts or abrasions, fruit stem or peduncle scars, and other openings provide localized areas that have lower diffusion resistances than the majority of the product surface. Natural surface coatings of epicuticular waxes and cutin tend to increase the diffusive resistance to oxygen, carbon dioxide and water movement into and out of the tissue.[65] When gas diffusion is sufficiently restricted, the internal concentration of carbon dioxide increases and oxygen decreases. For example, apples of the 'Granny Smith' cultivar held in air had an internal oxygen concentration of 17% at 7°C and 2% at 29°C, while the respective internal carbon dioxide concentrations were 2% and 17%.[102] The surface of the tomato fruit restricts all but around 5% of the total gas exchange between the interior and the exterior, the primary path of exchange being *via* the stem scar.

In some postharvest products, it is advantageous to apply an artificial coating of waxes or similar material on the surface. Citrus fruit, apples, cucumbers, pineapples, rutabagas, cassava and dormant rose plants are commonly waxed. This not only alters the internal gas concentration of the product[32] but has the additional advantage of decreasing water loss. In many cases, the waxes also enhance the appearance of the product by imparting a shiny gloss to the surface

j. Preharvest cultural and postharvest handling conditions

Preharvest factors can significantly influence the respiratory behavior of a harvested product. The nutrient composition of the harvested product is strongly affected by the nutrition of the parent plant. Preharvest nutrition alters not only the elemental composition of the product but also the relative amount of many organic compounds.[83] Plant tissues low in potassium and

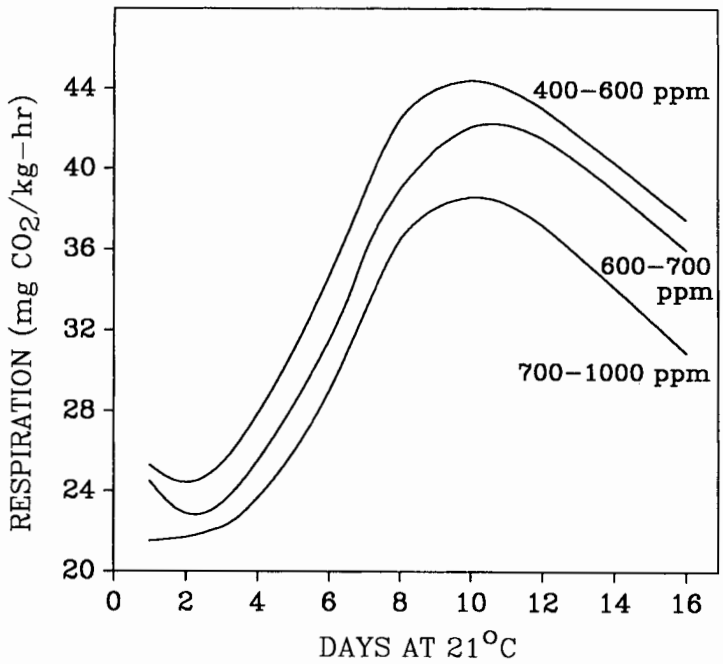


Figure 3.21. Respiratory climacteric in apple fruit (*Malus sylvestris* Mill. cv. Baldwin) with varying peel calcium contents (*redrawn from Bramlage et al.*).[20]

calcium often have substantially higher respiratory rates.[1,2,34,44] This is illustrated by the correlation between the peel calcium content of apples and their subsequent postharvest respiratory rate (Figure 3.21). Apples with a low calcium concentration have higher respiratory rates at the preclimacteric stage, the climacteric peak and during the postclimacteric period than apples with a higher calcium content.[20] High tissue nitrogen concentration (apples, strawberries) is also correlated with elevated respiration; in the case of apple fruit the effect of high nitrogen is pronounced only when the fruit calcium concentration is low.[34,87]

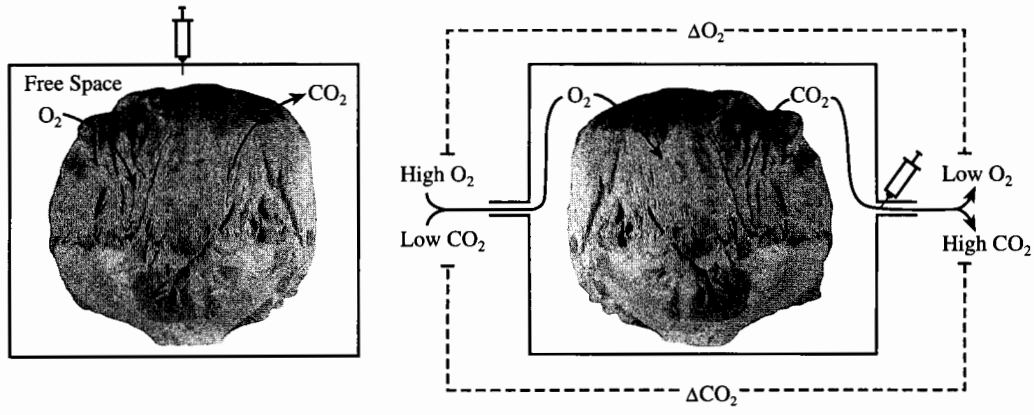
Other factors such as preharvest sprays,[97] rough handling,[74] orchard temperature,[80] production year[20] and acclimatization can also significantly influence postharvest respiratory responses.

3.1.5. Methods of Measuring Respiration

The respiratory rate of a stored product can be used as an indicator for adjusting the storage conditions to maximize the longevity of the commodity. As a consequence, it is often desirable to measure respiration in commercial storage houses. These measures can also be used in many cases as a general index of the potential storage life of the product. In addition, the rate of respiration can be used to calculate the loss of dry matter from the product during storage, the rate of oxygen removal from storage room air, and the heat generated during storage.

As discussed in section 3.1.1. of this chapter, respiration consumes oxygen from the surrounding environment and substrate from the commodity. Carbon dioxide, water and energy (both chemical and heat) are produced.

$$\text{Substrate} + O_2 \rightarrow CO_2 + H_2O + \text{energy (chemical and heat)}$$



ΔCO_2= concentration time 2 - concentration time 1

ΔO_2= concentration time 1 - concentration time 2

ΔCO_2= concentration out - concentration in

ΔO_2= concentration in - concentration out

$$\frac{(\Delta\ \%\ \text{x}\ 10)(\text{free space volume of container in liters})}{(\text{product fwt in kg})(\text{time container is closed in hours})} = \text{ml kg}^{-1}\ \text{hr}^{-1}$$

$$\frac{(\Delta\ \%\ \text{x}\ 10)(\text{air flow rate in ml/min x } 60)/1000}{(\text{product fwt in kg})} = \text{ml kg}^{-1}\ \text{hr}^{-1}$$

* Milliliters of gas are normally converted to milligrams to remove the effect of temperature on the volume of gas so that direct comparisons can be made. To do this, a temperature correction must be used.

One mole of gas is equal to 22.4 L at O°C at 1 atmosphere, therefore, its volume (V_1) at the temperature of the product can be calculated with the following equation:

$$V_1 = 22.4\ (1 + \frac{\text{Temperature of product in °C)}}{273°\ \text{Kelvin}}$$

For example, the volume of 1 mole of CO_2 at 25°C = 24.45 L. The volume of gas per gram is calculated by dividing the correct volume by the molecular weight of the gas (CO_2 = 44, O_2 = 32), i.e. 24.45 L/44 = .556 L/g or 556 ml/1000 mg (the volume of CO_2 at 25°C divided by its molecular weight). Then the weight of gas from the respiration sample can be calculated by:

$$\frac{556\ \text{ml}}{1000\ \text{mg}} = \frac{\text{measured ml from sample}}{\text{x}}$$

Corrections for common temperatures are:

0°C = 509 ml CO_2/1000 mg or 700 ml O_2/1000 mg
5°C = 518 ml CO_2/1000 mg or 712 ml O_2/1000 mg
10°C = 528 ml CO_2/1000 mg or 726 ml O_2/1000 mg
15°C = 537 ml CO_2/1000 mg or 738 ml O_2/1000 mg
20°C = 546 ml CO_2/1000 mg or 751 ml O_2/1000 mg
25°C = 556 ml CO_2/1000 mg or 764 ml O_2/1000 mg
30°C = 565 ml CO_2/1000 mg or 777 ml O_2/1000 mg

Figure 3.22. Techniques for collecting respiratory gas samples and calculating respiratory rates of harvested products.

Theoretically, changes in any of these reactants or products could be used as a measure of respiration. In general practice, however, measurement of carbon dioxide production is used due to its relative ease of measurement and accuracy. Oxygen is more difficult to accurately detect in that relatively small changes in concentration are against a high background oxygen concentration (21%) in air, while changes in carbon dioxide are large compared to the background (0.033%). Since the respiratory reactions take place in an aqueous medium, the small quantity of water produced in relation to the total volume of water present in the tissue cannot be accurately measured. Similarly, relatively large rates of respiration over a short measurement period result in only small total substrate or dry matter changes. Energy production, whether chemically trapped or liberated as heat, is also difficult to measure precisely. As a consequence, either the production of carbon dioxide or the utilization of oxygen is almost invariably used to monitor respiration.

