# 2B. Respiration

## 1. Introduction

A large portion of the carbohydrates that a plant assimilates each day are expended in respiration in the same period (Table 1). If we seek to explain the carbon balance of a plant and to understand plant performance and growth in different environments, it is imperative to obtain a good understanding of respiration. **Dark respiration** is needed to produce the energy and carbon skeletons to sustain plant growth; however, a significant part of respiration may proceed via a **nonphosphorylating pathway** that is cyanide resistant and generates less ATP than the **cytochrome pathway**, which is the primary energy-producing pathway in both plants and animals. We present several hypotheses in this chapter to explore why plants have a respiratory pathway that is not linked to ATP production.

The types and rates of plant respiration are controlled by a combination of **respiratory capacity**, **energy demand**, **substrate availability**, and **oxygen supply** (Covey-Crump et al. 2002, 2007). At low levels of $O_2$, respiration cannot proceed by normal aerobic pathways, and fermentation starts to take place, with **ethanol** and **lactate** as major end-products. The ATP yield of fermentation is considerably less than that of normal aerobic respiration. In this chapter, we discuss the control over respiratory processes, the demand for respiratory energy, and the significance of respiration for the plant's carbon balance, as these are influenced by species and environment.

## 2. General Characteristics of the Respiratory System

### 2.1 The Respiratory Quotient

The respiratory pathways in plant tissues include **glycolysis**, which is located both in the cytosol and in the plastids, the **oxidative pentose phosphate pathway**, which is also located both in the plastids and the cytosol, the **tricarboxylic acid** (TCA) or **Krebs cycle**, in the matrix of mitochondria, and the **electron-transport pathways**, which reside in the inner mitochondrial membrane.

The **respiratory quotient** (RQ, the ratio between the number of moles of $CO_2$ released and that of $O_2$ consumed) is a useful index of the types of substrates used in respiration and the subsequent use of respiratory energy to support biosynthesis. In the absence of biosynthetic processes, the RQ of respiration is expected to be 1.0, if sucrose is the only substrate for respiration and is fully oxidized to $CO_2$ and $H_2O$. When **leaves** of *Phaseolus vulgaris* (common bean) are exposed to an extended dark period or to high temperatures, their RQ declines, due to a shift from **carbohydrates** as the main substrate for

**Table 1. Utilization of photosynthates in plants, as dependent on the nutrient supply.***

| Item | Utilization of photosynthates % of C fixed | |
|---|---|---|
| | Free nutrient availability | Limiting nutrient supply |
| Shoot growth | 40*–57 | 15–27* |
| Root growth | 17–18* | 33*–35 |
| Shoot respiration | 17–24* | 19–20* |
| Root respiration | 8–19* | 38*–52 |
| – Growth | 3.5–4.6* | 6*–9 |
| – Maintenance | 0.6–2.6* | ? |
| – Ion acquisition | –13* | ? |
| Volatile losses | 0–8 | 0–8 |
| Exudation | <5 | <23 |
| $N_2$ fixation | Negligible | 5–24 |
| Mycorrhiza | Negligible | 7–20 |

*Source*: Van der Werf et al. (1994).
* inherently slow-growing species; ? no information for nutrient-limited conditions.

**Table 2. The respiratory quotient (RQ) of root respiration of a number of herbaceous species.***

| Species | RQ | Special Remarks |
|---|---|---|
| *Allium cepa* | 1.0 | Root tips |
| | 1.3 | Basal parts |
| *Dactylis glomerata* | 1.2 | |
| *Festuca ovina* | 1.0 | |
| *Galinsoga parviflora* | 1.6 | |
| *Helianthus annuus* | 1.5 | |
| *Holcus lanatus* | 1.3 | |
| *Hordeum distichum* | 1.0 | |
| *Lupinus albus* | 1.4 | |
| | 1.6 | $N_2$-fixing |
| *Oryza sativa* | 1.0 | $NH_4^+$-fed |
| | 1.1 | |
| *Pisum sativum* | 0.8 | $NH_4^+$-fed |
| | 1.0 | |
| | 1.4 | $N_2$-fixing |
| *Zea mays* | 1.0 | Fresh tips |
| | 0.8 | Starved tips |

*Source*: Various authors, as summarized in Lambers et al. (2002).
*All plants were grown in nutrient solution, with nitrate as the N-source, unless stated otherwise. The *Pisum sativum* (pea) plants were grown with a limiting supply of combined N, so that their growth matched that of the symbiotically grown plants.

respiration to **fatty acids** (Tcherkez et al. 2003). For **roots** of young seedlings, measured in the absence of an N source, values close to 1.0 have been found, but most experimental RQ values differ from unity (Table 2). RQ values for germinating **seeds** depend on the storage compounds in the seeds. For seeds of *Triticum aestivum* (wheat), in which **carbohydrates** are major storage compounds, RQ is close to unity, whereas for the **fat**-storing seeds of *Linum usitatissimum* (flax) RQ values as low as 0.4 are found (Stiles & Leach 1936).

Both the nature of the respiratory substrate and biosynthetic reactions strongly influence RQ. The RQ can be greater than 1, if **organic acids** are an important substrate, because these are more oxidized than sucrose, and, therefore, produce more $CO_2$ per unit $O_2$. On the other hand, RQ will be less than 1, if compounds that are more reduced than sucrose (e.g., **lipids** and **protein**) are a major substrate, as occurs during starvation of leaves and excised root tips (Table 2). In **shoots** of *Hordeum vulgare* (barley) that receive $\mathbf{NH_4^+}$ as their sole N source respiratory fluxes of $O_2$ equal those of $CO_2$. By contrast, shoots exposed to $\mathbf{NO_3^-}$ show a higher $CO_2$ evolution than $O_2$ consumption in the dark (RQ = 1.25). These results show that a substantial portion of respiratory electron transport generates reductant for $NO_3^-$ assimilation, producing an additional two molecules of $CO_2$ per molecule of $NO_3^-$ reduced to $NH_4^+$ (Bloom et al. 1989). Substrates available to support root respiration depend on processes occurring throughout the plant. For example, organic acids (malate) that are produced during the reduction of $NO_3^-$ in leaves can be transported and decarboxylated in the roots, releasing $CO_2$ and increasing RQ (Ben Zioni et al. 1971). If $\mathbf{NO_3^-}$ **reduction** proceeds in the roots, then the RQ is also expected to be greater than 1. Values of RQ are therefore lower in plants that use $NH_4^+$ as an N source than in plants grown with $NO_3^-$ or, symbiotically, with $N_2$ (Table 2).

Biosynthesis influences RQ in several ways. Carboxylating reactions consume $CO_2$, reducing RQ, whereas decarboxylating reactions produce $CO_2$ and, therefore, increase RQ. In addition, synthesis of oxidized compounds such as organic acids decreases RQ, whereas the production of reduced compounds such as lipids leads to higher RQ values. The average molecular formula of the biochemical compounds typical of plant biomass is more reduced than sucrose, so RQ values influenced by biosynthesis should be greater than 1, as generally observed (Table 2; for further information, see Table 5.11 in Sect. 5.2.2).

RQ values of root respiration increase with increasing potential **growth rate** of a species (Fig. 1). This results from high rates of biosynthesis, relative to rates of ATP production; as explained above, ATP production associated with sucrose breakdown is

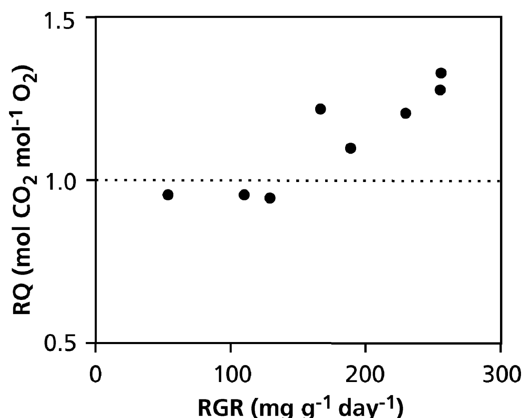


**FIGURE 1. The respiratory quotient of a number of fast- and slow-growing grass species, grown with free access to nutrients and with nitrate as the source of N (Scheurwater et al. 1998).**

associated with an RQ of 1.0, whereas biosynthesis yields RQ values greater than 1.0 (Scheurwater et al. 2002).

In summary, the patterns of RQ in plants clearly demonstrate that in roots it depends on the plant's growth rate. For all organs, it depends on the predominant respiratory substrate, integrated whole-plant processes, and ecological differences among species.

## 2.2 Glycolysis, the Pentose Phosphate Pathway, and the Tricarboxylic (TCA) Cycle

The first step in the production of energy for respiration occurs when glucose (or starch or other storage carbohydrates) is metabolized in glycolysis or in the oxidative pentose phosphate pathway (Fig. 2). **Glycolysis** involves the conversion of glucose, via phospho*enol*pyruvate (PEP), into malate and pyruvate. In contrast to mammalian cells, where virtually all PEP is converted into pyruvate, in plant cells malate is the major end-product of glycolysis and thus the major substrate for the mitochondria. Key enzymes in glycolysis are controlled by adenylates (AMP, ADP, and ATP), in such a way as to speed up the rate of glycolysis when the demand for metabolic energy (ATP) increases (Plaxton & Podestá 2006).

Oxidation of one glucose molecule in glycolysis produces two **malate** molecules, without a net production of ATP. When **pyruvate** is the end product, there is a net production of two ATP molecules in glycolysis. Despite the production of NADH in one step in glycolysis, there is no net production of NADH when malate is the end product, due to the need for NADH in the reduction of oxaloacetate, catalyzed by malate dehydrogenase.

Unlike glycolysis, which is predominantly involved in the breakdown of sugars and ultimately in the production of ATP, the **oxidative pentose phosphate pathway** plays a more important role in producing intermediates (e.g., amino acids, nucleotides) and NADPH. There is no evidence for a control of this pathway by the demand for energy.

The malate and pyruvate that are formed in glycolysis in the cytosol are imported into the mitochondria, where they are oxidized in the **tricarboxylic acid (TCA) cycle**. Complete oxidation of one molecule of malate, yields three molecules of $CO_2$, five molecules of NADH and one molecule of $FADH_2$, as well as one molecule of ATP (Fig. 2). NADH and $FADH_2$ subsequently donate their electrons to the electron-transport chain (Sect. 2.3.1).

## 2.3 Mitochondrial Metabolism

The malate formed in glycolysis in the cytosol is imported into the mitochondria and oxidized partly via **malic enzyme**, which produces pyruvate and $CO_2$, and partly via **malate dehydrogenase**, which produces oxaloacetate. Pyruvate is then oxidized so that malate is regenerated (Fig. 2). In addition, pyruvate can be produced in the cytosol and imported into the mitochondria. Oxidation of malate, pyruvate, and other NAD-linked substrates is associated with complex I (Sect. 2.3.1). In mitochondria there are four major complexes associated with **electron transfer** and one associated with **oxidative phosphorylation**, all located in the inner mitochondrial membrane. In addition, there are two small redox molecules, **ubiquinone** (Q) and **cytochrome** ***c***, which play a role in electron transfer. In plant mitochondria there is also a cyanide-resistant, nonphosphorylating, **alternative oxidase**, located in the inner membrane (Fig. 3). Finally, there are additional NAD(P)H dehydrogenases in the inner mitochondrial membrane that allow electron transport without ATP formation as well as **uncoupling proteins** that converts energy that could have been used for ATP production into heat.

In the mitochondrial **matrix** the imported substrates are oxidized in a cyclical process (**Krebs** or **TCA cycle**), releasing three $\mathbf{CO_2}$ molecules per pyruvate in each cycle and generating reducing power (**NADH** and $\mathbf{FADH_2}$) in several reactions (Fig. 2). Pyruvate decarboxylase (PDC), which converts pyruvate into acetylCoA, which then reacts with oxaloacetate to produce citrate, is a major control point for entry of carbon into the TCA cycle.

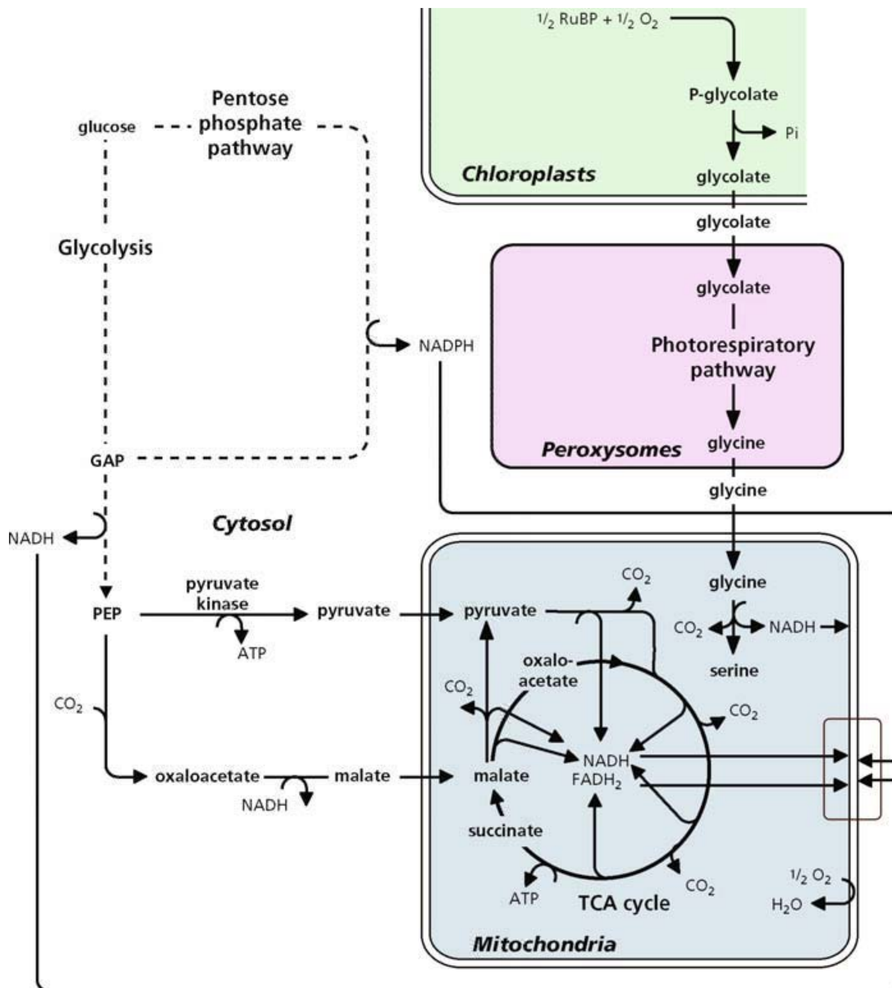


**Figure 2. The major substrates for the electron transport pathways. Glycine is only a major substrate in photosynthetically active cells of $C_3$ plants when photorespiration plays a role.**

### *2.3.1 The Complexes of the Electron-Transport Chain*

**Complex I** is the main entry point of electrons from NADH produced in the **TCA cycle** or in **photorespiration** (glycine oxidation). Complex I is the **first coupling site** or **site 1** of proton extrusion from the matrix into the intermembrane space which is linked to ATP production. Succinate is the only intermediate of the TCA cycle that is oxidized by a membrane-bound enzyme: succinate dehydrogenase (Fig. 3). Electrons enter the respiratory chain via complex II and are transferred to ubiquinone. NAD(P)H that is produced outside the mitochondria also feeds its electrons into the chain at the level of ubiquinone (Fig. 3). As with complex II, the external dehydrogenases are not connected with the translocation of $H^+$ across the inner mitochondrial membrane. Hence less ATP is produced per $O_2$ when succinate or NAD(P)H are oxidized in comparison with that of glycine, malate, or citrate, which enter at complex I. Complex III transfers electrons from ubiquinone to cytochrome c, coupled to the extrusion of protons to the intermembrane space and is therefore **site 2** of proton extrusion from the matrix into the intermembrane space. Complex IV is the terminal oxidase of the cytochrome pathway, accepting electrons from cytochrome c and donating these to $O_2$. It also generates a proton-motive force (i.e., an electrochemical potential gradient across a membrane), which makes complex IV **site 3** of proton extrusion.

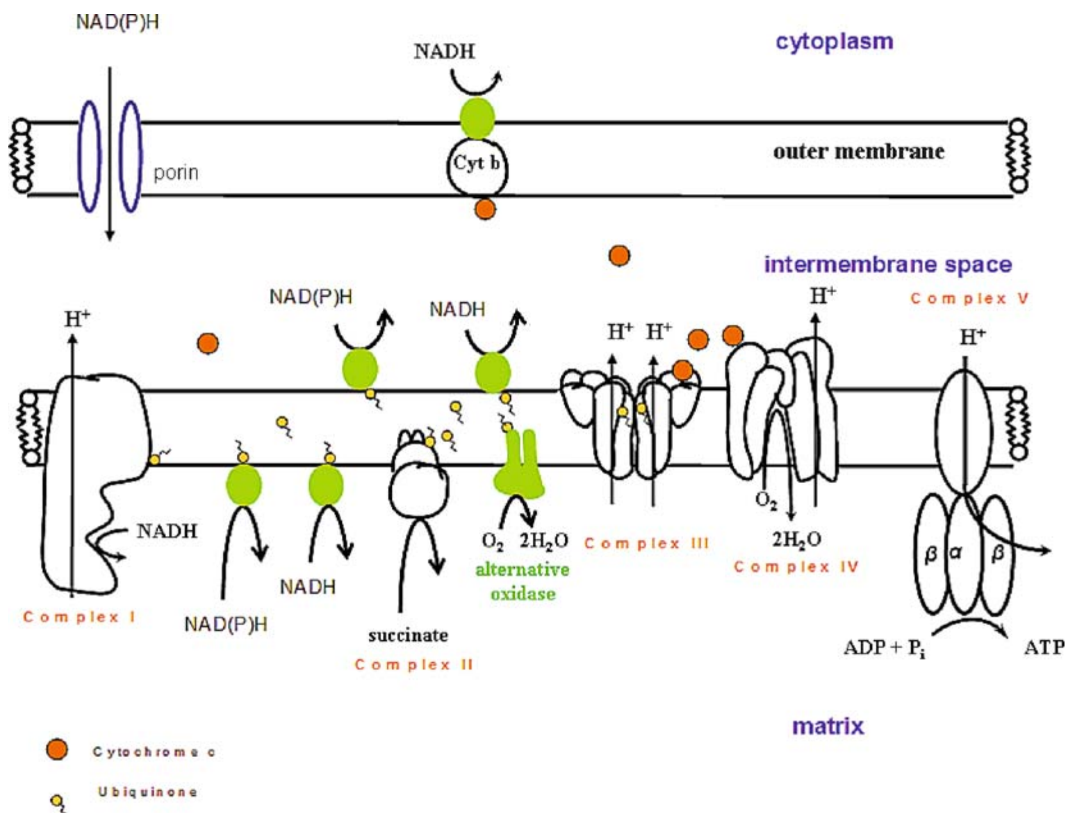


FIGURE 3. The organization of the electron-transporting complexes of the respiratory chain in higher plant mitochondria. All components are located in the inner mitochondrial membrane. Some of the components are membrane spanning, others face the mitochondrial matrix or the space between the inner and the outer mitochondrial membrane. Q (ubiquinone) is a mobile pool of quinone and quinol molecules. Alternative NAD(P)H dehydrogenases and the alternative oxidase are shown in green (Rasmusson et al. 2004). Reprinted, with permission, from the *Annual Review of Plant Biology*, Volume 55 ©2004 by Annual Reviews www.annualreviews.org.

### *2.3.2 A Cyanide-Resistant Terminal Oxidase*

Mitochondrial respiration of many tissues from higher plants is not fully inhibited by inhibitors of the cytochrome path (e.g., KCN). This is due to the presence of a cyanide-resistant, alternative electron-transport pathway, consisting of one enzyme, the **alternative oxidase**, firmly embedded in the inner mitochondrial membrane. The branching point of the alternative path from the cytochrome path is at the level of ubiquinone, a component common to both pathways. Transfer of electrons from ubiquinone to $O_2$ via the alternative path is not coupled to the extrusion of protons from the matrix to the intermembrane space. Hence, the transfer of electrons from NADH produced inside the mitochondria to $O_2$ via the alternative path bypasses two sites of proton extrusion, and therefore yields only one third of the amount of ATP that is produced when the cytochrome path is used.

### *2.3.3 Substrates, Inhibitors, and Uncouplers*

Figure 2 summarizes the major substrates for mitochondrial $O_2$ uptake as well as their origin. Oxidation of glycine is of quantitative importance only in tissues exhibiting **photorespiration**. Glycolysis may start with glucose, as depicted here, or with starch, sucrose, or any major transport carbohydrate or sugar alcohol imported via the phloem (Sect. 2 of Chapter 2C on long-distance transport).

A range of respiratory inhibitors have helped to elucidate the organization of the respiratory pathways. To give just one example, **cyanide** effectively blocks complex IV and has been used to demonstrate the presence of the alternative path.

**Uncouplers** make membranes, including the inner mitochondrial membrane, permeable to protons and hence prevent oxidative phosphorylation. Many compounds that inhibit components of the respiratory chain or have an uncoupling activity occur naturally as **secondary compounds** in plant and fungal tissues; they may protect these tissues from being grazed or infected by other organisms or be released from roots and act as allelochemicals (Sects 2 and 3.1 of Chapter 9B on ecological biochemistry). A more recent addition to the complexity of the plant mitochondrial electron-transport chain is the discovery of **uncoupling protein** (UCP) (Hourton-Cabassa et al. 2004). UCP is a homologue of thermogenin, a protein responsible for thermogenesis in mammalian brown fat cells. Both uncoupling protein and thermogenin allow protons to diffuse down their concentration gradient from the intermembrane space into the matrix, circumventing the ATP synthase complex and thus uncoupling electron transport from ATP production (Plaxton & Podestá 2006).

### *2.3.4 Respiratory Control*

To learn more about the manner in which plant respiration responds to the demand for metabolic energy, we first describe some experiments with **isolated mitochondria**. Freshly isolated intact mitochondria in an appropriate buffer that lacks substrates, a condition referred to as "state 1", do not consume an appreciable amount of $O_2$; in vivo they rely on a continuous import of respiratory substrate from the cytosol (Fig. 4). Upon addition of a respiratory substrate ("state 2") there is some, but still not much $O_2$ uptake; for rapid rates of respiration to occur in vivo, import of additional metabolites is required. As soon as ADP is added, a rapid consumption of $O_2$ can be measured. This "state" of the mitochondria is called "state 3". In vivo, rapid supply of ADP will occur when a large amount of ATP is required to drive biosynthetic and transport processes. Upon conversion of all ADP into ATP ("state 4"), the respiration rate of the mitochondria declines again to the rate found before addition of ADP (Fig. 4). Upon addition of

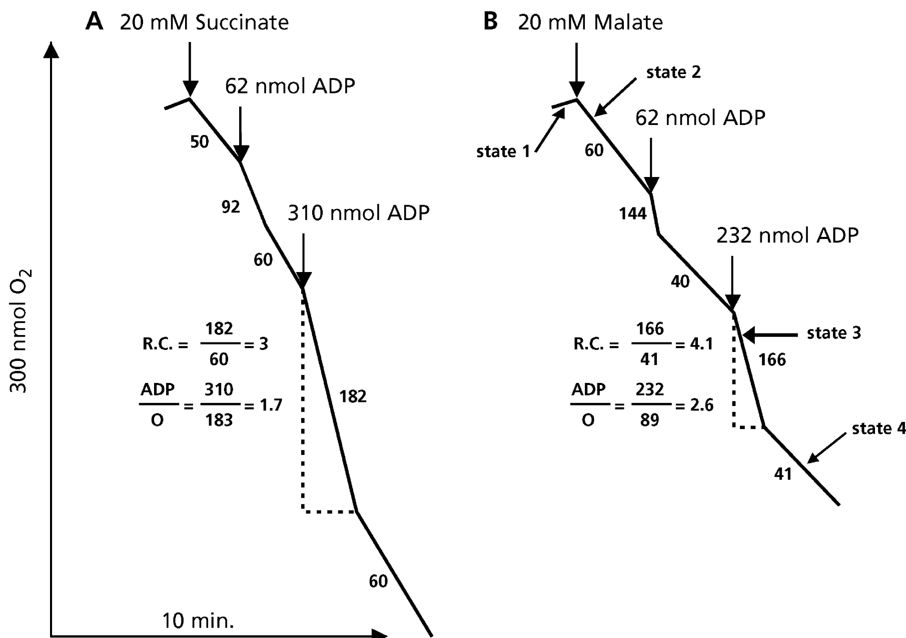


FIGURE 4. The "states" of isolated mitochondria. The ADP:O ratio (also called ATP:O ratio or P:O ratio) is calculated from the $O_2$ consumption during the phosphorylation of a known amount of added ADP (state 3). The amount of ADP consumed equals the amount that has been added to the cuvette (310 and 232 in A and B, respectively); since the total amount of $O_2$ in the cuvette is known (300 nmol), the amount consumed during the consumption of the added ADP can be derived (dashed vertical lines, with values of 183 and 89 nanomoles of O atoms in A and B, respectively). The respiratory control ratio (RC) is the ratio of the rate of $O_2$ uptake (in nmol $O_2$ $mg^{-1}$ protein $min^{-1}$; values written along the slopes) in state 3 and state 4. State 1 refers to the respiration in the absence of respiratory substrate and ADP, and state 2 is the respiration after addition of respiratory substrate, but before addition of ADP (based on unpublished data from A.M. Wagner, Free University of Amsterdam).

more ADP, the mitochondria go into state 3 again, followed by state 4 upon depletion of ADP. This can be repeated until all $O_2$ in the cuvette is consumed. Thus the respiratory activity of isolated mitochondria is effectively controlled by the availability of ADP: **respiratory control**, quantified in the "respiratory control ratio" (the ratio of the rate at substrate saturation in the presence of ADP and that under the same conditions, but after ADP has been depleted; Fig. 4). The same respiratory control occurs in intact tissues and is one of the mechanisms ensuring that the rate of respiration is enhanced when the demand for ATP increases.

## 2.4 A Summary of the Major Points of Control of Plant Respiration

We briefly discussed the control of glycolysis by "energy demand" (Sect. 2.2) and a similar control by "energy demand" of mitochondrial electron transport, termed respiratory control (Sect. 2.3.4). The effects of **energy demand** on dark respiration are a function of the metabolic energy that is required for **growth**, **maintenance**, and **transport** processes; therefore, when tissues grow quickly, take up ions rapidly and/or have a fast turnover of proteins, they generally have a high rate of respiration. At low levels of **respiratory substrate supply** (carbohydrates, organic acids), however, the activity of respiratory pathways may be substrate-limited. When substrate levels increase, the respiratory capacity is enhanced and adjusted to the high substrate input, through the transcription of specific genes that encode respiratory enzymes. Figure 5 summarizes these and several other points of control. Plant respiration is clearly quite flexible and responds rapidly to the demand for respiratory energy as well as the supply of respiratory substrate. The production of ATP which is coupled to the oxidation of substrate, may also vary widely, due to the presence of both nonphosphorylating and phosphorylating paths [alternative oxidase and NAD(P) dehydrogenases other than complex I] as well as the activity of an uncoupling protein.

## 2.5 ATP Production in Isolated Mitochondria and In Vivo

The rate of $O_2$ consumption during the phosphorylation of ADP can be related to the total ADP that must be added to consume this $O_2$. This allows calculation of the **ADP:O ratio** in vitro. This ratio is around 2.5 for NAD-linked substrates (e.g., malate, citrate) and around 1.5 for succinate and external NAD(P)H. Nuclear Magnetic Resonance (NMR) spectroscopy has been used to estimate ATP production in intact tissues, as outlined in Sect. 2.5.2.

### *2.5.1 Oxidative Phosphorylation: The Chemiosmotic Model*

During the transfer of electrons from various substrates to $O_2$ via the cytochrome path, protons are extruded into the space between the inner and outer mitochondrial membranes. This generates a **proton-motive force** across the inner mitochondrial membrane which drives the synthesis of ATP. The basic features of this **chemiosmotic model** are (Mitchell 1966, Nicholls & Ferguson 1992):

1. Protons are transported outwards, coupled to the transfer of electrons, thus giving rise to both a **proton gradient** ($\Delta$pH) and a **membrane potential** ($\Delta\Psi$)
2. The inner membrane is **impermeable to protons** and other ions, except by special transport systems
3. There is an **ATP synthetase** (also called ATPase), which transforms the energy of the electrochemical gradient generated by the proton-extruding system into ATP

The pH gradient, $\Delta$pH, and the membrane potential $\Delta\Psi$, are interconvertible. It is the combination of the two which forms the **proton-motive force** ($\Delta p$), the driving force for ATP synthesis, catalyzed by an ATPase:

$$\Delta p = \Delta\Psi - 2.3\ RT/F\Delta \mathrm{pH} \quad (1)$$

where $R$ is the gas constant (J $mol^{-1}$ $K^{-1}$), $T$ is the absolute temperature (K) and $F$ is Faraday's number (Coulomb). Both components in the equation are expressed in mV. Approximately one ATP is produced per three protons transported.