Several techniques may be used for collecting gas samples from a respiring product. The product may be placed in a closed (gas tight) container and the decrease in oxygen or increase in carbon dioxide measured over a known period of time. Small samples are withdrawn from the enclosed atmosphere and either or both gases are measured (Figure 3.22). By measuring the change (Δ) in concentration as a function of time (i.e., concentration of oxygen at $time_1$ minus the concentration of oxygen at $time_2$ divided by $time_2$ minus $time_1$ gives Δoxygen/unit of time), the volume of free space in the container and the weight of the enclosed product, the respiratory rate can be expressed as weight of gas/weight of product unit of time (see Figure 3.22). In closed systems, care must be taken not to leave the product enclosed for too long, since the decreasing oxygen and increasing carbon dioxide concentrations will begin to affect the rate of respiration of the product. For many products, it is not desirable to let the carbon dioxide concentration increase to much above 0.5%. A second technique employs a continuous flow of air or gas of known composition through the container holding the product (Figure 3.22). The difference (Δ) between the concentration of oxygen and/or carbon dioxide going into the container and that leaving the container is used to calculate the respiratory rate. In addition to the difference in gas concentration, the rate of air flow through the container and the weight of the product must be known. Care must be taken to adjust the air flow rate through the container to an appropriate level. An excessively high flow rate results in extremely small differences between incoming and exiting gases, making measurement with an acceptable level of accuracy difficult. Air flow rates that are too slow result in the same problem that can be encountered with a closed system, the buildup of carbon dioxide or depletion of oxygen altering the rate of respiration. Care should also be taken to allow the system sufficient time to develop a steady-state equilibrium before measurements are made.

The oxygen and/or carbon dioxide concentrations in gas samples from either system can be measured by utilizing any of a number of different methods (Table 3.6).

Table 3.6. Comparison of Techniques Available for Measuring Respiration of Plants.

Technique	*Gas measured*	*Sample Type*	*Sample size*	*Initial Expense*	*Recurring Expense*	*Requirement for Electricity*
Gas chromatography	CO_2 and/or O_2	Discrete	0.2–5.0 ml	Very high	Medium	Yes
Infrared	CO_2	Continuous flow		Very high	Low	Yes
Paramagnetic	O_2	Continuous flow		High	Very low	Yes
Titration/colorimetry	CO_2	Continuous flow		Low	Low	No
Kitagawa	CO_2 or O_2	Discrete		Very low	Very low	No
Pressure/volume changes	CO_2 or O_2	Discrete	100 ml	Medium	Low	No
Polarography	O_2	Continuous flow		Medium	Low	Yes

a. Gas chromatography

Carbon dioxide and oxygen can be measured with gas chromatographs equipped with thermal conductivity detectors and dual columns. Gas chromatographic analysis is used widely because of its accuracy and the small gas samples (i.e., 0.1–5 mL) needed.

b. Infrared gas analyzer for carbon dioxide

This instrument is used to measure carbon dioxide in a continuous flow of air and has the advantage over many techniques of being extremely accurate at very low carbon dioxide concentrations. The molecules of carbon dioxide in the sample absorb infrared radiation at a specific wavelength, and this absorption is used as a measure of the carbon dioxide concentration in the air stream.

c. Paramagnetic oxygen analyzers

Oxygen is strongly paramagnetic, and since no other gases commonly present in the air exert a magnetic influence, this characteristic can be monitored and used as a measure of the oxygen concentration in a continuous stream of air.

d. Titration/colorimetry for carbon dioxide

When a stream of air is passed through a sodium hydroxide or calcium hydroxide solution, the carbon dioxide is absorbed, and sodium or calcium carbonate is formed, decreasing the solution's pH. The change in alkalinity (decrease in pH) is used to determine the quantity of carbon dioxide absorbed,[27] a technique that can measure carbon dioxide concentrations up to 1.0%. The pH change is measured either by titration or colormetrically with the addition of bromthymol blue[91] and monitored with a spectrophotometer. As with other continuous flow systems, the flow rate of air needs to be known to calculate the final respiratory rate. This technique has the advantage of being relatively inexpensive and requiring only a limited amount of equipment.

e. Kitagawa detectors

Carbon dioxide, oxygen and a number of other gases can be measured quickly, relatively accurately and without significant expense using Kitagawa detectors. Gas is pulled into a reaction tube (specific for each gas monitored) where it is absorbed and reacts with a chemical reagent. The color change produced is used as a measure of the concentration of the gas. Carbon dioxide can be accurately measured between 0.01 and 2.6% and oxygen from 2 to 30%.

f. Pressure/volume changes

Samples of gas are placed in sealed containers of known volume and either carbon dioxide or oxygen is absorbed by a suitable reactant. The change in the internal pressure of the container or in the volume of gas within the container is used as a measure of the concentration of the respective gas.[29] The absorption principle is similar to that used in the titration/colorimetry method; however, instead of measuring changes in the absorbing material (e.g., pH), pressure or volume changes within the chamber are determined. Relatively large gas samples (e.g., 100 mL) are required; however, analyses are accurate to approximately 0.5%.

g. Polarography

The oxygen concentration in a gas sample can be measured with a polarographic oxygen electrode. The differential in electrical potential across a pair of electrodes is measured.

3.1.6. Respiratory Patterns

In the 1920's, Kidd and West studied the respiratory patterns of sunflower plants and their component parts during an entire growing season (Figure 3.23). In general they found that respiration closely paralleled the rate of plant growth (i.e., young, rapidly metabolizing cells have the highest respiratory rates). The high demand for energy and carbon compounds in actively growing cells results in a stimulation of respiration. As the age of the plant or individual organ (stem, leaves, flowers) increases, the respiratory rate decreases. This decline in respiration of the whole plant could not be attributed simply to an increased percentage of non-respiring structural material in the plant, since the initial respiratory rate of successive new leaves also decreased with the age of the plant. Hence, internal factors have a pronounced influence on respiration.

A number of external (environmental) and internal (commodity) factors have a pronounced influence on the rate of respiration of plant tissues (section 3.1.4). While environmental factors such as temperature are routinely studied with each postharvest product, considerable effort has been directed toward understanding the more elusive commodity factors that influence respiration. Changes in respiratory rate during growth, development and senescence of a plant or plant part under standard conditions display distinctive patterns, and these can often be related to other functional processes that occur concurrently.

Fruits are typically classed into one of two groups based on their pattern in respiratory

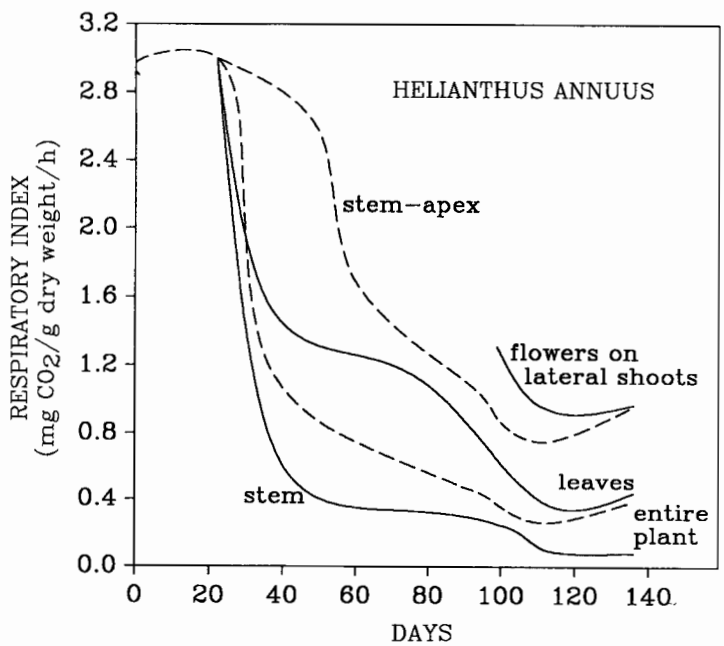


Figure 3.23. Changes in the respiratory rate of sunflower plants and selected plant parts during development (*data from Kidd et al.*).[61]

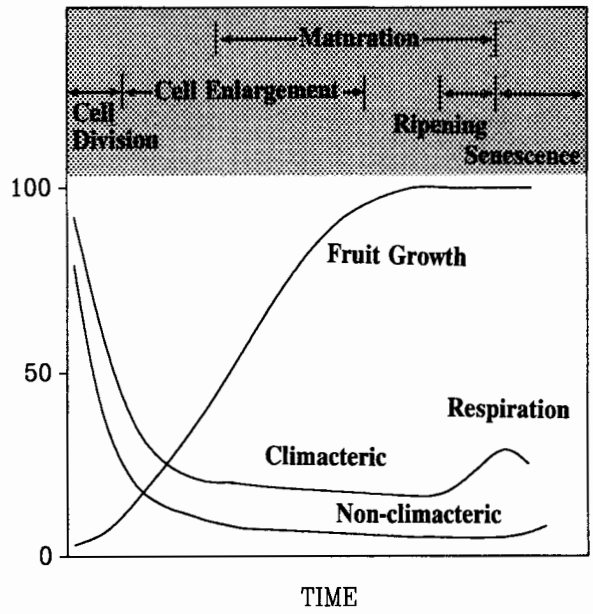


Figure 3.24. General respiratory pattern of climacteric and non-climacteric fruits during development, maturation, ripening and senescence.

behavior during the final stages of ontogeny of the organ (e.g., fruit ripening, Figure 3.24). Fruits classified as having a **respiratory climacteric** exhibit a marked upsurge in respiratory activity at the end of the maturation phase. These marked changes in the rate of respiration of climacteric fruit have long fascinated postharvest biologists. Although climacteric fruit represent an extremely small percentage of the plant products handled in agriculture, the dramatic shift in respiration during ripening has stimulated research into the function and control of respiratory patterns. The respiratory climacteric represents a transition between maturation and senescence. Nonclimacteric fruit (Figure 3.24) do not exhibit an upsurge in respiration, but rather a progressive, slow decline during senescence until microbial or fungal invasion.