### *2.5.2 ATP Production* In Vivo

ATP production in vivo can be measured using **NMR spectroscopy**. This technique relies on the fact that certain nuclei, including $^{31}P$, possess a permanent magnetic moment, because of nuclear spin. Such nuclei can be made "visible" in a strong external magnetic field, in which they orient their nuclear spins in the same direction. It is just like the orientation of a small magnet in response to the presence of a strong one. NMR spectroscopy allows one to monitor the absorption of radiofrequency by the oriented spin population in the strong magnetic

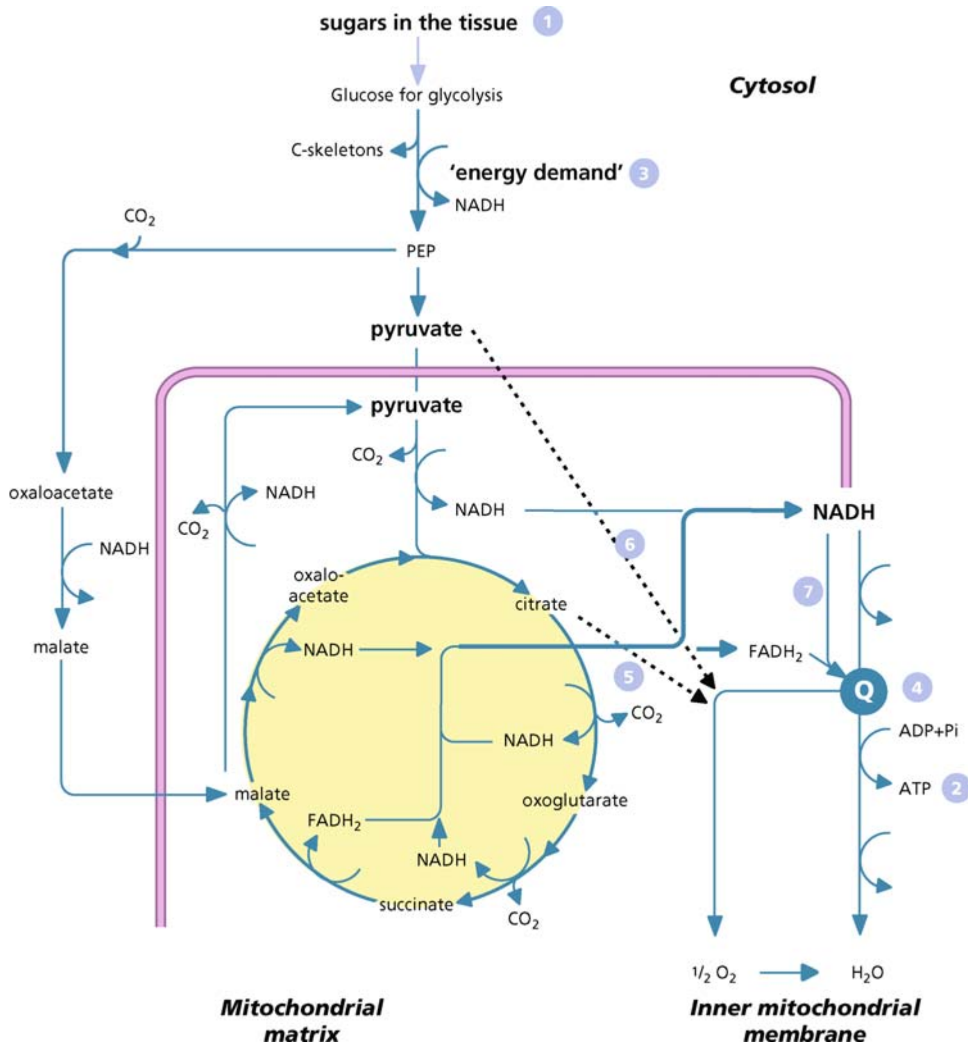


Figure 5. A simplified scheme of respiration and its major control points. Controlling factors include the concentration of respiratory substrate [e.g., glucose (1)] and adenylates (2, 3). Adenylates may exert control on electron transport via a constraint on the rate of oxidative phosphorylation (2) as well as on glycolysis, via modulation of the activity of key enzymes in glycolysis, phosphofructokinase and pyruvate kinase ("energy demand", 3). When the input of electrons into the respiratory chain is very high, a large fraction of ubiquinone becomes reduced and the alternative path becomes more active (4). When the rate of glycolysis is very high, relative to the activity of the cytochrome path, organic acids may accumulate (5, 6). The accumulation of citric acid may lead to the reduction of the sulfide bonds of the alternative oxidase and thus enhance the capacity of the alternative path (5). Accumulation of pyruvate or other α-keto acids may increase the $V_{max}$ of the alternative oxidase and, hence, allow it to function at a low level of reduced ubiquinone (6). There is increasing evidence that the nonphosphorylating rotenone-insensitive bypass (7) operates in concert with the alternative path, when the concentration of NADH is very high.

field. The location of the peaks in a NMR spectrum depends on the molecule in which the nucleus is present and also on the "environment" of the molecule (e.g., pH). Figure 6 illustrates this point for a range of phosphate – containing molecules (Roberts 1984).

The resonance of specific P – containing compounds can be altered by irradiation with radiofrequency power. If this irradiation is sufficiently strong ("saturating"), then it disorientates the nuclear spins of that P – containing compound, so that its peak disappears from the spectrum.

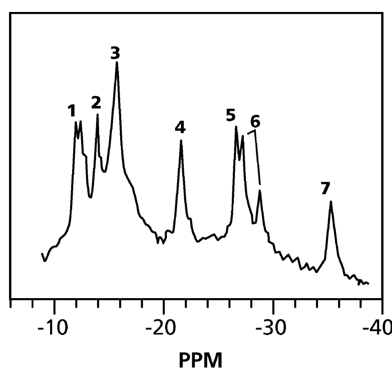


Figure 6. NMR spectrum of root tips of *Pisum sativum* (pea), showing peaks of, for example, glucose-6-phosphate (1), ($P_i$) (2, 3), and ATP (4, 5) in a living plant cell. The exact radiofrequency at which a phosphate-containing compound absorb depends on the pH. This explains why there are two peaks for $P_i$: a small one for the cytosol (2), where the pH is approximately 7, and a larger one for the vacuole (3), where the pH is lower (Roberts 1984). Reprinted, with permission, from the *Annual Review of Plant Physiology,* Volume 35 ©1984 by Annual Reviews www.annualreviews.org.

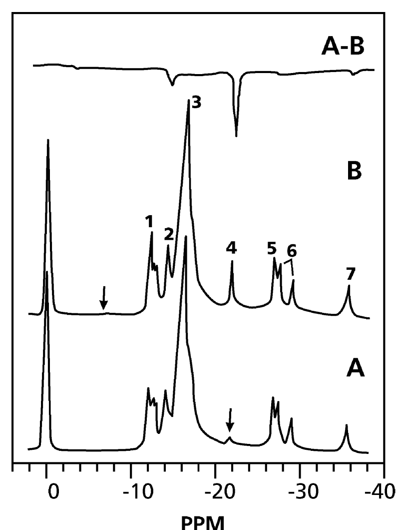


Figure 7. Saturation transfer from γ-ATP phosphate to cytosolic $P_i$ in root tips of *Zea mays* (maize). Spectrum A was obtained with selective presaturation of the γ-ATP peak. Spectrum B was obtained with selective presaturation of a point equidistant from the cytosolic $P_i$ peak, Spectrum A–B gives the difference between the two spectra, showing the transfer of saturation from γ-ATP to cytosolic $P_i$ (after Roberts et al. 1984a). Copyright American Society of Plant Biologists.

Figure 7A illustrates this for the γ-ATP P-atom, the P atom that is absent in ADP. Upon hydrolysis of ATP, the γ-ATP P atom becomes part of the cytoplasmic inorganic phosphate ($P_i$) pool. For a brief period, therefore, some of the $P_i$ molecules also contain disoriented nuclear spins; specific radiation of the γ-ATP peak decreases the $P_i$ peak. This phenomenon is called "saturation transfer" (Fig. 7). Saturation transfer has been used to estimate the rate of ATP hydrolysis to ADP and $P_i$ in vivo.

If the rate of disappearance of the saturation in the absence of biochemical exchange of phosphate between γ-ATP and $P_i$ is known, then the rate of ATP hydrolysis can be derived from the rate of loss of saturation. This has been done for root tips for which the $O_2$ uptake was measured in parallel experiments. In this manner ADP:O ratios in *Zea mays* (maize) root tips exposed to a range of conditions have been determined (Table 3).

The ADP:O ratios for the root tips supplied with 50 mM glucose are remarkably close to those expected when glycolysis plus TCA cycle are responsible for the complete oxidation of exogenous glucose, provided the alternative path does not contribute to the $O_2$ uptake (Table 3). KCN decreases the ADP:O ratio of glucose oxidation by two-thirds in a manner to be expected from mitochondrial studies. SHAM, an inhibitor of the alternative path, has no effect on the rate of ATP production. So far, maize root tips are the only intact plant material used for the determination of ADP:O ratios in vivo. We cannot assume, therefore, that the ADP:O ratio in vivo is invariably 3. In fact, the ratio under most circumstances is probably far less than 3 (Sect. 2.6.2).

## 2.6 Regulation of Electron Transport via the Cytochrome and the Alternative Paths

The existence of two respiratory pathways, both transporting electrons to $O_2$, in higher plant mitochondria, raises the question if and how the **partitioning of electrons** between the two paths is regulated. This is important because the cytochrome path is coupled to proton extrusion and the production of ATP, whereas transport of electrons via the alternative path is not, at least not beyond the point where both pathways branch to $O_2$ (Millenaar & Lambers 2003).

### *2.6.1 Competition or Overflow?*

Under specific conditions, the activity of the cytochrome path *in vitro* increases linearly with the

TABLE 3. The in vivo ADP:O ratios in root tips of *Zea mays* (corn) determined with the saturation transfer $^{31}P$ NMR technique and $O_2$ uptake measurements.

| Exogenous substrate | $O_2$ concentration | Inhibitor | Rate of $O_2$ uptake | Rate of ATP production | ADP:O ratio |
|---|---|---|---|---|---|
| Glucose | 100 | None | 22 | 143 | 3.2 |
| Glucose | 0 | None | 0 | <20 | – |
| None | 100 | None | 15 | 93 | 3.0 |
| Glucose | 100 | KCN | 14 | 26 | 1.0 |
| Glucose | 100 | KCN+SHAM | 4 | <20 | – |
| Glucose | 100 | SHAM | 21 | 137 | 3.2 |

*Source*: Roberts et al. (1984a).
* The $O_2$ concentration was either that in air (100) or zero. Rates of ATP production and $O_2$ consumption are expressed as nmol $g^{-1}$ FM $s^{-1}$. Exogenous glucose was supplied at 50 mM. The concentration of KCN was 0.5 mM and that of SHAM was 2 mM; this is sufficiently high to fully block the alternative path in maize root tips.

fraction of ubiquinone (Q, the common substrate with the alternative path) that is in its reduced state ($Q_r/Q_t$). By contrast, the alternative path shows no appreciable activity until a substantial (30–40%) fraction of the Q is in its reduced state, and then the activity increases exponentially (Fig. 8). This would suggest that the alternative path functions as an "energy overflow"; however, recent experimental results suggest that this is an over-simplification, as outlined below.

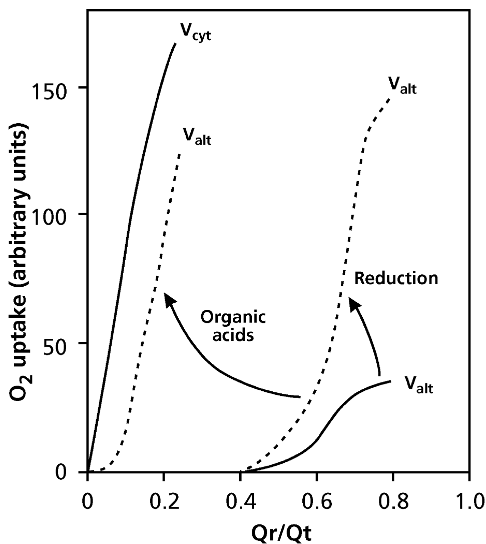


FIGURE 8. Dependence of the activity of the cytochrome path and of the alternative path on the fraction of ubiquinone that is in its reduced state ($Q_r/Q_t$). When the alternative oxidase is in its "reduced" (higher-activity) configuration, it has a greater capacity to accept electrons. In its reduced state, the alternative oxidase can be affected by α-keto acids, which enhance its activity at low levels of $Q_r$. [Based on Dry et al. (1989), Umbach et al. (1994), Day et al. (1995), and Hoefnagel et al. (1997)].