The climacteric rise in respiration was described as early as 1908[84] in apple and pear fruit. Later, Kidd and West[60] detailed the relationship between changes in respiratory rate and changes in quality attributes occurring during the climacteric period. The dark respiration of an unripe fruit declines to what is termed the **preclimacteric minimum** just before the climacteric rise in respiration (Figure 3.24). Subsequently, respiration increases dramatically, often to levels 2 to 4 times that of the preclimacteric minimum. A similar trend occurs if the fruit is allowed to ripen on the tree,[64] although the respiratory pattern is modified somewhat [e.g., the rate at which it proceeds (slower) and the peak value (higher)]. Interpretation of the overall response is complicated by the fact that photosynthesis and photorespiration are occurring concurrently. An exception is found in some avocado cultivars, where the respiratory upsurge is inhibited while the fruit remains attached to the tree.

The respiratory climacteric is substantially altered by temperature. At both low and high temperatures, the climacteric can be suppressed. As storage temperature decreases from around 25°C, the duration of the climacteric rise is prolonged and the rate of respiration at the climacteric peak depressed. In addition, the ambient oxygen and carbon dioxide concentration can markedly alter the respiratory climacteric. Low oxygen and high carbon dioxide (up to approximately 10%) can prolong the length of time to the climacteric peak in a number of fruit, thus extending storage life. In many nonclimacteric fruit, respiration can also be depressed by low oxygen and high carbon dioxide concentrations. There are exceptions, however. For example, high carbon dioxide tends to stimulate the respiration of lemon fruit,[14]

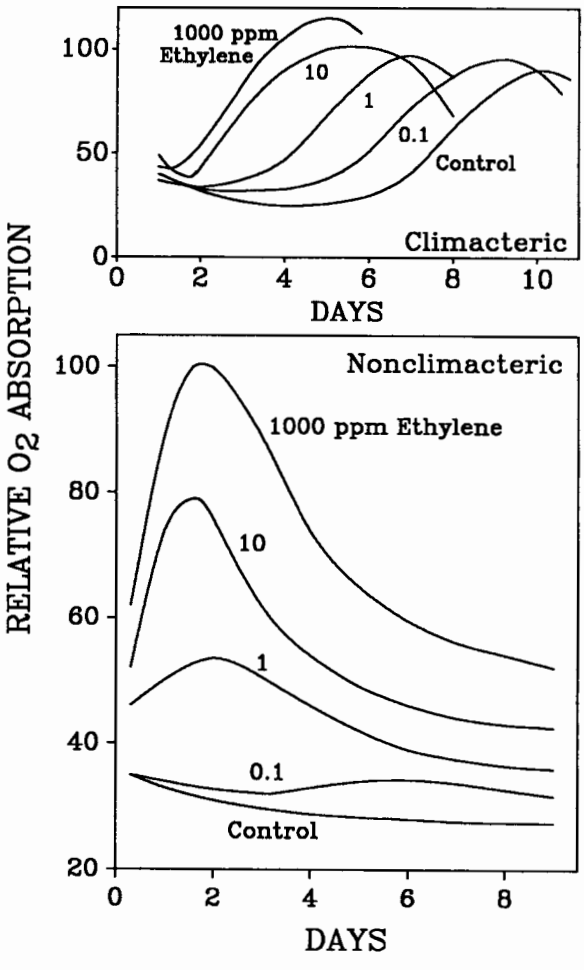


Figure 3.25. Oxygen uptake by climacteric and non-climacteric fruits exposed to varying levels of ethylene (*redrawn from Biale*).[13]

probably through the fixation and incorporation of the molecule into organic acids utilized in the tricarboxylic acid cycle.

The unsaturated hydrocarbon gas ethylene stimulates the respiration of a wide range of plant tissues. The response differs for climacteric and nonclimacteric fruit (Figure 3.25). Exposure of climacteric fruits to relatively low levels of ethylene decreases the preclimacteric period without a substantial effect on the rate of respiration at the climacteric peak. The concentration required for maximum acceleration varies with different fruits (e.g., 1 $\mu L \cdot L^{-1}$ for banana, 10 $\mu L \cdot L^{-1}$ for avocado). The shortening of the preclimacteric period is approximately proportional to the logarithm of the concentration applied.[22] Once ripening is initiated, removal of the external ethylene has no effect on the subsequent respiratory rate or pattern. Respiration in nonclimacteric fruit is likewise stimulated by ethylene; however, upon removal of ethylene the respiratory rate returns to near the value found prior to treatment (Figure 3.25).

Ethylene is known to be a natural product of fruit ripening.[36] The synthesis and the internal concentration of ethylene increases in most climacteric fruits around the same time the upsurge in respiration. Ethylene is thought to accelerate the outset of ripening and to coordinate ripening in the whole fruit. Exposure to an external source of ethylene results in an increased synthesis of ethylene. This stimulated synthesis is due to the effect of ethylene on the *de novo* synthesis of ACC synthase, a critical enzyme in the ethylene synthesis pathway.[6] There

are at least five ACC synthase genes involved in tomato ripening[6] that are expressed in a specific order and at different stages of fruit ripening. Exogenous ethylene, therefore, can be substituted for the endogenous ethylene normally produced by the plant to initiate the respiratory climacteric and ripening response, allowing earlier ripening of the fruit. Ethylene application (exogenous) is most widely used to commercially ripen banana fruit.

Theories as to the precise cause of the respiratory climacteric have been numerous. In 1928, Blackman and Parija[15] proposed that the increase in respiration was due to the loss of organizational resistance between enzymes and substrates. Several other theories have enjoyed popularity, including: 1) the presence of "active" substrate,[59] 2) availability of phosphate acceptors, 3) availability of cofactors, 4) uncouplers of oxidation and phosphorylation,[81] 5) shifts in metabolic pathways[54] and 6) an increase in mitochondria content and/or activity.[49] After 90 years of research, the precise cause of the respiratory climacteric in fruit has yet to be elucidated, although our understanding of the physiological, chemical and enzymatic changes occurring has increased tremendously.

Leaves undergo distinct changes in their respiratory behavior at certain stages of their development.[99] Generally, there is an increase in respiration during the early stages of senescence (the period of chlorophyll degradation) followed by a steady decline in the later stages. The respiratory increase, although not universal, occurs in a wide range of species in both attached[45] and detached leaves. Severing the leaf from the plant enhances the rate of senescence;[95] however, the timing of the respiratory increase relative to other biochemical and physical changes occurs at essentially the same stage in the senescence process. Low light (100–200 lux) delays senescence in detached oat leaves and thus can influence the respiratory strategy utilized.[97] When leaves are held in the dark, approximately 25% of the respiratory increase could be accounted for by increases in free amino acids and sugars from catabolic events. The remaining respiration (~75%) appears to be due to a partial uncoupling of respiration from phosphorylation.

Many flowers also undergo marked changes in respiration rate, the pattern and control of which has many parallels with the respiratory changes in climacteric fruits. In fact, the term respiratory climacteric is often used in studies on flower storage and senescence. Respiration in many species of cut flowers declines after harvest and then increases as the flowers begin to senesce.[86] This trend, however, is not universal. For example, cut roses progressively decline in respiration following harvest (Figure 3.26). In flowers that exhibit a postharvest respiratory rise, the increase in respiration, like that in many climacteric fruits, appears to be closely tied to the endogenous synthesis of ethylene by the flower (Figure 3.27). There is an autocatalytic synthesis of ethylene in flowers like the carnation, and the increase in ethylene precedes changes in membrane permeability and other senescence-related phenomena.[77] Chemicals such as aminovinylglycine, aminooxyacetic acid, Ag^{2+} ions, and 1-methylcyclopropene (MCP) that inhibit ethylene synthesis or action, can delay senescence in a wide range of ethylene sensitive flowers.

As the flower proceeds toward the final stages of senescence, there is a gradual reduction in respiration that may reflect a decline in respiratory substrate availability. Carbohydrates are known to be transported from the petals into the ovary during this period, with the reallocation stimulated by ethylene.

In summary, many plant products undergo substantial changes in their respiratory pattern after harvest. These changes often reflect significant alterations in metabolism and concurrent physical and chemical alterations within the tissues. Changes in respiration are of interest from an applied point of view, in that specific handling strategies may be required. Changes in respiration in part reflect the physiological state of the commodity, which can help in predicting the product's storage potential and life expectancy.

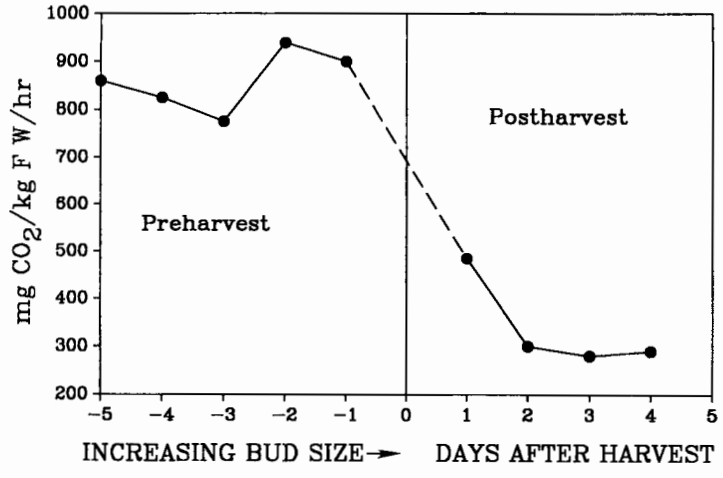


Figure 3.26. The effect of time of harvest on the respiratory rate of 'Velvet Times' roses held at 21°C (*redrawn from Coorts et al.*).[28] Zero time corresponds to the stage of development that the cultivar would be commercially harvested.