### *2.6.2 The Intricate Regulation of the Alternative Oxidase*

Depending on metabolic state, the activity of the alternative pathway changes, so that it competes with the cytochrome pathway for electrons. When embedded in the inner mitochondrial membrane, the alternative oxidase exists as a **dimer**, with the two subunits linked by **disulfide bridges**. These sulfide bridges may be oxidized or reduced. If they are reduced, then the alternative oxidase is in its **higher-activity state**, as opposed to the **lower-activity state** when the disulfide bridges are oxidized. Roots of soybean seedlings (*Glycine max*) initially have a very high respiration rate, and almost all of this respiration occurs *via* the cytochrome path (Fig. 9A). At this stage, the activity of the alternative path is very low and the enzyme is in its oxidized (lower-activity) state. Within a few days, the growth rate and the cytochrome oxidase activity decline about fourfold, and the contribution of the alternative path to root respiration increases to more than 50%. At that stage, all the dimers are in their reduced (higher-activity) state, suggesting that the transition from partly oxidized to fully reduced is responsible for the increased alternative oxidase activity (Millar et al. 1998). A similar change from oxidized to reduced occurs in leaves of *Alocasia odora* (Japanese taro) upon exposure to high-light stress, as discussed in Section 4.4. In intact roots of *Poa annua* (annual meadow-grass) and several other grasses, however, the alternative oxidase is invariably in its reduced, higher-activity configuration (Millenaar et al. 1998, 2000). There is, therefore, no clear evidence that changes in redox state of the alternative

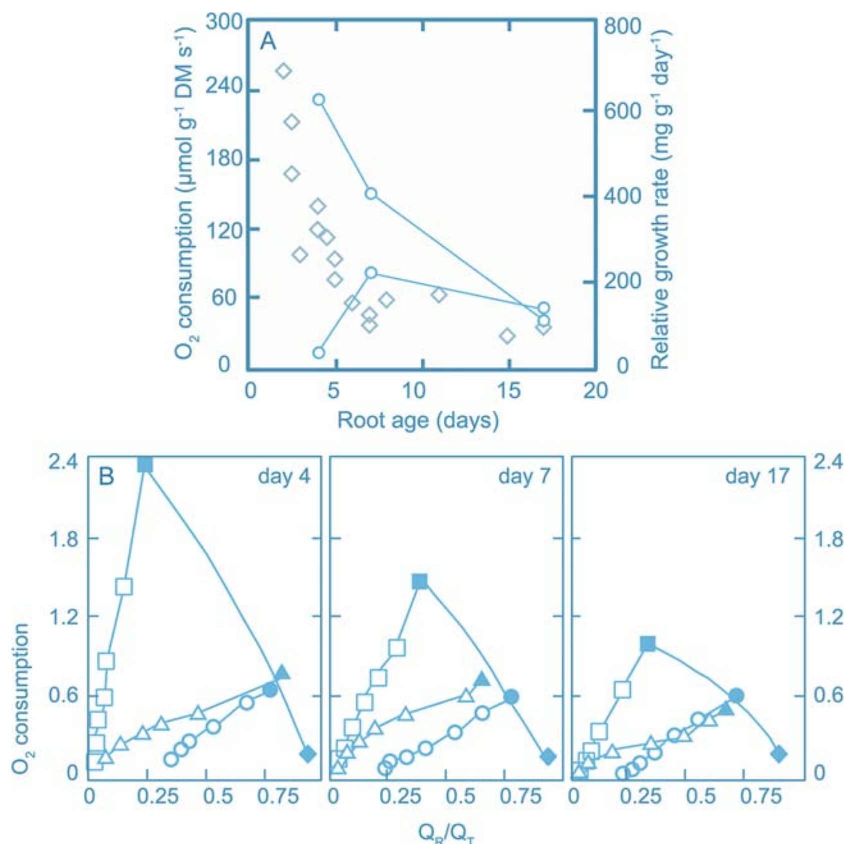


FIGURE 9. Root respiration, growth and the activity of isolated mitochondria for young *Glycine max* (soybean) seedlings. (*Top*) $O_2$ consumption via the cytochrome path (*circles*) and the alternative path (*squares*), and the relative growth rate (*diamonds*). (*Bottom*) Succinate-dependent $O_2$ consumption and Q-pool reduction state by isolated mitochondria (17-days old seedlings). Succinate was the respiratory substrate (10 mM); myxothiazol was used to inhibit the cytochrome path (2 µM); 1 mM pyruvate was added to activate the alternative oxidase. Modified after Millar et al. (1998). Copyright American Society of Plant Biologists.

oxidase play an important regulatory role in vivo during plant development (Hoefnagel & Wiskich 1998).

The alternative oxidase's capacity to oxidize its substrate ($Q_r$) also increases in the presence of **pyruvate** and other **α–keto acids** (Millar et al. 1996, Hoefnagel et al. 1997). As a result, in the presence of the potent activator pyruvate the alternative path shows significant activity even when less than 30% of ubiquinone is in its reduced state, when the cytochrome pathway is not fully saturated (Fig. 7). In intact tissues pyruvate levels appear to be sufficiently high to fully activate the alternative oxidase. That is, changes in the level of keto acids probably do not play a regulatory role in vivo (Hoefnagel & Wiskich 1998, Millenaar et al. 1998).

Whenever the alternative oxidase is in its higher activity state and active at low levels of $Q_r$, there will be competition for electrons between the two pathways, both in vitro (Hoefnagel et al. 1995, Ribas-Carbó et al. 1995) and in vivo (Atkin et al. 1995). **Competition** for electrons between the two pathways is the rule, rather than an exceptional situation, as was initially thought.

Does competition for electrons between the two pathways really occur at the levels of $Q_r$ that are commonly found in vivo (about 55% reduced; Millar et al. 1998)? In vitro studies with mitochondria isolated from tissues of which we know that the alternative path contributes to respiration can provide the answer (Fig. 8B). In the presence of succinate, but no ADP (state 4; Fig. 4), most of Q is reduced. Upon addition of ADP (state 3; Fig. 4), Q becomes more oxidized, until ADP is depleted. Activation of the alternative oxidase by pyruvate oxidizes Q to a level similar to that found in vivo.

Blocking the cytochrome path leads to Q being more reduced again. Since the alternative oxidase contributes substantially to root respiration at a $Q_r$ level of 55%, the activation mechanisms must operate. Because $Q_r$ levels in vivo are similar to those in state 4, Fig. 8B also suggests that mitochondrial electron transport in roots is probably restricted by ADP (Fig. 5).

#### *2.6.3 Mitochondrial NAD(P)H Dehydrogenases That Are Not Linked to Proton Extrusion*

In addition to the alternative oxidase (Sect. 2.3.2) and the uncoupling proteins (Sect. 2.3.3), there are **NAD(P)H dehydrogenases** that allow electron transport without proton extrusion (Møller 2001, Rasmusson et al. 2004; Fig. 3). Addition of $NO_3^-$ to N-limited seedlings of *Arabidopsis thaliana* (thale cress) decreases the transcript abundance of NAD(P)H dehydrogenase and **alternative oxidase** genes, while addition of $NH_4^+$ decreases the expression of the same gene families. Switching between $NO_3^-$ and $NH_4^+$ in the absence of N stress leads to very similar results. Corresponding changes in alternative respiratory pathway capacities are exhibited in seedlings supplied with either $NO_3^-$ or $NH_4^+$ as an N source and in mitochondria purified from the seedlings (Escobar et al. 2006). The parallel changes in both respiratory bypass pathways suggests that the NAD(P) dehydrogenases play a similar role as the alternative oxidase (Sect. 3).

## 3. The Ecophysiological Function of the Alternative Path

Why should plants produce and maintain a pathway that supports nonphosphorylating electron transport in mitochondria? Do they really differ fundamentally from animals in this respect, or do animals have functional alternatives? Perhaps it is merely a relict or an "error" in the biochemical machinery that has not yet been eliminated by natural selection. On the other hand, there may be situations where respiration in the absence of ATP production could serve important physiological functions. This Section discusses the merits of hypotheses put forward to explain the presence of the alternative path in higher plants. Testing of these hypotheses will require the use of transgenics lacking alternative path activity, some of which are now available.

### 3.1 Heat Production

An important consequence of the lack of coupling to ATP production in the alternative pathway is that the energy produced by oxidation is released as **heat**. More than 200 years have passed since Lamarck described heat production in *Arum italicum* (Italian arum) and more than 70 years since **thermogenesis** was linked to **cyanide-resistant respiration** (Laties 1998). Thermogenesis has been reported for species in the Annonaceae, Araceae, Arecaceae, Aristolochiaceae, Cycadaceae, Nymphaeaceae, Winteraceae, Illiciaceae, Magnoliaceae, Rafflesiaceae, and Nelumbonaceae (Seymour, 2001). This **heat production** is ecologically important in, e.g., *Aymplocarpus renifoliusm* (Asian skunk cabbage), which blooms in early spring when effective pollinators are inactive (Sect. 3.3.5 of Chapter 8 on life cycles). During the female flowering phase, the spadices produce heat 24 hours per day, until the beginning of the male phase. The spadices are visited by small numbers of invertebrate **pollinators** throughout the flowering season, attracted by the stench of **amines** that are volatilized by the elevated spadix temperature (Uemura et al. 1993). During heat production the respiration of the spadix is largely cyanide-resistant. If the alternative pathway is indeed responsible for a major fraction of the spadix respiration, then this would contribute to the heat production, as the lack of proton extrusion coupled to electron flow allows a large fraction of the energy in the substrate to be released as heat. This regulated thermogenic activity in inflorescences is functionally analogous, but differs in biochemical mechanism, to the uncoupled respiration that occurs in thermogenic tissues (brown fat) of some **mammals** under cold conditions.

Heat production also occurs in the flowers of several South American *Annona* species, *Victoria amazonica* (Amazon water lily) and *Nelumbo nucifera* (sacred lotus), clearly linked to activity of the alternative path (Fig. 10). These flowers regulate their temperature with remarkable precision (Seymour et al. 1998). When the air temperature varies between 10 and 30°C, the flowers remain between 30 and 35°C. The stable temperature is a consequence of increasing respiration rates in proportion to decreasing temperatures. Such a phenomenon of thermoregulation in plants is known for only a few species, e.g., *Philodendron selloum* (heart-leaf philodendron), *Symplocarpus foetidus* (skunk cabbage) (Knutson 1974, Seymour 2001). It has been suggested that the heat production in lotus is an energetic reward for **pollinating beetles**. These are trapped overnight, when they

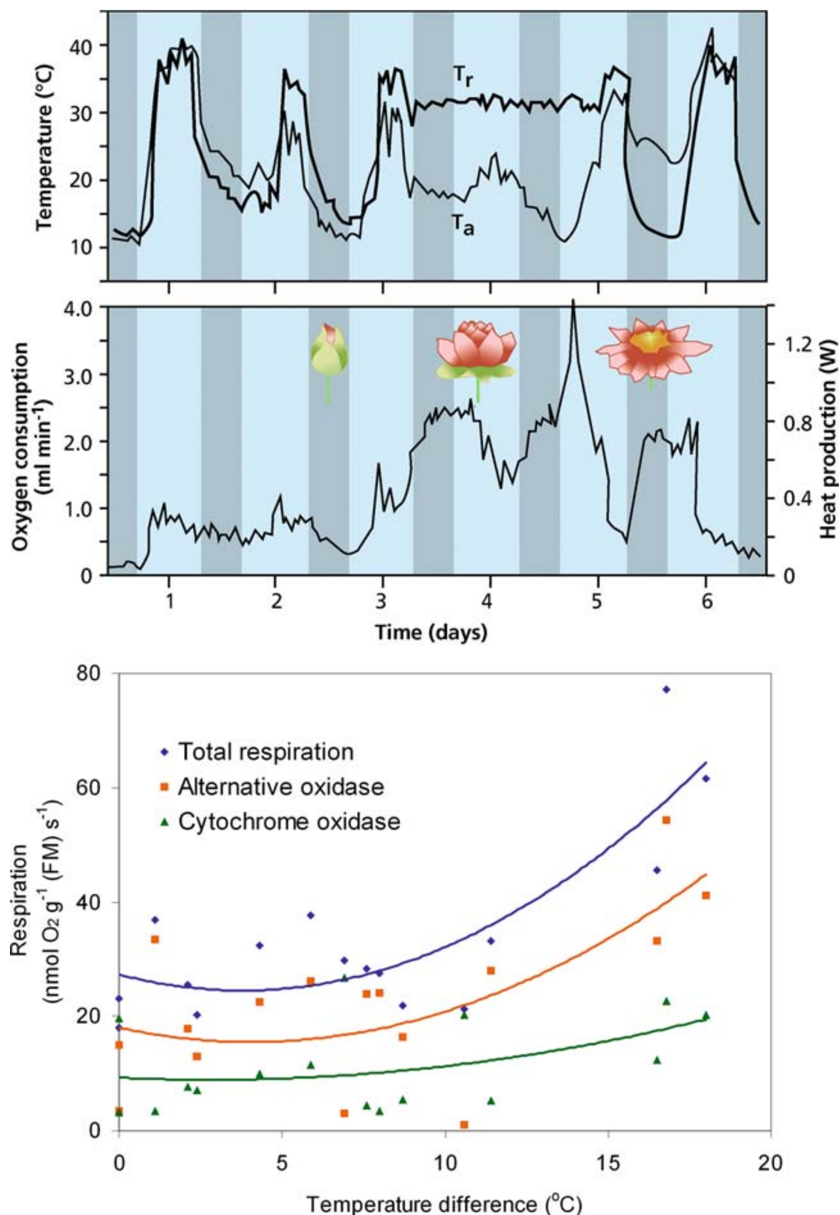


**FIGURE 10.** (*Top*) Temperature of the receptacle ($T_r$) and ambient air ($T_a$) and (*Middle*) rates of $O_2$ consumption throughout the thermogenic phase in *Nelumbo nucifera* (sacred lotus). $O_2$ consumption is converted to heat production assuming 21.1 J $ml^{-1}$ of $O_2$. Shaded areas indicate the night period (Seymour & Schultze-Motel 1996). Reprinted with permission from Nature copyright 1996 MacMillan Magazines Ltd. (*Bottom*) Total respiratory flux and fluxes through the alternative and cytochrome pathways, in lotus receptacle tissues as a function of the difference between receptacle temperature and temperature of an adjacent nonheating receptacle. Partitioning of electron transport between the two respiratory pathways was determined on the basis of $^{18}O$-isotope fractionation of intact tissues, as described in Box 2B.1 (modified after Watling et al. 2006). Copyright American Society of Plant Biologists.

feed and copulate, and then carry the pollen away (Seymour & Schultze-Motel 1996).

Can the alternative oxidase also play a significant role in increasing the temperature of leaves, for example during exposure to low temperature? There is indeed some evidence for increased heat production (7–22% increase) in low-temperature resistant plants (Moynihan et al. 1995). It can readily be calculated, however, using an approach outlined in Chapter 4A on the plant's energy balance, that such an increase in heat production *cannot* lead to a significant temperature rise in leaves (less that 0.1°C), and hence is unlikely to play a role in any cold-resistance mechanism. To explain the contribution of the alternative path in respiration of nonthermogenic organs other ecophysiological roles must be invoked.

## 3.2 Can We Really Measure the Activity of the Alternative Path?

Does the alternative path also play a role in the respiration of "ordinary" tissues, such as roots and leaves? The application of specific inhibitors of the

**Table 4. A comparison of the KCN resistance of respiration of intact tissues of a number of species and of $O_2$ uptake by mitochondria isolated from these tissues.***

| | | Cyanide-resistance (%) | |
|---|---|---|---|
| Species | Tissue | Whole tissue | Mitochondria |
| *Gossypium hirsutum* | Roots | 36 | 22 |
| *Phaseolus vulgaris* | Roots | 61 | 41 |
| *Spinacia oleracea* | Roots | 40 | 34 |
| *Triticum aestivum* | Roots | 38 | 35 |
| *Zea mays* | Roots | 47 | 32 |
| *Pisum sativum* | Leaves | 39 | 30 |
| *Spinacia oleracea* | Leaves | 40 | 27 |

*Source*: Lambers et al. (1983).

* The percentage KCN resistance of intact tissue respiration was calculated from the rate measured in the presence of 0.2 mM KCN and that measured in the presence of 0.1 μM FCCP, an uncoupler of the oxidative phosphorylation; this was done to obtain a rate of electron transfer through the cytochrome path closer to the state 3 rate (Fig. 4). KCN-resistance of isolated mitochondria was calculated from the rate in the presence and absence of 0.2 mM KCN. Mitochondrial substrates were 10 mM malate plus 10 mM succinate and a saturating amount of ADP. KCN-resistant $O_2$ uptake by isolated mitochondria was fully inhibited by inhibitors of the alternative path; in the presence of both KCN and SHAM approximately 10% of the control respiration proceeded in some of the tissues ("residual respiration").

alternative path suggests that the alternative path does contribute to the respiration of roots and leaves of at least some species (Tables 4 and 5). The decline in respiration, however, upon addition of an inhibitor of the alternative path tends to underestimate the actual activity of the alternative path. If the two pathways compete for electrons, then the inhibition is less than the activity of the alternative path (Table 5). Thus, any observed inhibition of respiration following the addition of an alternative pathway inhibitor indicates that some alternative pathway activity was present prior to inhibition, but provides no quantitative estimate of its activity (Day et al. 1996).

**Stable isotopes** can be used to estimate alternative path activity without the complications caused by use of inhibitors, because the alternative oxidase and cytochrome oxidase discriminate to a different extent against the heavy isotope of $O_2$ (Box 2B.1). The discrimination technique shows that the alternative pathway may account for over 40% of all respiration. The role of the alternative path in roots and leaves cannot be that of heat production. What might be its role in these tissues?

**Table 5. KCN-resistance, expressing the total respiratory electron flow through the alternative path under the conditions of measurement, and SHAM-inhibition of root respiration.***

| Species | KCN resistance | SHAM inhibition |
|---|---|---|
| *Carex diandra* | 66 | 29 |
| *Festuca ovina* | 53 | 1 |
| *Hordeum distichum* | 34 | 0 |
| *Pisum sativum* | 40 | 11 |
| *Phaseolus vulgaris* | 57 | 4 |
| *Plantago lanceolata* | 53 | 45 |
| *Poa alpina* | 41 | 1 |
| *Poa costiniana* | 61 | 0 |

*Source*: Atkin et al. (1995).

* Values are expressed in percentage of the control rate of respiration. KCN and SHAM (salicylhydroxamic acid) are specific inhibitors of the cytochrome path and the alternative path, respectively. Only if the cytochrome path is saturated, SHAM inhibition would equal the activity of the alternative path. Since the cytochrome path is rarely saturated, SHAM-inhibition is usually less than the activity of the alternative path; in fact its activity may be as high as the KCN-resistant component of root respiration. Because the two pathways generally compete for electrons, inhibitors cannot provide information on the actual activity of the two pathways in root respiration.

## 3.3 The Alternative Path as an Energy Overflow

The activity of the alternative path might increase when the production of organic acids is not matched by their oxidation, so that they accumulate. This observation led to the "energy overflow hypothesis" (Lambers 1982). It states that respiration via the alternative path only proceeds in the presence of high concentrations of respiratory substrate. It considers the alternative path as a **coarse control** of carbohydrate metabolism, but not as an alternative to the finer control by adenylates (Sects. 2.1 and 2.2).

The continuous employment of the alternative oxidase under normal "nonstress" conditions may ensure a rate of carbon delivery to the root that enables the plant to cope with "stress". According to the energy overflow hypothesis, if the carbon demand of a tissue suddenly increases, there is sufficient carbon transport to the tissue to meet these demands, if respiration were to switch entirely to supporting ATP synthesis. For example, a decrease in soil water potential increases the roots' carbon demand for synthesis of compatible solutes for osmotic adjustment. Similarly, attack by parasites

## Box 2B.1
## Measuring Oxygen-Isotope Fractionation in Respiration

Plants have a cyanide-insensitive respiratory pathway in addition to the cytochrome pathway (Sect. 2.3). Unlike the cytochrome pathway, the transport of electrons from ubiquinol to $O_2$ through the alternative path is not linked to proton extrusion, and therefore not coupled to energy conservation. The alternative oxidase and cytochrome oxidase discriminate to a different extent against the heavy isotope of oxygen ($^{18}O$) when reducing $O_2$ to produce water (Guy et al. 1989). This allows calculation of the partitioning of electron flow between the two pathways in the absence of added inhibitors, also in intact tissues. For many years, studies of electron partitioning between the two respiratory pathways were performed using specific inhibitors of the two pathways [e.g., cyanide for the cytochrome path, and SHAM (salicylhydroxamic acid) for the alternative path]. It was thought that electrons were only available to the alternative pathway when the cytochrome pathway was either saturated or inhibited; however, we now know that both pathways compete for electrons (Sect. 2.6.1). The only reliable technique to study electron partitioning between the cytochrome and alternative pathway is by using oxygen-isotope fractionation (Day et al. 1996). Although the methodology employed has changed dramatically in the last decade, the theoretical basis of the oxygen-isotope fractionation technique remains that described by Guy et al. (1989).

The origin of the oxygen-fractionation methodology can be found in Bigeleisen & Wolfsberg (1959) and Mariotti et al. (1981). Oxygen-isotope fractionation is measured by examining the isotope fractionation of the substrate $O_2$ as it is consumed in a closed, leak-tight cuvette. The energy needed to break the oxygen-oxygen bond of a molecule containing $^{18}O$ is greater than that to break the molecule $^{16}O{=}O^{16}$. Therefore, both terminal oxidases of the plant mitochondrial electron-transport chain react preferentially with $^{32}O_2$, but they produce different isotope effects (Hoefs 1987). This allows determining the relative flux through each terminal oxidase. If $\alpha$ is the ratio of the rate of the reaction with $^{18}O$ to that with $^{16}O$, then:

$$R_p = R\alpha \tag{1}$$

where $R_p$ is the $^{18}O/^{16}O$ ratio of the product ($H_2O$), and $R$ is that of the substrate ($O_2$). Since $\alpha$ generally differs from unity by only a few percent, fractionation is usually given by $D$, where

$$D = (1 - \alpha) \times 1000 \tag{2}$$

and the units of $D$ are parts per mil (‰). $D$ is generally obtained directly from Equation (1) by measurements of the isotope ratio of the substrate and product, but since the product of both mitochondrial oxidases is $H_2O$, which is either the solvent for these reactions (liquid phase) or very difficult to obtain (gas phase), this is not feasible in this case. Instead, changes in the isotope ratio of the $O_2$ in the substrate pool are measured (Fig. 1). If there is any isotopic fractionation during respiration, the oxygen-isotope ratio ($R$) of the remaining $O_2$ increases as the reaction proceeds. The respiratory isotope fractionation can be obtained by measuring $R$, and the fraction of molecular $O_2$ remaining at different times during the course of the reaction.

Therefore, if we define the following terms:

$R_o$ = initial $^{18}O/^{16}O$
$R$ = $^{18}O/^{16}O$ at time t
$f$ = fraction of remaining oxygen at time $t : f = [o_2]/[o_2]_0$

then the change in $R$ through time is:

$$\delta R/\delta t = \frac{[^{16}o(\delta^{18}o/\delta t - ^{18}o(\delta^{16}o/\delta t]}{(^{16}o)^2} \tag{3}$$

Since

$$\delta^{18}o/\delta t = R\alpha(\delta^{16}o/\delta t) \tag{4}$$

we obtain:

$$\delta R/R = \delta^{16}o/^{16}o(1 - \alpha) \tag{5}$$

which, upon integration, yields

$$\ln R/R_0 = \ln^{16} o/^{16}o_0(1 - \alpha) \tag{6}$$

Since only 0.4% of the $O_2$ contains $^{18}O$, the ratio $^{16}O/^{16}O_o$ is a good approximation of $[O_2]/[O_{2o}]$ ($f$), and hence we may write

*continued*

**Box 2B.1** *Continued*

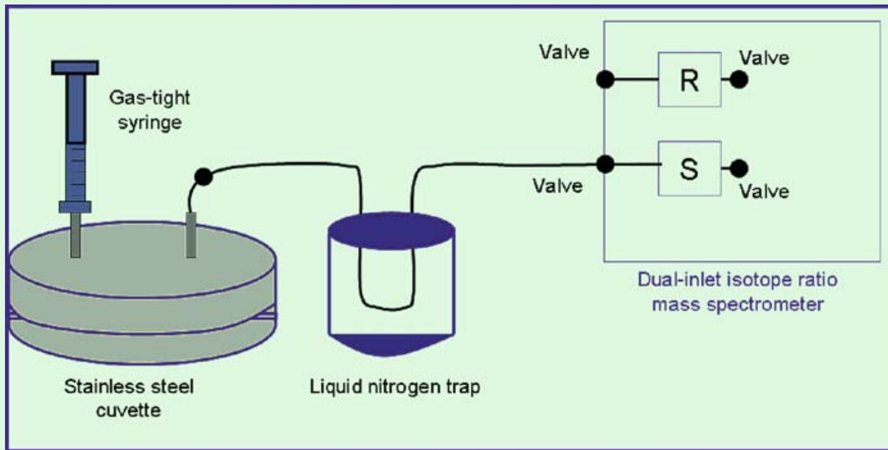


FIGURE 1. Diagram of an on-line oxygen-isotope fractionation system, with a gas-tight syringe, a stainless steel cuvette, a liquid nitrogen trap to remove $CO_2$ and $H_2O$, a reference bellow, and a sample bellow (Ribas-Carbó et al. 2005b).

$$D = \ln(R/R_0)/-\ln f \quad (7)$$

and $D$ can be determined by the slope of the linear regression of a plot of ln $R/R_o$ vs. –ln $f$, without forcing this line through the origin (Henry et al. 1999). The standard error (SE) of the slope is determined as

$$\mathrm{SE} = \frac{D(1-r^2)^{1/2}}{r(n-2)^{1/2}} \quad (8)$$

and indicates the precision of the measurement of isotopic fractionation ($D$). This error should be less than 0.4%, because the fractionation differential between the cytochrome pathway (18–20%) and the alternative pathway (24–31%) is between 6% and 12%, for roots and green tissues, respectively (Robinson et al. 1995). In most cases, accurate determinations of $D$ can be achieved with experiments comprising six measurements, providing the $r^2$ of the linear regression is 0.995 or higher (Ribas-Carbó et al. 1995, Henry et al. 1999). Because it is common practice in the plant literature to express isotope fractionation in "Δ" notation, the fractionation factors, $D$, are converted to Δ:

$$\Delta = \frac{D}{1-(D/1000)} \quad (9)$$

The partitioning between the cytochrome and the alternative respiratory pathways ($\tau_a$) is (Ribas-Carbó et al. (1997):

$$\tau_a = \frac{\Delta n - \Delta c}{\Delta a - \Delta c}$$

where $\Delta n$ is the oxygen-isotope fractionation measured in the absence of inhibitors, and $\Delta c$ and $\Delta a$ are the fractionation by the cytochrome and alternative pathway, respectively. These "end points" for purely cytochrome or alternative pathway respiration are established for each experimental system using inhibitors of the alternative oxidase and cytochrome oxidase, respectively. The cytochrome oxidase consistently gives a $\Delta c$ between 18% and 20%, while $\Delta a$ is more variable, with values ranging from 24 to 25% in roots and nongreen tissues, and 30–32% in cotyledons and green leaves (Ribas-Carbó et al. 2005b).

and pathogens may suddenly increase carbon demands for tissue repair and the mobilization of plant defenses. The alternative oxidase activity may also prevent the production of superoxide and/or hydrogen peroxide under conditions where electron transport through the cytochrome path is impaired (e.g., due to low temperature or desiccation injury). This is partly due to a reaction of ubisemiquinone

with molecular $O_2$ (Purvis & Shewfelt 1993, Møller 2001). **Superoxide**, like other **reactive oxygen species** (**ROS**), can cause severe metabolic disturbances. So far, the various interpretations of the physiological function of an "energy overflow" remain speculative.

## 3.4 NADH Oxidation in the Presence of a High Energy Charge

If cells require a large amount of carbon skeletons (e.g., oxoglutarate or succinate) but do not have a high demand for ATP, then the operation of the alternative path could prove useful in oxidizing the NADH that would otherwise accumulate; considering the pool size of NADH, this would then stop respiration within minutes. However, can we envisage such a situation in vivo? Whenever the rate of carbon skeleton production is high, there tends to be a great need for ATP to further metabolize and incorporate these skeletons. When plants are infected by pathogenic microorganisms, however, they tend to produce **phytoalexins** (Sect. 3 of Chapter 9C on effects of microbial pathogens). This generates substantial amounts of NAD(P)H without major ATP requirements, and hence might require engagement of the alternative path (Sect. 4.8).

There are also other circumstances where the production of carbon skeletons does not entail a need for ATP. **Cluster roots** of *Hakea prostrata* (harsh hakea) accumulate large amounts of carboxylates (e.g., citrate), which they subsequently release to mobilize sparingly available P in the rhizosphere (Sect. 2.2.5 of Chapter 6 on mineral nutrition). During the phase of rapid carboxylate synthesis, the alternative path is up-regulated, presumably allowing re-oxidation of NADH that is produced during citrate synthesis (Shane et al. 2004).