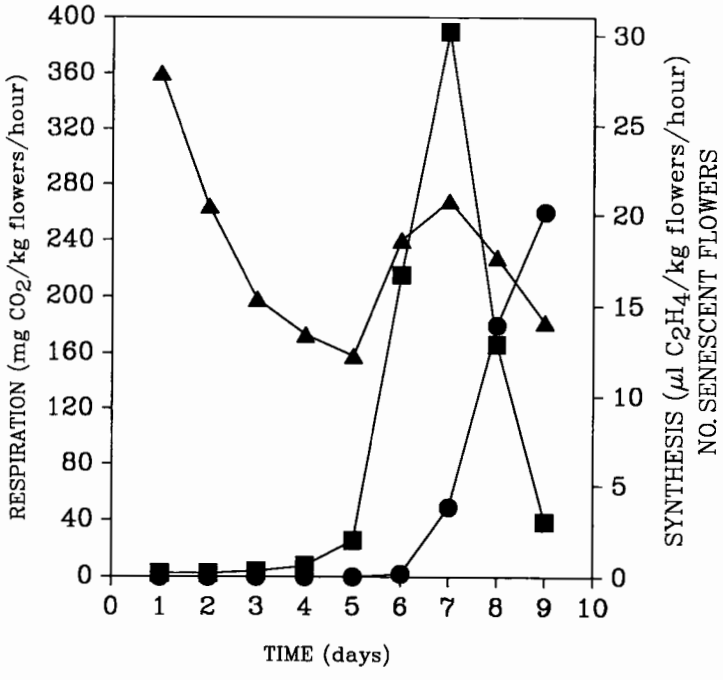


Figure 3.27. Changes in the rate of respiration (▲) and ethylene synthesis (■) with the development of senescence (●) after harvest of 'Improved White Sims' carnation flowers at 20°C (*redrawn from Maxie et al.*).[76]

3.3. Photosynthesis

The potential for photosynthesis and the rate at which it proceeds in harvested products varies widely, influenced by both internal and external factors. Many plant products are devoid of chlorophyll, or they are held after harvest under environmental conditions (low light and temperature) that are not conducive to photosynthesis. The importance of photosynthesis in detached chlorophyll containing tissues is probably minimal, but it should not be ignored.

A significant number of postharvest products not only have the potential for photosynthesis but need to photosynthesize to maintain the product's existing level of quality. Intact plants such as actively growing herbaceous and woody ornamentals, vegetable transplants and rooted cuttings are typical examples of postharvest products that normally photosynthesize during the handling and marketing period. For some protea flowers, photosynthesis can minimize postharvest leaf blackening, and illumination of broccoli plantlets during storage, even at the light compensation point, maintains their dry weight and subsequent growth potential.[68] The rate at which these plants photosynthesize after removal from the production area is governed by a number of internal and external factors. While the internal factors, such as stomatal number, photosynthetic pathway and others, influence the rate of photosynthesis, it is not possible to alter them. However, a number of external factors such as light, temperature, moisture, carbon dioxide and exogenous chemicals that influence photosynthesis can be altered. Manipulation of these external factors, therefore, provides the potential to exert a significant level of control over the rate of photosynthesis in many harvested products.

3.3.1. Tissue Type and Condition

The ability to photosynthesize and the rate of photosynthesis vary considerably among plant species as well as types of tissue. Chloroplasts, which carry out photosynthesis in the cell, are found primarily in leaf tissue; however, they also occur in petioles, stems, specialized floral parts of many plants and the epidermis of certain fruit. Roots, tubers and other structures, normally devoid of chloroplasts and hence chlorophyll, are capable of synthesizing chlorophyll when exposed to sufficient light. The pre- or postharvest formation of chlorophyll in some products (e.g., potato, Jerusalem artichoke) is detrimental to quality and needs to be avoided. The contribution of chloroplasts in organs other than leaves to the total assimilation of carbon is generally small due in part to the low number or absence of stomates, reducing carbon dioxide availability. In some instances, however, such as the corticular tissue of dormant dogwoods, photosynthesis by non-leaf structures may offset a significant portion of respiratory loss of carbon. The capacity of a fruit's epidermal cells to carry out photosynthesis declines with ripening, with the majority of photosynthate being supplied from the plant leaves.[89]

Photosynthetic rate is affected by leaf age (Figure 3.28). Rates are commonly highest when leaves first mature and shortly thereafter but tend to decline gradually with age.[66] The effect of leaf age on photosynthesis is a general phenomenon, found in annuals and perennials, including evergreen species.

3.3.2. Light

Whether from the sun or from an artificial source, light provides the energy needed by plants to fix carbon from carbon dioxide, allowing them to offset respiratory losses incurred. Light is one of the most important postharvest external variables affecting photosynthesis. During

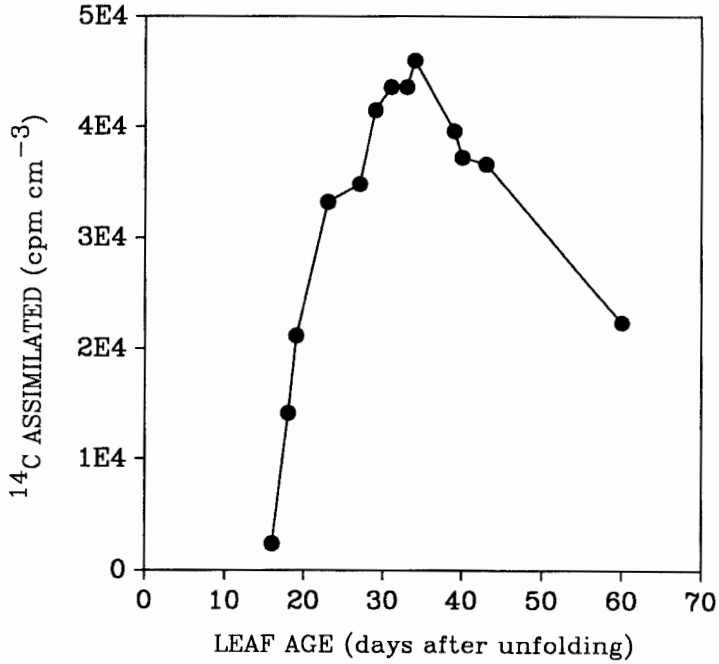


Figure 3.28. The effect of leaf age on the rate of photosynthesis ($^{14}CO_2$ assimilated) of grape leaves (*redrawn from Kriedemann et al.*).[66]

the postharvest handling of plants, especially intact plants, light intensity, quality and duration are crucial in determining storage potential.

Individual leaves, when exposed to increasing light intensity, exhibit a typical light response pattern (Figure 3.29). As the intensity of the light is increased, the **light compensation point** is reached. At this point, the amount of carbon dioxide trapped is equal to the amount of carbon dioxide lost from the tissue due to respiratory processes. Additional increases in intensity result in a proportional increase in photosynthetic rate of carbon fixed, eventually reaching a point at which photosynthesis becomes light saturated. At this point, additional increases in light intensity have only a slight effect on increasing the carbon fixation rate. Light saturation of individual leaves of full sun plants is often only 1/4 to 1/2 that of full sunlight; however, with entire plants, saturation is seldom reached because of mutual shading of the leaves within the canopy. With further increases in light, the point of maximum photosynthesis is reached, and additional increases result in a decrease in carbon fixation and sometimes damage (Figure 3.29).

Light is necessary for the formation of chlorophyll in plants, and there is a continuous turnover (synthesis and degradation) of chlorophyll molecules under normal conditions. Insufficient light, therefore, can result in the net loss of leaf chlorophyll. With prolonged exposure, an indirect loss occurs through abscission of leaves, decreasing the plant's surface area of photosynthetic tissue. Normally, abscission progresses from the oldest leaves to the youngest, with leaves that are shaded by their position at the bottom or interior of the canopy being more susceptible to abscission.

Plant species vary widely in their tolerance to light, and excess light may present a serious postharvest problem. For example, prolonged exposure of full shade plants such as the African violet to full sunlight may result in chlorophyll degradation, leaf burning and a decrease in net photosynthesis. The effect of excess light can also be seen in heliotropic responses,

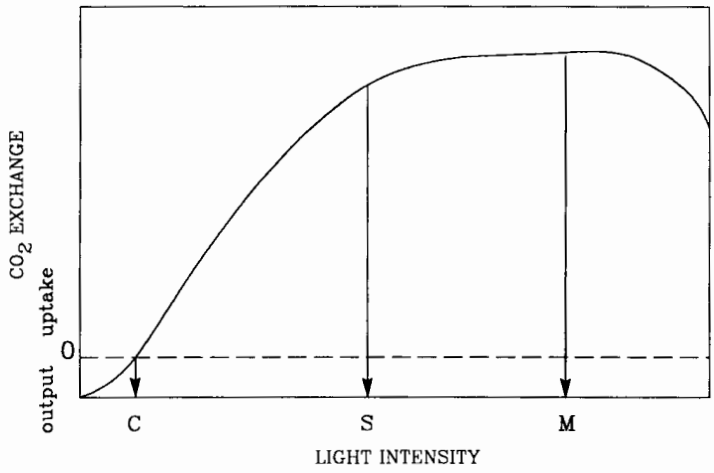


Figure 3.29. The relationship between light intensity and the rate of carbon uptake by photosynthesizing leaves (C—light compensation point; S—light saturation intensity; M—maximum photosynthetic rate) (*redrawn from Rabinowitch*).[93] At the light compensation point (C), the amount of carbon fixed by the leaf is equal to the amount lost through respiration. At very high light intensities, photosynthesis is inhibited.

where the plant's growth pattern is altered in the direction of the light, which may potentially be in an undesirable orientation.

It is normally desirable to maintain the product in the same qualitative condition as at harvest (removal from the production zone for intact plants), and hence the net carbon balance (acquisition vs. utilization) is important. The precise postharvest photosynthetic requirements for individual species at various stages in their life cycle are not currently known. It is probable, however, that the requirements for photosynthetic carbon input needed to maintain the existing condition of the plant are slightly above the plant's gross respiratory utilization, due to non-respiratory uses of carbon in maintenance reactions. Photosynthetic acquisition of carbon can be maintained at or above this critical maintenance point with a range of light intensity and duration combinations. When some species are exposed to prolonged periods at higher light intensities, the chloroplasts are unable to store the additional starch formed, and photosynthesis is inhibited.

Of the total spectrum of radiant energy, plants utilize light only from a region between 400 and 700 nm for photosynthesis (Figure 3.30). Peak photosynthesis and absorption of light by chlorophyll and other pigments come from the red and blue portions of the spectrum. In natural canopies, light entering the upper leaves is selectively absorbed from the red and blue regions, with less photosynthetically active light being transmitted to the lower and inner leaves. As the spectral distribution of the light shifts toward a greater percentage of green light, the rate of photosynthesis per photon of photosynthetically active light declines.

Changes in the amount of energy at particular wavelengths (light quality) a plant receives after harvest are often also dramatically altered. These changes occur primarily from the use of artificial light that does not have the same spectral quality as sunlight and in the use of shading material that selectively absorbs light from certain regions of the spectrum. While photomorphogenic changes in the plant due to light quality are normally of greater postharvest importance than changes in the net photosynthetic rate, prolonged exposure of plants to light that is not spectrally suited for photosynthesis will compromise product quality maintenance.

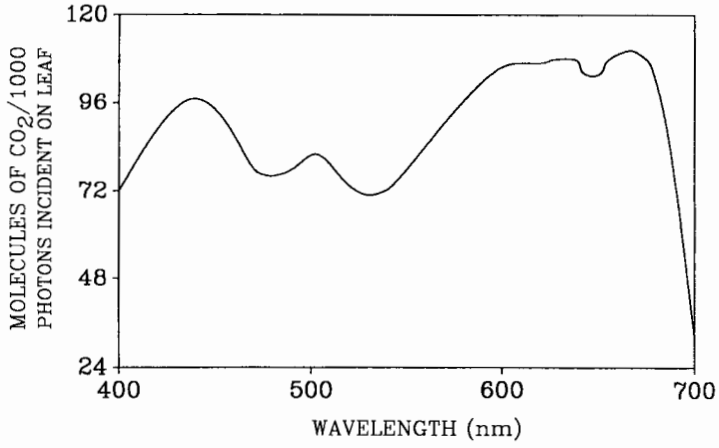


Figure 3.30. The change in photosynthetic activity with changes in wavelength of the light striking the leaf (*redrawn from Balegh and Biddulph*).[5]

3.3.3. Temperature

The rate of photosynthesis is highly dependent upon temperature. This dependence varies widely, with some species capable of photosynthesizing at temperatures near 0°C, while others require substantially higher temperatures. Generally, net photosynthesis (total photosynthesis minus respiration) increases with temperature until reaching a maximum and then declines (Figure 3.14). The decline is probably mediated by several factors, one of which is an elevation in respiration that occurs with increasing temperature. As temperature is progressively increased, respiratory losses will eventually be greater than the carbon fixed through photosynthesis, giving a net loss of carbon. During the postharvest handling of photosynthetically active products, both excessively low and high temperatures present potential problems in maintaining an adequate carbon input-output balance. At very low temperatures, photosynthesis is insufficient, while at high temperatures, respiratory losses are greater than the carbon dioxide fixed.

3.3.4. Moisture Stress

The availability of water is an important factor governing the rate of photosynthesis (Figure 3.31). Upon removal of plants from the production zone, the potential for moisture stress increases substantially due in part to the altered environmental conditions to which the plants are exposed. In addition, water management often becomes less organized and structured. Lack of precise control over the moisture balance in postharvest products can result in either water deficit or excess; each can substantially impede photosynthesis. Leaf water content has a direct effect on water's chemical role in photosynthesis and an indirect effect on hydration of the protoplasm and closure of stomates. Since the total amount of water that participates directly in the biochemical reactions of photosynthesis is extremely small, indirect effects on stomatal aperture appear to present the greater hindrance to photosynthesis under conditions of low moisture. The degree of inhibition of photosynthesis due to water deficit depends to a large extent upon both the level of stress imposed and the species involved. Wilted sunflower leaves are photosynthetically as much as 10 times less efficient than turgid leaves. As a conse-

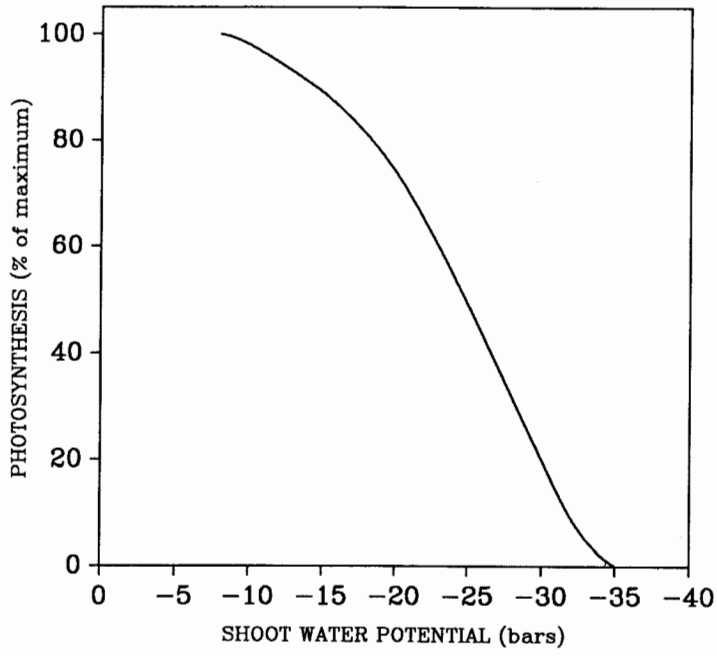


Figure 3.31. The effect of shoot water potential on the photosynthetic rate of Douglas fir trees (*redrawn from Brix*).[21]

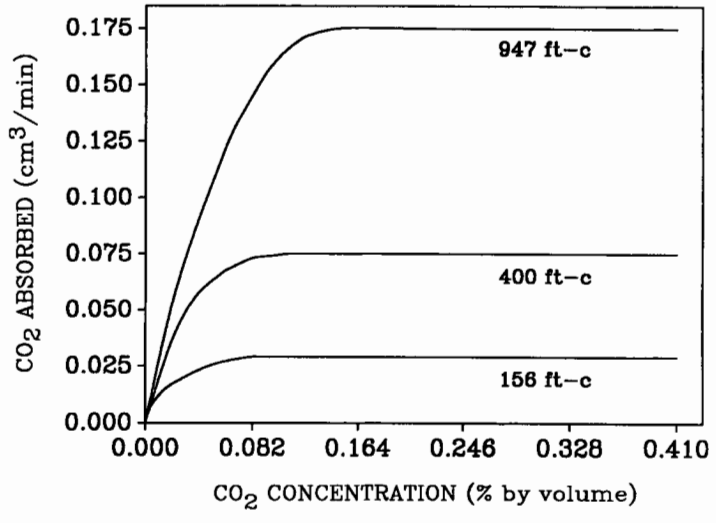


Figure 3.32. The effect of carbon dioxide concentration on photosynthetic rate (CO_2 absorbed) at varying light intensities (*redrawn from Hoover et al.*).[52]

quence, prolonged closure of stomates can result in a serious deficit in carbohydrates within the plant.

3.3.5. Carbon Dioxide

Photosynthesis in plants exposed to sufficient light is limited primarily by the low carbon dioxide concentration in the ambient atmosphere (~330 $\mu L \cdot L^{-1}$). As the carbon dioxide concentration increases up to 1000 to 1500 $\mu L \cdot L^{-1}$, photosynthesis also increases in many species. At high light intensities, the effect of additional carbon dioxide is even greater (Figure 3.32).

Elevated carbon dioxide concentration has been used successfully to increase the growth rate of a number of agricultural crops grown in controlled environment conditions (e.g., greenhouses).[107] To date, the beneficial effect of elevated carbon dioxide concentration on the photosynthetic rate of plants has been used only in the production zone for these crops and not during the postharvest handling and sales period.