There may also be a need for a nonphosphorylating path to allow rapid oxidation of malate in plants exhibiting crassulacean acid metabolism (**CAM plants**) during the day (Sect. 10.2 of Chapter 2A on photosynthesis). Unfortunately, there are no techniques available to assess alternative path activity in the light. If measurements are made in the dark, however, during the normal light period, then malate decarboxylation in CAM plants is indeed associated with increased engagement of the alternative path (Table 6). Malate decarboxylation, however, naturally occurs in the light (Sect. 10.2 of Chapter 2A on photosynthesis). It therefore remains to be confirmed that the alternative path plays a vital role in CAM.

**TABLE 6. Respiration, oxygen-isotope discrimination and partitioning of electrons to the cytochrome and the alternative pathway in leaves of *Kalanchoe daigremontiana*.**

| Parameter | Acidification | De-acidification |
|---|---|---|
| Respiration $\mu mol\ O_2\ m^{-2}\ s^{-1}$ | 1.8 | 2.6 |
| Discrimination o/oo | 22.4 | 25.0 |
| Cytochrome path $\mu mol\ O_2\ m^{-2}\ s^{-1}$ | 1.3 | 1.4 |
| Alternative path $\mu mol\ O_2\ m^{-2}\ s^{-1}$ | 0.5 | 1.2 |

*Source*: Robinson et al. 1992.
Note: Measurements were made in the dark, during the normal dark period (acidification phase) and the normal light period (de-acidification phase, when rapid decarboxylation occurs).

## 3.5 NADH Oxidation to Oxidize Excess Redox Equivalents from the Chloroplast

In illuminated leaves, mitochondria are thought to play a role in optimizing photosynthesis. Inhibition of either the cytochrome or the alternative path, using specific inhibitors (Sect. 2.3.3), reduces photosynthetic $O_2$ evolution and the redox state of the photosynthetic electron transport chain in *Vicia faba* (broad bean) leaves under various light intensities. Under saturating photosynthetic photon flux density, inhibition of either pathway causes a decrease in the steady-state levels of the photosynthetic $O_2$ evolution rate and the PSII quantum yield. Obviously, both two respiratory pathways are essential for maintenance of high photosynthetic rates at saturating light. At low light intensity, however, only inhibition of the alternative path lowers the photosynthetic rate. This suggests that inhibition of the alternative path causes over-reduction of the photosynthetic electron transport chain, even at low light levels.

It has been suggested that an important function of the alternative oxidase is to prevent chloroplast over-reduction through efficient dissipation of excess reducing equivalents (Noguchi et al. 2005). This hypothesis was tested using *Arabidopsis thaliana* (thale cress) mutants defective in cyclic electron flow around PSI, in which the reducing equivalents accumulate in the chloroplast stroma due to an unbalanced ATP/NADPH production ratio. These mutants show enhanced activities of the enzymes needed to export the reducing equivalents from the

chloroplasts. Interestingly, the amounts of alternative oxidase protein and cyanide-resistant respiration in the mutants are also higher than those in the wild type. After high-light treatment, the alternative oxidase, even in the wild type, is up-regulated concomitant with the accumulation of reducing equivalents in the chloroplasts and an increase in the activities of enzymes needed to export reducing equivalents. These results indicate that the alternative oxidase can dissipate excess reducing equivalents that are exported from the chloroplasts, and that it plays a role in photosynthesis (Yoshida et al. 2007).

## 3.6 Continuation of Respiration When the Activity of the Cytochrome Path Is Restricted

Naturally occurring **inhibitors** of the cytochrome path (e.g., cyanide, sulfide, carbon dioxide, and nitric oxide) may reach such high concentrations in the tissue that respiration via the cytochrome path is partially or fully inhibited (Palet et al. 1991, Millar & Day 1997). Similarly, mutants of that lack **complex I** (Karpova et al. 2002) and hence must use the non-phosphorylating bypass, produce less ATP than the wild type, if respiring at the same rate. Under these circumstances the alternative pathway may be important in providing energy, even though it yields only a third as much ATP as the cytochrome path. This has indeed been shown to be the case for a *Nicotiana sylvestris* (flowering tobacco) mutant that lacks complex I, using the oxygen-isotope fractionation technique (Box 2B.1; Vidal et al. 2007).

Dry seeds, including those of *Cucumis sativus* (cucumber), *Hordeum vulgare* (barley), *Oryza sativa* (rice), and *Xanthium pennsylvanicum* (cocklebur) contain **cyanogenic** compounds, such as cyanohydrin, cyanogenic glycosides, and cyanogenic lipids. Such compounds liberate free HCN after hydrolysis during imbibition. Upon imbibition and triggered by ethylene, seeds containing these cyanogenic compounds produce a mitochondrial β-cyano-alanine synthase that detoxifies HCN (Hagesawa et al. 1995). Despite this detoxifying mechanism, some HCN is likely to be present in the mitochondria of germinating seeds, and hence there is a need for a cyanide-resistant path.

Some plants produce **sulfide** (e.g., species belonging to the Cucurbitaceae) (Rennenberg & Filner 1983). Sulfide is also produced by anaerobic sulfate-reducing microorganisms. It may occur in high concentrations in the phyllosphere of aquatic plants or the rhizosphere of flooded plants. In such flooded soils, **carbon dioxide** levels also increase. Since both sulfide and high concentration of carbon dioxide inhibit the cytochrome path (Palet et al. 1991), there may be a need for the alternative path under these conditions also.

When the activity of the cytochrome path is restricted by **low temperature**, the alternative path might also increase in activity to provide energy needed for metabolism. In fact, sustained exposure to low temperature enhances the amount of alternative oxidase in mitochondria of *Zea mays* (corn) (Stewart et al. 1990) and *Nicotiana tabacum* (tobacco) (Vanlerberghe & McIntosh 1992). Such an induction also occurs when the activity of the cytochrome path is restricted in other ways [e.g., by application of inhibitors of mitochondrial protein synthesis (Day et al. 1995), or of inhibitors of the cytochrome path (Wagner et al. 1992)]. Interestingly, only those inhibitors of the cytochrome path that enhance superoxide production lead to induction of the alternative oxidase, suggesting that the prevention of damage by reactive oxygen species is a particularly important role of the alternative path. Moreover, superoxide itself can also induce expression of the alternative oxidase. This has led to the suggestion that **reactive oxygen species**, including $H_2O_2$, are part of the signal(s) communicating cytochrome path restriction in the mitochondria to the nucleus, thus inducing alternative oxidase synthesis (Rhoads et al. 2006). The key question is, of course, if enhanced *expression* of the alternative oxidase leads to greater *activity* of the alternative path. In *Vigna radiata* (mung bean) this appears to be the case, but such a response is not found in *Glycine max* (soybean) (Gonzàlez-Meler et al. 1999).

In the absence of an alternative oxidase, inhibition or restriction of the activity of the cytochrome path would inexorably lead to the accumulation of fermentation products, as found in transgenic plants lacking the alternative oxidase (Vanlerberghe et al. 1995). In addition, it might cause the ubiquinone pool to become highly reduced which might lead to the formation of reactive oxygen species and concomitant damage to the cell (Purvis & Shewfelt 1993, Møller 2001). Further work with transgenics lacking the alternative path is an essential avenue of future research on the ecophysiological role of the alternative path in plant functioning.

## 3.7 A Summary of the Various Ecophysiological Roles of the Alternative Oxidase

The alternative oxidase is widespread and can serve a wide variety of physiological functions, ranging from providing ATP when the cytochrome pathway is

restricted (which can occur under a wide variety of circumstances) to acting as an overflow to balancing the physiological rates of a range of processes (e.g., organic acid synthesis and ATP production) to prevent metabolism from getting severely unbalanced. In higher plants, the alternative pathway is just as much entrained in all aspects of metabolism as is the cytochrome path. In addition to the alternative path, plants have a bypass of complex I and uncoupling proteins. The reason why most animals do not have an alternative respiratory path is probably that they entirely depend on uncoupling proteins. In addition, animals may have less need to balance different metabolic functions.

# 4. Environmental Effects on Respiratory Processes

## 4.1 Flooded, Hypoxic, and Anoxic Soils

Plants growing in flooded soil are exposed to **hypoxic** (low-$O_2$) or **anoxic** (no-$O_2$) conditions in the root environment, and experience a number of conditions, including an insufficient supply of $O_2$ and accumulation of $CO_2$ (Sect. 4.7), and changes in plant water relations (Sect. 3 of Chapter 3 on plant water relations).

### *4.1.1 Inhibition of Aerobic Root Respiration*

The most immediate effect of soil **flooding** on plants is a decline in the $O_2$ concentration in the soil. In water-saturated soils the air that is normally present in the soil pores is almost completely replaced by water. The **diffusion** of gases in water is approximately 10000 times slower than in air. In addition, the **concentration** of $O_2$ in water is much less than that in air (at 25°C approximately 0.25 mmol $O_2$ dissolves per liter of water, whereas air contains approximately 10 mmol). The $O_2$ supply from the soil, therefore, decreases to the extent that aerobic root respiration, and hence ATP production, is restricted. Under these conditions the synthesis of RNA and proteins is strongly suppressed, but that of specific m-RNAs and **anaerobic polypeptides** is induced. Among these "anaerobic polypeptides" is the fermentative enzyme **alcohol dehydrogenase** (Andrews et al. 1993).

### *4.1.2 Fermentation*

When insufficient $O_2$ reaches the site of respiration, such as in seeds germinating under water and submerged rhizomes, ATP may be produced through **fermentative processes**. These tissues generate energy in **glycolysis**, producing ethanol, and sometimes lactate. **Lactat**e tends to be the product of fermentation immediately after the cells are deprived of $O_2$. Lactate accumulation decreases the pH in the cytosol (Sect. 4.1.3), which inhibits lactate dehydrogenase and activates the first enzyme of ethanol fermentation: pyruvate decarboxylase. When lactate accumulation does not stop, **cytosolic acidosis** may lead to cell death (Rivoal & Hanson 1994).

It was initially believed that root metabolism cannot continue in flooded conditions, due to the production of toxic levels of **ethanol**. Ethanol, however, does not really inhibit plant growth until concentrations are reached that far exceed those found in flooded plants (Table 7), and hence ethanol plays only a minor role in flooding injury to roots and shoots (Jackson et al. 1982). As long as there is no accumulation of **acetaldehyde**, which is the product of pyruvate decarboxylase and the substrate for alcohol dehydrogenase, which reduces acetaldehyde to ethanol, alcoholic fermentation is unlikely to cause plant injuries. If **acetaldehyde** does accumulate, however, for example upon reaeration, then this may cause injury, because acetaldehyde is a potent toxin, giving rise to the formation of **reactive oxygen species** (Blokhina et al. 2003). It is the low potential for **ATP production** and its metabolic consequences, rather than the

**Table 7. The effect of supplying ethanol in aerobic and anaerobic nutrient solutions to the roots of *Pisum sativum* (garden pea) at a concentration close to that found in flooded soil (i.e., 3.9 mM) or greater than that.**

| | Aerobic control | Aerobic + ethanol | Anaerobic control | Anaerobic + ethanol |
|---|---|---|---|---|
| Ethanol in xylem sap (mM) | 37 | 540 | 90 | 970 |
| Stem extension (mm) | 118 | 108 | 94 | 74 |
| Final fresh mass (g) | | | | |
| shoot | 11.9 | 11.9 | 10.7 | 11.4 |
| roots | 7.8 | 9.7 | 5.7 | 6.1 |

***Source*: Jackson et al. (1982).**

toxicity of the products of fermentative metabolism that constrain the functioning of plants under anoxia (Sect. 4.1.3).

Continued fermentation requires the mobilization of a large amount of reserves, such as starch. Seeds of most species fail to germinate under anoxia, but those of *Oryza sativa* (rice) are an exception (Perata & Alpi 1993). In contrast to cereals like *Triticum aestivum* (wheat) and *Hordeum vulgare* (barley), rice seeds produce $\alpha$-amylase and sucrose-metabolizing enzymes under anoxia; these enzymes allows the degradation and further metabolism of starch, and therefore sustain a rapid fermentative metabolism (Perata et al. 1996).

The **energetic efficiency** of ethanol formation is low, producing only two molecules of ATP in glycolysis ("substrate phosphorylation") per molecule of glucose. This is considerably less than that of aerobic respiration, which produces around 36 molecules of ATP per molecule of glucose, if the most efficient mitochondrial electron-transport pathways are used and not taking into account the costs for transport of metabolites across the inner mitochondrial membrane (Sects. 2.2 and 2.3). Moreover, a large fraction of the **lactate** may be secreted into the rhizosphere [e.g., in some *Limonium* (statice) species]. Although such secretion prevents acidification of the cytosol, it also represents a substantial carbon loss to the plant (Rivoal & Hanson 1993).

### *4.1.3 Cytosolic Acidosis*

A secondary effect of the decline in root respiration and ATP production in the absence of $O_2$ is a decrease in the pH of the cytosol (**cytosolic acidosis**), due in part to accumulation of organic acids in fermentation and the TCA cycle. Moreover, in the absence of $O_2$ as a terminal electron acceptor, ATP production decreases, so there is less energy available to maintain ion gradients within the cell. Acidification of the cytosol reduces the activity of many cytosolic enzymes, whose pH optimum is around 7 and hence severely disturbs the cell's metabolism, so that protons leak from the vacuole to the cytosol. Cytosolic acidosis also reduces the activity of aquaporins (Sect. 5.2 of Chapter 3 on plant water relations). The extent of this cytosolic acidification is less in the presence of $NO_3^-$ (Fig. 11). $NO_3^-$ reduction leads to the formation of hydroxyl ions (Sect. 2.2.6.1 of Chapter 6 on mineral nutrition), which partly neutralize the protons and prevent severe acidosis. Moreover, $NO_3^-$ reduction requires the oxidation of NADH, producing NAD. This allows the continued

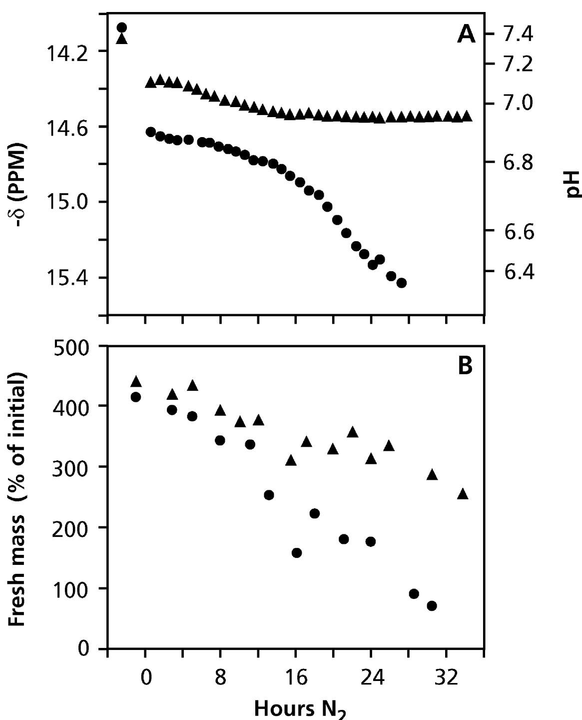


**FIGURE 11. The effect of hypoxia on root tips of *Zea mays* (maize), in the presence (*triangles*) and absence (*circles*) of nitrate. (A) The effect on the pH of the cytosol, as measured in experiments using $^{31}$P-NMR spectroscopy; (B) the increase in fresh mass during 48 hours in air, after the indicated period of hypoxia. The location of the inorganic phosphate ($P_i$) peaks in an NMR spectrum depends on the "environment" of the molecule (e.g., pH) (Fig. 6 in this chapter). NMR spectroscopy can therefore be used to determine the peak wavelength at which $P_i$ absorbs the magnetic radiation and hence the pH in the cytosol as well as in the vacuole (after Roberts et al. 1985). Copyright American Society of Plant Biologists.**

oxidation of organic acids in the TCA cycle, thus preventing their accumulation and associated drop in pH.