3.3.6. Chemicals

A number of chemical compounds applied directly to plants (e.g., pesticides) or found in the ambient atmosphere (air pollutants), can depress photosynthesis. The response may be due to a direct effect on the biochemical reactions of photosynthesis or indirect effects such as increases in the diffusive resistance of carbon dioxide into the leaf, changes in the optical properties of the leaf, changes in the leaf's thermal balance, or the loss of photosynthetic surface area. The common air pollutants that can reduce photosynthesis are sulfur dioxide, ozone, fluorides, ethylene and particulate matter such as dusts. Brief exposure to sulfur dioxide (4.1 mg $\cdot m^{-3}$ for 2 hours) has been shown to inhibit photosynthesis by as much as 80%. While the effect of chemicals on postharvest photosynthesis may be pronounced, more direct effects such as the formation of lesions, chlorosis, discoloration, and leaf and flower drop are often economically much more significant than impaired photosynthesis in compromising product quality.

ADDITIONAL READING

Barber, J. (ed.). 1992. *The Photosystems: Structure, Function and Molecular Biology.* Elsevier, Amsterdam.

Borochov, A., and R. Woodson. 1990. Physiology and biochemistry of flower petal senescence. *Hort. Rev.* 11:15–43.

Boyer, P.D. 1997. The ATP synthase: A splendid molecular machine. *Annu. Rev. Biochem.* 66:717–749.

Buchanan, B.B., W. Gruissem and R.L. Jones. 2000. *Biochemistry & Molecular Biology of Plants.* American Soc. Plant Physiologists, Rockville, MD.

Buchanan-Wollaston, V. 1997. The molecular biology of leaf senescence. *J. Exp. Bot.* 48:181–199.

Burton, W.G. 1982. *Postharvest Physiology of Food Crops.* Longman, New York.

Chitnis, P.R. 1996. Photosystem I. *Plant Physiol.* 111:661–669.

Cohen, Y., S. Yalovsky and R. Nechushtai. 1995. Integration and assembly of photosynthetic protein complexes in chloroplast thylakoid membranes. *Biochem. Biophys. Acta* 1241:1–30.

Davies, D.D. (ed.). 1988. *Biochemistry of Metabolism.* Vol. 11. *The Biochemistry of Plants.* P.K. Stumpf and E.E. Conn (eds.). Academic Press, London.

Davies, D.D. (ed.). 1988. *Physiology of Metabolism.* Vol. 12. *The Biochemistry of Plants.* P.K. Stumpf and E.E. Conn (eds.). Academic Press, London.

Day, D.A., J. Whelan, A.H. Millar, J.N. Siedow and J.T. Wiskich. 1995. Regulation of the alternative oxidase in plants and fungi. *Aust. J. Plant Physiol.* 22:497–509.

Dennis, D.T., Y. Huang and F.B. Negm. 1996. Glycolysis, the pentose phosphate pathway and anaerobic respiration. Pp.105–123. In: *Plant Metabolism.* D.T. Dennis, D.H. Turpin, D.D. Lefebvre and D.B. Layzell (eds.), Longman Press, Harlow, UK.

Emes, M.J., and D.T. Dennis. 1997. Regulation by compartmentation. Pp.69–80. In: *Plant Metabolism.* D.T. Dennis, D.H. Turpin, D.D. Lefebvre and D.B. Layzell (eds.), Longman Press, Harlow, UK.

Fluhr, R., and A.K. Mattoo. 1996. Ethylene-biosynthesis and reception. *Critical Rev. Plant Sci.* 15:479–523.

Gan, S., and R.M. Amasino. 1997. Making sense of senescence. *Plant Physiol.* 113:313–319.

Gutteridge, S., and A.A. Gatensby. 1995. Rubisco synthesis, assembly, mechanism and regulation. *Plant Cell* 7:809–819.

Halevy, A.H., and S. Mayak. 1979. Senescence and postharvest physiology of cut flowers. Part I. *Hort. Rev.* 1:204–236.

Hartman, F.C., and M.R. Harpel. 1994. Structure, function, regulation and assembly of D-ribulose-1, 5-bisphosphate carboxylase/oxygenase. *Annu. Rev. Biochem.* 63:197–234.

Horton, T., A.V. Ruban and R.G. Walters. 1996. Regulation of light harvesting in green plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47:655–684.

James, W.O. 1953. *Plant Respiration.* Clarendon Press, Oxford, England.

Kostychev, S. 1927. *Kostychev's Plant Respiration.* Translated by C.J. Lyon, Blakiston's Son & Co., Philadelphia, PA.

Kozaki, A., and G. Takeba. 1996. Photorespiration protects C_3 plants from photooxidation. *Nature* 384:557–560.

Kromer, S. 1995. Respiration during photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46:45–70.

Lambers, H. 1985. Respiration in intact plants and tissues. Its regulation and dependence on environmental factors, metabolism and invaded organisms. Pp.418–473. In: *Higher Plant Cell Respiration.* Vol. 18. *Encyclopedia of Plant Physiology.* (new series), R. Douce and D.A. Day (eds.), Springer, Berlin.

Long, S.P., S. Humphries and P.G. Falkowski.1994. Photoinhibition of photosynthesis in nature. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45:633–662.

Mackenzie, S., and L. McIntosh. 1999. Higher plant mitochondria. *Plant Cell* 11:571–586.

Mir, N., and R. Beaudry. 2002. Atmospheric control using oxygen and carbon dioxide. Pp. 122–156. In: *Fruit Quality and its Biological Basis.* M. Knee (ed.), CRC Press, Boca Raton, FL.

Moller, I.M., P. Gardestom, K. Glimelius and E. Glaser. 1998. *Plant Mitochondria: From Gene to Function.* Backhuys Pub., Leiden, The Netherlands.

Nugent, J.H.A. 1996. Oxygenic photosynthesis: Electron transfer in photosystem I and photosystem II. *Eur. J. Biochem. Sci.* 237:519–531.

Ort, D.R., and C.F. Yocum (eds.). 1996. *Oxygenic Photosynthesis: The Light Reactions.* Vol. 4. *Advances in Photosynthesis.* Kluwer, Dordrecht, The Netherlands.

Pakrasi, H.B. 1995. Genetic analysis of the form and function of Photosystem I and Photosystem II. *Annu. Rev. Genet.* 29:755–776.

Plaxton, W.C. 1996. The organization and regulation of plant glycolysis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47:185–214.

Rogner, M., E.J. Boekema and J. Barber. 1996. How does photosystem 2 split water? The structural basis of efficient energy conversion. *Trends Biochem. Sci.* 21:44–49.

Seymour, G.B., J.E. Taylor and G.A. Tucker (eds.). 1993. *Biochemistry of Fruit Ripening.* Chapman & Hall, London.

Siedow, J.N. 1995. Bioenergetics: The plant mitochondrial electron transfer chain. Pp.281–312. In: *The Molecular Biology of Plant Mitochondria.* C.S. Levings III and I. Vasil (eds.), Kluwer, Dordrecht, The Netherlands.

Siedow, J.N., and A.L. Umbach. 1995. Plant mitochondrial electron transfer and molecular biology. *Plant Cell* 7:821–831.

Taiz, L., and E. Zeiger. 1998. *Plant Physiology.* Benjamin/Cummins Pub., Redwood City, CA.

Vanlerberghe, G.C., and L. McIntosh. 1997. Alternative oxidase: From gene to function. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:703–734.

Wagner, A.M., and K. Krab. 1995. The alternative respiration pathway in plants: Role and regulation. *Physiol. Plantarum* 95:318–325.

Whitehouse, D.G., and A.L. Moore. 1995. Regulation of oxidative phosphorylation in plant mitochondria.

Pp.313–344. In: *The Molecular Biology of Plant Mitochondria,* C.S. Levings III and I. Vasil (eds.), Kluwer, Dordrecht, The Netherlands.