### *4.1.4 Avoiding Hypoxia: Aerenchyma Formation*

In wetland plants, including crop species such as *Oryza sativa* (rice), mechanisms have evolved to prevent the problems associated with flooded soils. The most important adaptation to flooded soils is the development of a functional **aerenchyma**, a continuous system of air spaces in the plant that allows diffusion of $O_2$ from the shoot or the air to the roots (Jackson & Armstrong 1999). Aerenchyma avoids inhibition of respiration due to lack of $O_2$ which is inevitable for plants that are not adapted to wet soils (Colmer 2003b). In many species, other special structures allow the diffusion of $O_2$ from the air into the plant: the pneumatophores of mangroves, lenticels in the bark of many wetland trees, and, possibly, the knee roots of *Taxodium distichum* (bald cypress). The mechanisms that maintain the intercellular spaces filled with gas rather than water are not fully understood. Inward radial gradients in water potential created by transpiration in combination with water-impermeable apoplastic barriers such as the exodermis may offer an explanation (Jackson & Armstrong 1999).

Because there is a gradient in partial pressure within the aerenchyma, $O_2$ will move by **diffusion** to the roots. In aquatic plants, however, like *Nuphar lutea* (yellow water lily) and *Nelumbo nucifera* (sacred lotus) there is also a **pressurized flow-through** system, which forces $O_2$ from young emergent leaves to the roots and rhizomes buried in the anaerobic sediment (Dacey 1980, 1987). Such a mass flow requires a difference in atmospheric pressure between leaves and roots. The diurnal pattern of the mass flow of air to the roots suggests that the energy to generate the pressure comes from the sun; however, it is not the photosynthetically active component of radiation, but the long-wave region (heat), which increases the atmospheric pressure inside young leaves by as much as 300 Pa. How can these young leaves draw in air against a pressure gradient? To understand this we have to realize that the atmosphere inside the leaf is saturated with water vapor and that movement of gases occurs by **diffusion**, along a gradient in partial pressure, and by **mass flow**, depending on the **porosity** of the pathway. The porosity of the young emergent leaves is such that gas flux by diffusion (i.e., down a concentration gradient) is more important than a mass flux due to a difference in atmospheric pressure. The concentration gradient is due to the evaporation from the cells inside the leaf, which dilutes the other gases in the intercellular spaces, thus creating a gradient allowing diffusion between the atmosphere and the intercellular spaces. The slightly higher atmospheric pressure inside young leaves forces air, which has been enriched in $O_2$ by photosynthesis, to move along a pressure gradient from young leaves to roots and rhizomes. Some of the air from roots and rhizomes, which is enriched with $CO_2$ from respiration, is then forced to older leaves. Isotope studies show that much of this $CO_2$ is subsequently assimilated in photosynthesis. The reason that only young leaves show this internal ventilation is the higher porosity of the older leaves which does not allow them to draw in more air through diffusion than is lost via mass flow. The quantity of air flow through a single petiole is enormous: as much as 22 liters per day, with peak values as high as 60 ml per minute and rates of 50 cm per minute. The transport of $O_2$ from the shoot by convective gas flow is also likely to contribute to the flow of $O_2$ to roots of other species growing in an anaerobic soil (Armstrong et al. 1997). Pressurized flow of $O_2$ plays a role in the $O_2$ supply to the roots and rhizosphere of many **emergent macrophytes**. The vital element is that a compartment exists surrounded by walls with sufficiently small pores to allow diffusion to occur at greater rates than mass flow (Colmer 2003a,b).

Aerenchymatous plants often transport more $O_2$ to the roots than is consumed by root respiration. The **outward diffusion of $O_2$** into the rhizosphere implies a loss of $O_2$ for root respiration. Plants adapted to flooded conditions, e.g., *Oryza sativa* (rice), *Phragmitis australis* (common reed) and *Glyceria maxima* (reed mannagrass) develop a **flooding-induced $O_2$ barrier** in basal root zones, thus reducing radial $O_2$ loss (Colmer 2003a, Soukup et al. 2007). On the other hand, outward diffusion of $O_2$ also allows the oxidation of potentially harmful compounds (Colmer 2003b). This can readily be seen when excavating a plant from a reduced substrate. The bulk substrate itself is black, due to the presence of FeS, but the soil in the immediate vicinity of the roots of such a plant will be brown or red, indicating the presence of oxidized iron ($Fe^{3+}$, "rust"), which is less soluble than the reduced $Fe^{2+}$.

Aerenchyma and induction of a barrier reducing radial $O_2$ loss are not unmitigated benefits to plants. Aerenchymatous roots characteristically have a large diameter, and therefore a small surface area per unit biomass. Because plant nutrient

uptake is strongly affected by root diameter and surface area, a likely cost associated with aerenchyma is a reduced rate of nutrient uptake per unit root biomass. The basal $O_2$ barrier, which involves both quantitative and qualitative differences in **suberin** composition and distribution within exodermal cell walls (Soukup et al. 2007) probably also decreases the roots' capacity for nutrient and water uptake.

Aerenchyma also serves as a conduit of soil gases to the atmosphere, including methane, ethylene, and carbon dioxide. **Methane** ($CH_4$) is a bacterial product commonly produced in anaerobic soils. In rice paddies and natural wetlands most $CH_4$ is transported to the atmosphere through plant aerenchyma. Experimental removal of sedges from wetland substantially reduces $CH_4$ flux and causes $CH_4$ to accumulate in soils (Fig. 12). $CH_4$ production and transport to the atmosphere is a topic of current concern, because $CH_4$ is a "**greenhouse gas**" that absorbs infrared radiation 20 times more effectively than does $CO_2$. Recent increases in atmospheric $CH_4$ have contributed approximately 20% of the warming potential of the atmosphere that has caused recent global warming (Ramaswamy et al. 2001). The expansion of rice agriculture and associated $CH_4$ transport via aerenchyma from the soil to the atmosphere is an important contributor to atmospheric $CH_4$. There is no firm evidence that plants themselves generate significant amounts of $CH_4$, and suggestions in the literature that planting trees might contribute to a major extent to global warming due to their aerobic production of $CH_4$ have not been substantiated (Dueck et al. 2007, Kirschbaum et al. 2007).

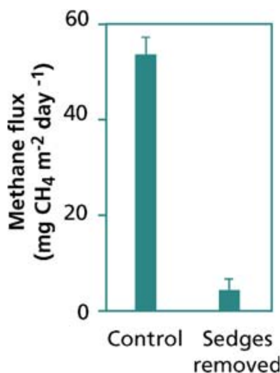


FIGURE 12. Methane flux and soil methane concentration in a tundra wetland in which sedges are present (control) or have been experimentally removed (data from Torn & Chapin 1993).

## 4.2 Salinity and Water Stress

Sudden exposure of sensitive plants to salinity or water stress often enhances their respiration. For example, the root respiration of *Hordeum vulgare* (barley) increases upon exposure to 10 mM NaCl (Bloom & Epstein 1984). This may either reflect an increased **demand for respiratory energy** or an increased activity of the alternative path, when carbon use for growth is decreased more than carbon gain in photosynthesis (Sect. 5.3 of Chapter 7 on growth and allocation). Long-term exposure of sensitive plants to salinity or desiccation gradually decreases respiration as part of the general decline in carbon assimilation and overall metabolism associated with slow growth under these conditions (Galmés et al. 2007; Sect. 5.3 of Chapter 7 on growth and allocation). Generally, specific rates of leaf respiration at 25°C are highest in plants growing in hot, dry habitats, reflecting acclimation and/or adaptation to such habitats (Wright et al. 2006). Additional declines in root respiration of *Triticum aestivum* (wheat) plants upon exposure to dry soil may reflect a specific decline in the alternative path. The decline correlates with the accumulation of **osmotic solutes**, reducing the availability of sugars and hence providing less "grist for the mill" of the alternative path.

Leaves also show a decline in respiration, as leaf water potential declines. The decline is most likely associated with a decrease in the energy requirement for growth or the export of photoassimilates. In *Glycine max* (soybean) net photosynthesis decreases by 40% under mild and by 70% under severe water stress, whereas the total respiratory $O_2$ uptake is not significantly different at any water-stress level. However, severe water stress causes a significant shift of electrons from the cytochrome to the alternative pathway. The electron partitioning through the alternative pathway increases from about 11% under well watered or mild water-stress conditions to near 40% under severe water stress (Fig. 13). Consequently, the calculated rate of mitochondrial ATP synthesis decreases by 32% under severe water stress (Ribas-Carbó et al. 2005a).

Species differ in their respiratory response to water stress, primarily due to differences in sensitivity of growth to desiccation. When salt-adapted plants are exposed to mild salinity stress, they accumulate **compatible solutes**, such as sorbitol (Sect. 3 of Chapter 3 on plant water relations). Accumulation of these sugar alcohols requires glucose as a substrate but does not directly affect the concentration of carbohydrates or interfere with growth.

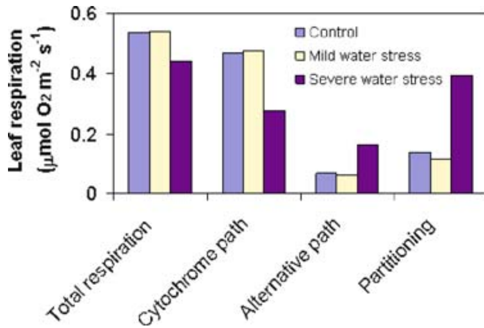


**FIGURE 13. Effect of different levels of water stress on total respiration ($V_t$), the activities of the cytochrome ($v_{cyt}$), and alternative ($v_{alt}$) pathways and the partitioning through the alternative pathway ($\tau_a$) (after Ribas-Carbó et al. 2005a). Copyright American Society of Plant Biologists.**

Studies of root respiration, using an inhibitor of the alternative path, suggested that sorbitol accumulation is associated with a reduction in activity of the nonphosphorylating alternative respiratory pathway. However, further experimentation using the oxygen-isotope fractionation technique (Box 2B.1) is required to confirm this. Interestingly, the amount of sugars that are "saved" by the decline in respiration is the same as that used as the substrate for the synthesis of sorbitol, suggesting that accumulation of compatible solutes by drought-adapted plants may have a minimal energetic cost (Lambers et al. 1981).

Prolonged exposure of salinity-adapted species (**halophytes**) to salt concentrations sufficiently low not to affect their growth has no effect on the rate of root respiration. This similarity in growth and respiratory pattern under saline and nonsaline conditions suggests that the respiratory costs of coping with mild salinity levels are negligible in salt-adapted species. The respiratory costs of functioning in a saline environment for adapted species that accumulate NaCl are also likely to be relatively small, because of the low respiratory costs of absorbing and compartmentalizing salt when grown in saline soils. For salt-excluding **glycophytes**, however, there may be a large respiratory cost associated with salt exclusion.

## 4.3 Nutrient Supply

Root respiration generally increases when roots are suddenly exposed to increased ion concentrations in their environment, a phenomenon known as **salt respiration**. The stimulation of respiration is at least partly due to the increased **demand for respiratory energy** for ion transport. The added respiration may also reflect a replacement of osmotically active sugars by inorganic ions, leaving a large amount of sugars to be respired via the **alternative path**.

When plants are grown at a low supply of N, their rate of **root respiration** is lower than that of plants well supplied with mineral nutrients (Atkinson et al. 2007). This is expected because their rates of growth and ion uptake are greatly reduced (Fig. 14). Rates of root respiration, however, per ion absorbed or per unit root biomass produced at a low $NO_3^-$ supply are relatively high, if we compare these rates with those of plants that grow and take up ions at a *much* higher rate. This suggests that **specific costs** of growth (that is cost per unit biomass produced), maintenance (cost per unit biomass to be maintained), or ion transport (cost per unit nutrient absorbed) must increase in plants grown at a limiting nutrient supply (Sect. 5.2.4).

There is also a correlation between **leaf respiration** and leaf N concentration (Loveys et al. 2003, Noguchi & Terashima 2006). Although the correlation between leaf respiration and leaf N concentration tends to be general, irrespective of the natural habitat of the species (Tjoelker et al. 1999, Reich et al. 2006), environment-mediated changes in the relationship between leaf respiration and leaf N can occur. For example, in a comparison of 70 Australian perennial species, the slope of leaf respiration (on a dry mass basis) vs. leaf N concentration is constant across sites, but there are differences in the intercept for sites differing in nutrient availability and rainfall (Wright et al. 2001). The physiological basis for such a difference in intercept remains to be explored.