REFERENCES

1. Alban, E.K., H.W. Ford and F.S. Nowlett. 1940. A preliminary report on the effect of various cultural practices with greenhouse tomatoes on the respiration rate of the harvested fruit. *Proc. Amer. Soc. Hort. Sci.* 52:385–390.
2. Amberger, A. 1953. Zur rolle des kaliums bei atmung svorgängen. *Biochem. Z.* 323:437–438.
3. Audis, L.J. 1935. Mechanical stimulation and respiration rate in the cherry laurel. *New Phytol.* 34:386–402.
4. Bailey, C.H. 1940. Respiration of cereal grains and flaxseed. *Plant Physiol.* 15:257–274.
5. Balegh, S.E., and O. Biddulph. 1970. The photosynthetic action spectrum of the bean plant. *Plant Physiol.* 46:1–5.
6. Barry, C.S., M.I. Llop-Tous and D. Grierson. 2000. The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. *Plant Physiol.* 123:979–9.
7. Beaudry, R.M., R.F. Severson, C.C. Black and S.J. Kays. 1989. Banana ripening: Implications of changes in glycolytic intermediate concentrations, glycolytic and gluconeogenic carbon flux, and fructose 2,6-bisphosphate concentration. *Plant Physiol.* 91:1436–1444.
8. Benoy, M.P. 1929. The respiration factor in the deterioration of fresh vegetables at room temperature. *J. Agr. Res.* 39:75–80.
9. Berard, J.E. 1821. Memoire sur la maturation des fruits. *Annales de Chimie et de Physique* XVI:152–183, 225–251.
10. Biale, J.B. 1946. Effect of oxygen concentration on respiration of the Fuerte avocado fruit. *Amer. J. Bot.* 33:363–373.
11. Biale, J.B. 1950. Postharvest physiology and biochemistry of fruits. *Annu. Rev. Plant Physiol.* 1:183–206.
12. Biale, J.B. 1954. Physiological requirements of citrus fruits. *Citrus Leaves* 34:6–7, 31–33.
13. Biale, J.B. 1960. Respiration of fruits. pp. 536–592. In: *Handbuch der Pflanzenphysiologie,* Vol. XII/2, W. Rukland (ed.). Springer-Verlag, Berlin.
14. Biale, J.B., and R.E. Young. 1962. The biochemistry of fruit maturation. *Endeavor* 21:164–174.
15. Blackman, F.F., and P. Parija. 1928. Analytic studies in plant respiration. II. The respiration of a population of senescent ripening apples. *Proc. Roy. Soc. Lond.,* Ser. B. 103:422–445.
16. Bloch, R. 1941. Wound healing in higher plants. *Bot. Rev.* 7:110–146.
17. Bloch, R. 1964. Wound healing in higher plants. *Bot. Rev.* 18:655–679.
18. Boehm, J.A. 1887. Üeber die respiration der kartoffel. *Bot Ztg.* 45:671–675, 680–691.
19. Bowyer, J.R., and R.C. Leegood. 1997. Photosynthesis. In: *Plant Biochemistry.* P.M. Dey and J.B. Harbone (eds.). Academic Press, New York.
20. Bramlage, W.J., M. Drake and J.H. Baker. 1974. Relationships of calcium content to respiration and seedlings. *Physiol. Plant.* 15:10–20.
21. Brix, H. 1962. The effect of water stress on the rates of photosynthesis and respiration on tomato plants and loblolly pine seedlings. *Plant Physiol.* 15:10–20.
22. Burg, S.P., and E.A. Burg. 1962. Role of ethylene in fruit ripening. *Plant Physiol.* 37:179–189.
23. Burlakow, G. 1898. Üeber athmung des keimes des weizens, *Triticum vulgare. Bot. Cent.* 74:323–324.
24. Burton, W.G. 1965. The sugar balance in some British potato varieties during storage. I. Preliminary observations. *European Potato J.* 8:80–91.
25. Chang, L.A., and S.J. Kays. 1981. Effect of low oxygen on sweet potato roots during storage. *J. Amer. Soc. Hort. Sci.* 106:481–483.
26. Childers, N.F., and D.G. White. 1942. Influence of submersion of the roots on transpiration, apparent photosynthesis, and respiration of young apple trees. *Plant Physiol.* 17:603–618.
27. Claypool, L.L., and R.M. Keefer. 1942. A colorimetric method for CO_2 determination in respiration studies. *Proc. Amer. Soc. Hort. Sci.* 40:177–186.

28. Coorts, G.D., J.B. Gartner and J.P. McCollum. 1965. Effect of senescence and preservative on respiration in cut flowers of *Rosa Hybrida,* 'Velvet Times'. *Proc. Amer. Soc. Hort. Sci.* 86:779–790.
29. deWild, H.P.J., and H.W. Peppelenbos. 2001. Improving the measurement of gas exchange in closed systems. *Postharv. Biol. Tech.* 22:111–119.
30. Denny, F.E. 1924. Hastening the coloration of lemons. *J. Agr. Res.* 27:757–771.
31. Denny, F.E. 1924. Effect of ethylene upon respiration of lemons. *Bot. Gaz.* (Chicago) 7:327–329.
32. Eaks, I.L., and W.A. Ludi. 1960. Effects of temperature, washing, and waxing on the composition of the internal atmosphere of orange fruits. *Proc. Amer. Soc. Hort. Sci.* 76:220–228.
33. Emmert, F.H., and F.W. Southwick. 1954. The effect of maturity, apple emanations, waxing and growth regulators on the respiration and red color development of tomato fruit. *Proc. Amer. Soc. Hort. Sci.* 63:393–401.
34. Faust, M., and C.B. Shear. 1972. The effects of calcium on respiration of apples. *J. Amer. Soc. Hort. Sci.* 97:437–439.
35. Foote, K.C., and M. Schaedle. 1976. Diurnal and seasonal patterns of photosynthesis and respiration by stems of *Populus tremuloides* Michx. *Plant Physiol.* 58:651–655.
36. Gane, R.1934. Production of ethylene by some ripening fruits. *Nature* (Lond.) 7:1465–1470.
37. Genevois, M.L. 1929. Sur la fermentation et sur la respiration chez les végétaux chlorophylliens. *Rev. Gen. Bot.* 41:252–271.
38. Godwin, H. 1935. The effect of handling on the respiration of cherry laurel leaves. *New Phytol.* 34:403–406.
39. Goeschl, J.D., L. Rappaport and H.K. Pratt. 1966. Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. *Plant Physiol.* 42:877–884.
40. Gortner, W.A., G.G. Dull and B. Krauss. 1967. Fruit development, maturation, ripening, and senescence: A biochemical basis for horticultural terminology. *HortScience* 2:141.
41. Green, W.P., W.V. Hukill and D.H. Rose. 1941. Calorimetric measurements of the heat of respiration of fruits and vegetables. *USDA Tech. Bull.* 771:1–21.
42. Greene, D.W., W.J. Lord and W.J. Bramlage. 1977. Mid-summer applications of ethephon and daminozide on apples. I. Effect on 'McIntosh'. *J. Amer. Soc. Hort. Sci.* 102:491–49.
43. Greene, D.W., W.J. Lord and W.J. Bramlage. 1977. Mid-summer applications of ethephon and daminozide on apples. II. Effect on 'Delicious'. *J. Amer. Soc. Hort. Sci.* 102:494–497.
44. Gregory, F.G., and F.J. Richards. 1929. Physiological studies in plant nutrition. I. The effect of manurial deficiency on the respiration and assimilation rate of barley. *Ann. Bot.* 43:119–161.
45. Hardwick, K., M. Wood and H.W. Woolhouse. 1968. Photosynthesis and respiration in relation to leaf age in *Perilla frutescens* (L.) Britt. *New Phytol.* 67:79–86.
46. Harris, G.H. 1929. Studies on tree root activities. I. An apparatus for studying root respiration and factors which influence it. *Sci. Agr.* 9:553–565.
47. Harris, G.H. 1930. Studies on tree root activities. II. Some factors which influence tree root respiration. *Sci. Agr.* 10:564–585.
48. Harvey, R.B. 1928. Artificial ripening of fruits and vegetables. *Minn. Agr. Exp. Sta. Bull.* 247.
49. Hatch, M.D., J.A. Pearson, A. Millerd and R.N. Robertson. 1959. Oxidation of Krebs cycle acids by tissue slices and cytoplasmic particles from apple fruit. *Aust. J. Biol. Sci.* 12:167–174.
50. Hill, G.R. 1913. Respiration of fruits and growing plant tissues in certain gases with reference to ventilation and fruit storage. *Cornell Agr. Exp. Sta. Bull.* 330:374–408.
51. Hinkle, M.A., A. Kumar, A. Resetar and D.L. Harris. 1991. Mechanistic stoichiometry of mitochondrial oxidative phosphorylation. *Biochemistry* 30:3576–3582.
52. Hoover, W.H., E.S. Johnston and F.S. Brackett. 1933. Carbon dioxide assimilation in a higher plant. *Smithsonian Inst. Misc. Coll.* 87:1–19.
53. Hulme, A.C. 1956. Carbon dioxide injury and the presence of succinic acid in apples. *Nature* (Lond.) 178:218–219.
54. Hulme, A.C., J.D. Jones and L.S.C. Wooltorton. 1963. The respiratory climacteric in apple fruits. *Proc. Roy. Soc. London,* Ser. B. 158:514–535.
55. Kahl, G. 1974. Metabolism in plant storage tissue slices. *Bot. Rev.* 40:263–314.
56. Kanai, R., and C.C. Black, Jr. 1972. Biochemical basis for net CO_2 assimilation in C_4-plants. Pp. 75–93. In: *Net Carbon Dioxide Assimilation in Higher Plants.* C.C. Black, Jr. (ed.). Symp. Southern Reg. Amer. Soc. Plant Physiol.

57. Kays, S.J., and J.E. Pallas, Jr. 1980. Inhibition of photosynthesis by ethylene. *Nature* 285:51–52.
58. Kidd, F. 1917. The controlling influence of carbon dioxide. Part III. The retarding effect of carbon dioxide on respiration. *Proc. Royal Soc. London* 89B:136–156.
59. Kidd, F. 1934. The respiration of fruits. *Royal Inst. of Great Britain* (as cited by Baile, 1960).
60. Kidd, F., and C. West. 1925. The course of respiratory activity throughout the life of an apple. *Great Britain Dept. Sci. Ind. Res., Food Invest. Bd. Rept.* 1924:27–33.
61. Kidd, F., C. West and G.E.A. Briggs. 1921. A quantitative analysis of the growth of *Helianthus annuus.* Part I. The respiration of the plant and of its parts throughout the life cycle. *Proc. Royal Soc. London,* B92:361–384.
62. Kirk, J.T., and R.A. Tilney-Bassett. 1967. *The Plastids; Their Chemistry, Structure, Growth, and Inheritance.* W.H. Freeman, San Francisco.
63. Knee, M. 1973. Effects of controlled atmosphere storage on respiratory metabolism of apple fruit tissue. *J. Sci. Food Agr.* 24:1289–1298.
64. Knee, M. 1995. Do tomatoes on the plant behave as climacteric fruits? *Physiol. Plant.* 95:211–216.
65. Kolattukudy, P.E. 1980. Cutin, suberin and waxes. Pp. 571–645. In: *Lipids: Structure and Function.* R.K. Stumpf (ed.). Vol. 4. *The Biochemistry of Plants.* Academic Press, New York.
66. Kriedemann, P.E., W.M. Kleiwer and J.M. Harris. 1970. Leaf age and photosynthesis in *Vitis vinifera* L. *Vitis* 9:97–104.
67. Kuc, R., and M. Workman. 1964. The relationship of maturity to the respiration and keeping quality of cut carnations and chrysanthemums. *Proc. Amer. Soc. Hort. Sci.* 84:575–581.
68. Kubota, C., and T. Kozai. 1995. Low-temperature storage of transplants at the light compensation point: Air temperature and light intensity for growth suppression and quality preservation. *Sci. Hort.* 61:193–204.
69. Lambers, H. 1980. The physiological significance of cyanide-resistant respiration in higher plants. *Plant Cell Environ.* 3:293–302.
70. Lange, D.L., and A.A. Kader. 1997. Effects of elevated carbon dioxide on key mitochondrial respiratory enzymes in 'Hass' avocado fruit and fruit disks. *J. Amer. Soc. Hort. Sci.* 122:238–244.
71. Lange, D.L., and A.A. Kader. 1997. Elevated carbon dioxide exposure alters intracellular pH and energy charge in avocado fruit tissue. *J. Amer. Soc. Hort. Sci.* 122:253–257.
72. Lipton, W.J. 1977. Toward an explanation of disorders of vegetables induced by high CO_2 and low O_2. *Proc. Second Nat. Controlled Atmos. Res. Conf.,* Mich. State Univ. Hort. Rept. 28:137–141.
73. Mack, W.B., and B.E. Livingstone. 1933. Relation of oxygen pressure and temperature to the influence of ethylene or carbon dioxide production and shoot elongation in very young wheat seedlings. *Bot. Gaz.* 94:625–687.
74. Massey, L.M., Jr., B.R. Chase and M.S. Starr. 1982. Effect of rough handling on CO_2 evolutions from 'Howes' cranberries. *HortScience* 17:57–58.
75. Mathooko, F.M. 1996. Regulation of respiratory metabolism in fruits and vegetables by carbon dioxide. *Postharv. Biol. Tech.* 9:247–264.
76. Maxie, E.C., D.S. Farnham, F.G. Mitchell, N.F. Sommer, R.A. Parsons, R.G. Snyder and H.L. Rae. 1973. Temperature and ethylene effects on cut flowers of carnations (*Dianthus caryophyllus* L.). *J. Amer. Soc. Hort. Sci.* 98:568–572.
77. Mayak, S., and A.H. Halevy. 1980. Flower senescence. Pp. 131–156. In: *Senescence in Plants.* K.V. Thimann (ed.). CRC Press, Boca Raton, FL.
78. McGlasson, W.B., and R.B.H. Wills. 1972. Effects of oxygen and carbon dioxide on respiration, storage life and organic acids of green bananas. *Aust. J. Biol. Sci.* 25:35–42.
79. Meeuse, B.J.D. 1975. Thermogenic respiration in Aroids. *Annu. Rev. Plant Physiol.* 26:117–126.
80. Mellenthin, W.M., and C.Y. Wang. 1976. Preharvest temperatures in relation to postharvest quality of d'Anjou pears. *J. Amer. Soc. Hort. Sci.* 101:302–305.
81. Millerd, A., J. Bonner and J.B. Biale. 1953. The climacteric rise in plant respiration as controlled by phosphorylative coupling. *Plant Physiol.* 28:521–531.
82. Morris, L.L., and A.A. Kader. 1977. Physiological disorders of certain vegetables in relation to modified atmospheres. *Proc. Second Nat. Controlled Atmos. Res. Conf.,* Mich. State Univ. Hort. Rept. 28:142–148.
83. Mulder, E.G. 1955. Effect of mineral nutrition of potato plants on respiration of the tubers. *Acta Bot. Neerlandica* 4:429–451.

84. Müller-Thurgan, H., and O. Schneider-Orelli. 1908. Reifevorgänge bei Kernobst früchten. *Landwirtsch. J. B. Schwei.* 22:760–774.
85. Nicholas, G. 1918. Contribution á l'etude des variatins de la respiration des végétaux avec l'age. *Rev. Gen. Bot.* 30:214–225.
86. Nichols, R. 1968. The response of carnations (*Dianthus caryophyllus*) to ethylene. *J. Hort. Soc.* 43:335–349.
87. Overholser, E.L., and L.L. Claypool. 1931. The relation of fertilizers to respiration and certain physical properties of strawberries. *Proc. Amer. Soc. Hort. Sci.* 28:220–224.
88. Peiris, K.H.S., J.L. Mallon and S.J. Kays. 1997. Respiration rate and vital heat of some specialty vegetables at various storage temperatures. *HortTechnology* 7:46–49.
89. Piechulla, B., R.E. Glick, H. Bahl, A. Melis and W. Gruissem. 1987. Changes in photosynthetic capacity and photosynthetic protein pattern during tomato fruit ripening. *Plant Physiol.* 84:911–917.
90. Paull, R.E. 1999. Effect of temperature and relative humidity on fresh commodity quality. *Postharv. Biol. Tech.* 15:263–277.
91. Pratt, H.K., and D.B. Merrdoza, Jr. 1979. Colorimetric determination of carbon dioxide for respiration studies. *HortScience* 14:175–176.
92. Purvis, A.C., and R.L. Shewfelt. 1993. Does the alternative pathway ameliorate chilling injury in sensitive plant tissues? *Plant Physiol.* 88:712–718.
93. Rabinowitch, E.I. 1951. Photosynthesis and related processes. Vol. II, Pt. I. *Spectroscopy and Fluorescence of Photosynthetic Pigments; Kinetics of Photosynthesis.* Interscience, New York.
94. Robinson, J.E., K.M. Browne and W.G. Burton. 1975. Storage characteristics of some vegetables and soft fruits. *Ann. Appl. Biol.* 81:399–408.
95. Simon, E.W. 1967. Types of leaf senescence. *Symp. Soc. Exp. Biol.* 21:215–230.
96. Siripanich, J., and A.A. Kader. 1986. Changes in cytoplasmic and vacuolar pH in harvested lettuce tissue as influenced by CO_2. *J. Amer. Soc. Hort. Sci.* 111:73–77.
97. Smock, R.M., L.J. Edgerton and M.B. Hoffman. 1954. Some effects of stop drop auxins and respiratory inhibitors on the maturity of apples. *Proc. Amer. Soc. Hort. Sci.* 63:211–219.
98. Solomos, T., and J.B. Biale. 1975. Respiration and fruit ripening. *Colloq. Int. C.N.R.S.* 238:221–228.
99. Thimann, K.V. 1980. The senescence of leaves. Pp. 85–115. In: *Senescence in Plants.* K.V. Thimann (ed.). CRC Press, Boca Raton, FL.
100. Thornton, N.C. 1933. Carbon dioxide storage. III. The influence of carbon dioxide on oxygen uptake by fruits and vegetables. *Contrib. Boyce Thompson Inst.* 5:371–402.
101. Tranquillini, W. 1955. Die bedeutung des lichtes und der temperatur fur die kohlensaureassimilation von *Pinus cembra* jungwuchs an einem hochalpinen standort. *Planta* 46:154–178.
102. Trout, S.A., E.G. Hall, R.N. Robertson, M.V. Hackney and S.M. Sykes. 1942. Studies in the metabolism of apples. *Aust. J. Exp. Biol. Med. Sci.* 20:219–231.
103. Uritani, I., and T. Asahi. 1980. Respiration and related metabolic activity in wounded and infected tissue. Pp. 463–487. In: *Metabolism and Respiration.* D.D. Davis (ed.). Vol. 2. *The Biochemistry of Plants.* Academic Press, New York.
104. van Iersel, M.W., and L. Seymour. 2000. Growth respiration, maintenance respiration, and carbon fixation of Vinca: A time series analysis. *J. Amer. Soc. Hort. Sci.* 125:702–706.
105. Wager, H.G. 1974. The effect of subjecting peas to air enriched with carbon dioxide. I. The path of gaseous diffusion, the content of CO_2 and the buffering of the tissue. *J. Exp. Bot.* 25:330–337.
106. Wager, H.G. 1974. The effect of subjecting peas to air enriched with carbon dioxide. II. Respiration and the metabolism of the major acids. *J. Exp. Bot.* 25:338–351.
107. Wittwer, S.H., and W. Robb. 1964. Carbon dioxide enrichment of greenhouse atmospheres for food crop production. *Econ. Bot.* 18:34–56.
108. Young, R.E., and J.B. Biale. 1956. Carbon dioxide fixation by lemons in a CO_2 enriched atmosphere. *Plant Physiol.* 32 (suppl.):23.
109. Zelitch, I. 1979. Photorespiration: Studies with whole tissues. Pp. 353–367. In: *Photosynthesis II.* M. Gibbs and E. Latzko (eds.). Vol. 6. *Encyclopedia of Plant Physiology.* Springer-Verlag, Berlin.