## 4.4 Irradiance

The respiratory response of plants to light and assimilate supply depends strongly on time scale. The immediate effect of low light is to reduce the **carbohydrate status** of the plant and, therefore, the supply of substrate available for respiration (Fig. 15A). Interestingly, in the shade species *Alocasia odora* (Asian taro) addition of sucrose does not increase the rate of leaf respiration of plants transferred to the shade (Fig. 15B), but addition of an uncoupler does increase respiration to a major extent (Fig. 15C) (Noguchi et al. 2001a). This shows that respiration is controlled by **energy**

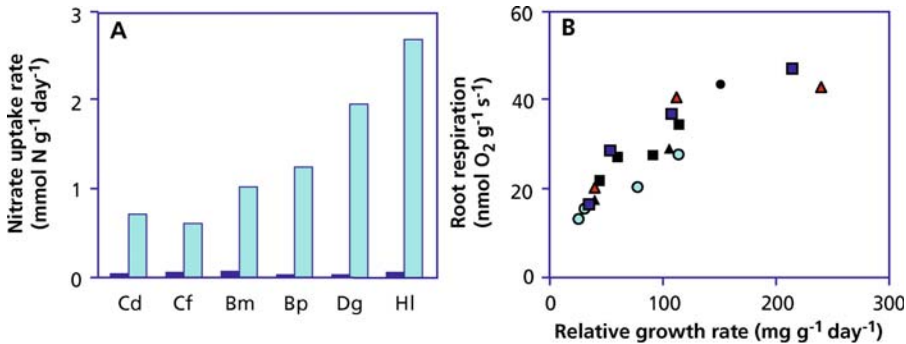


**FIGURE 14. (A) Rates of net inflow of nitrate of six grass species grown at two nitrogen addition rates, allowing a near-maximum relative growth rate (*open columns*) or a RGR well below $RGR_{max}$ (*black columns*). (B) Root respiration of the same inherently fast- and slow-growing grasses as shown in A, now compared at a range of nitrogen addition rates allowing a near-maximum relative growth rate or a relative growth rate below $RGR_{max}$, the lowest RGR being 38 mg $g^{-1}$ $day^{-1}$. Cd, *Carex diandra* (lesser panicled sedge) (*open circles*); Cf, *Carex flacca* (*blue sedge*) (*filled triangle*); Bm, *Briza media* (quacking grass) (*filled squares*); BP, *Brachypodium pinnatum* (Tor grass) (*filled circles*); DG, *Dactylis glomerata* (cocksfoot) (*open squares*); Hl, *Holcus lanatus* (common velvet grass) (*open triangles*) (Van der Werf et al. 1992a). Copyright SPB Academic Publishing.**

**demand**, rather than **substrate supply** (Sect. 2.4, Fig. 5), and that the energy demand is down-regulated in shade conditions. In the leaves of *Alocasia odora*, the contribution of the **alternative path** is less than 10% of the total respiratory rate, irrespective of growth irradiance. For the sun species *Spinacia oleracea* (spinach) and *Phaseolus vulgaris* (common bean) grown at high light intensity, the contribution of the alternative path in the leaves is about 40% early in the night, but decreases dramatically late in the night. When spinach is grown at low light intensity, however, the contribution of the alternative path in the leaves declines. The low activity of the alternative path in the leaves of the understory species *Alocasia odora* shows that the efficiency of ATP production (ADP:O ratio) of this species is high. This may be especially important in shade environments. In the leaves the of sun species, the ADP:O ratio changes depending on conditions (Noguchi et al. 2001a).

To further investigate why the understory species *Alocasia odora* (Asian taro) consistently shows low alternative path activity, Noguchi et al. (2005) grew *Alocasia odora* and *Spinacia oleracea* (spinach) plants under both high and low light intensities. On a mitochondrial protein basis, *Spinacia oleracea* leaves show a higher capacity of the cytochrome pathway than do *Alocasia odora* leaves. Despite a low in vivo activity of the alternative path, *Alocasia odora* has a higher capacity of the alternative oxidase on a mitochondrial protein basis. In the low-light environment, most of the alternative oxidase protein in *Alocasia odora* leaves is in its inactive, **oxidized dimer** form (Sect. 2.6.2), but it is converted to its **reduced**, active form when plants are grown under high light (Fig. 16). This shift may prevent over-reduction of the respiratory chain under photo-oxidative conditions.

Roots and leaves that are subjected to an increased or decreased carbohydrate supply gradually **acclimate** over several hours by adjusting their respiratory capacity. Upon transfer of *Poa annua* (annual meadow-grass) from high-light to low-light conditions, and at the same time from long-day to short-day conditions, the **sugar concentration** in the roots decreases by 90%. Both the rate of root respiration and the *in vitro* **cytochrome oxidase** capacity decrease by about 45%, relative to control values. The absolute rate of $O_2$ uptake via the **alternative pathway**, as determined using the isotope fractionation technique (Box 2B.1), does not change, but the cytochrome pathway activity decreases. Interestingly, there is no change in the concentration of the alternative oxidase protein or in the reduction state of the protein. Also, there is no change in the reduction state of the ubiquinone pool. These results show that neither the amount nor the activity of the alternative oxidase change under severe light deprivation (Millenaar et al. 2000), suggesting an important role for this apparently wasteful pathway; this role is most likely avoiding production of reactive oxygen species, as discussed in Sect. 3.3. The results also point to acclimation of respiration as a result of changes in

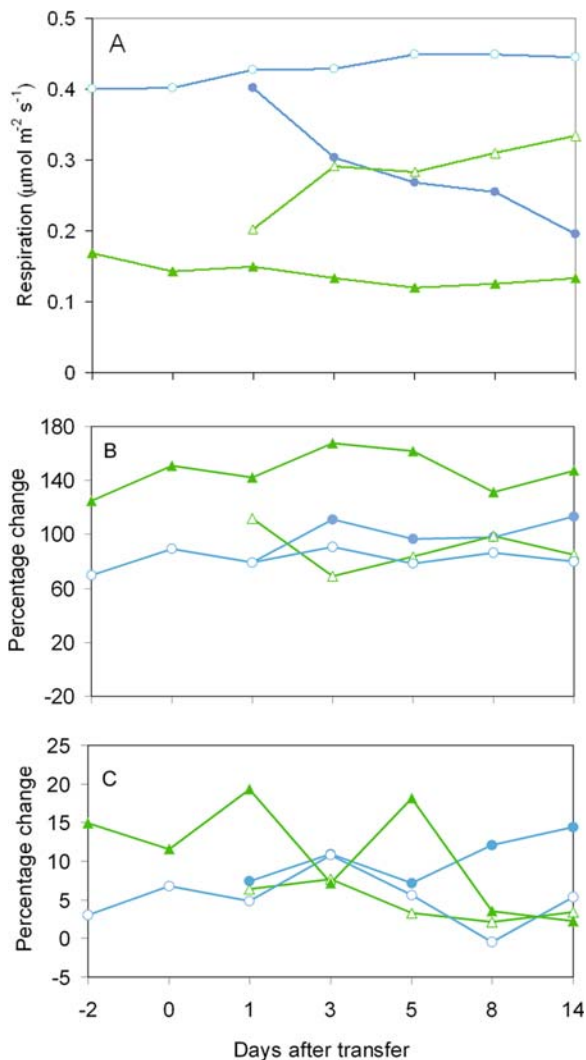


**Figure 15.** Leaf respiration in the shade species *Alocasia odora* (Asian taro) as dependent on light availability. (A) Changes in the rate of $O_2$ uptake. Effects of (B) the addition of an uncoupler (FCCP) and (C) a respiratory substrate (sucrose) on the rate of $O_2$ uptake. Plants that were originally grown in high light (*open green symbols*) or low light (*filled green triangles*) were subsequently transferred to high light (*filled circles*) or low light (*open triangles*) on day 0 (redrawn after Noguchi et al. 2001a).

**gene expression**. Also, after pruning of the shoot to one leaf blade, both the soluble sugar concentration and the respiration of the seminal roots decrease. These effects on respiration reflect the **coarse control** of the respiratory capacity upon pruning or sucrose feeding (Bingham & Farrar 1988, Williams & Farrar 1990). This illustrates the adjustment of the respiratory capacity to the root's carbohydrate level.

Changes in respiratory capacity induced by changes in **carbohydrate status** reflect acclimation of the respiratory machinery. The protein pattern of the roots of pruned plants is affected within 24 h (McDonnel & Farrar 1992, Williams et al. 1992). Glucose feeding to leaves enhances the activity of several glycolytic enzymes in these leaves, due to regulation of **gene expression** by carbohydrate levels (Krapp & Stitt 1994). Clearly, the capacity to use carbohydrates in respiration is enhanced when the respiratory substrate supply increases, and declines with decreasing substrate supply. The plant's potential to adjust its respiratory capacity to environmental conditions is ecologically significant. Individual plants acclimated to low light generally have low leaf respiration rates. Thus acclimation accentuates the short-term declines in respiration due to substrate depletion.

As with acclimation, species that are **adapted** to low light generally exhibit lower respiration rates than high-light adapted species. For example, the rainforests understory species of *Alocasia odora* (Asian taro) has lower rates of both photosynthesis

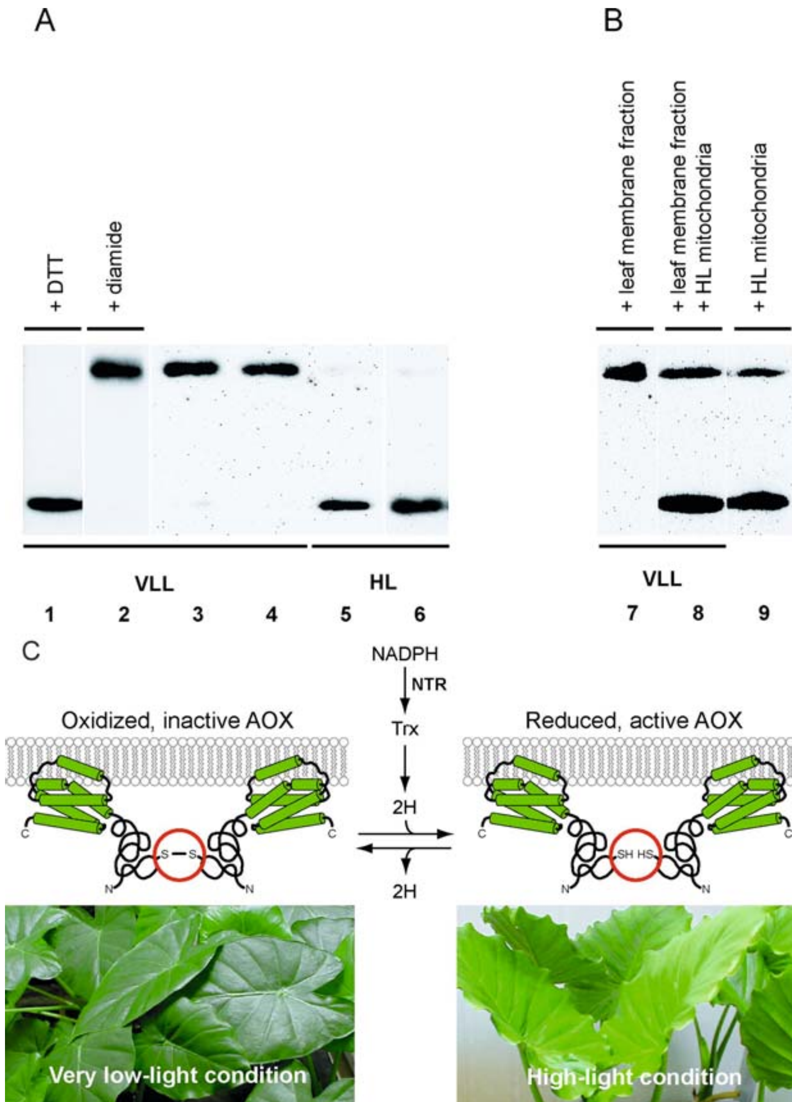


FIGURE 16. (A) Immunoblots of the alternative oxidase (AOX) in extracted membrane fractions isolated from *Alocasia odora* (Asian taro) leaves. Extractions were made very rapidly, so as to maintain the activation state of AOX and determine its in vivo state. Lane 1, a sample of leaves of plants grown at very low light intensities (VLL) treated in the presence of 50 mM DTT (dithiothreitol, which renders AOX in its reduced and active state, irrespective of its state in vivo). Lane 2, a sample of VLL leaves treated in the presence of 5 mM diamide (which oxidizes and inactivates the AOX dimer, irrespective of its state in vivo). Lanes 3 and 4, samples consisted of only VLL leaf membrane fractions; the immunoblots show that AOX was in its oxidized, inactive state in leaves of plants grown at very low light intensity. Lanes 5 and 6, samples consisted of only high-light grown (HL) leaf membrane fractions; these immunoblots show that AOX was in its reduced, active state in leaves of plants grown at high light intensity. (B) Immunoblots of AOX in rapidly extracted membrane fractions and/or mitochondria isolated from *Alocasia odora* leaves. Lane 7, a sample consisted of only VLL leaf membrane fractions; AOX was in its oxidized, inactive state. Lane 8, a sample of VLL leaf membrane fractions, added with a mitochondrial extract from HL leaves just before the extraction; Lane 9, mitochondrial sample isolated from HL leaves; during isolation some AOX is reduced and activated (Noguchi et al. 2005). (C) Under very low light conditions, the alternative oxidase is in its inactive, oxidized form (left). It is converted to its reduced, active form (right) when plants are exposed to high-light conditions. This shift may prevent over-reduction of the respiratory chain under photo-oxidative conditions. The structural model for AOX has been deduced from derived amino acid sequences and is reprinted with permission of the American Society of Plant Biologists. Photographs by K. Noguchi. Copyright Blackwell Science Ltd.

**Table 8. The daily carbon budget (mmol $g^{-1}$ $day^{-1}$) of the leaves of *Spinacia oleracea* (spinach), a sun species, and *Alocasia odora* (giant upright elephant ear), a shade species, when grown in different light environments.***

| | Photosynthesis | | Leaf respiration | | Net leaf carbon gain | |
|---|---|---|---|---|---|---|
| Irradiance | *Spinacia oleracea* | *Alocasia odora* | *Spinacia oleracea* | *Alocasia odora* | *Spinacia oleracea* | *Alocasia odora* |
| 500 | 26 | nd | 3.4 (13) | nd | 23 (87) | nd |
| 320 | 21 | 11 | 2.4 (12) | 1.1 (10) | 18 (88) | 9.4 (90) |
| 160 | 15 | 9 | 1.7 (11) | 0.82 (9) | 14 (89) | 8.2 (91) |
| 40 | nd | 4.5 | nd | 0.76 (17) | nd | 3.7 (83) |

*Source*: Noguchi et al. (1996), K. Noguchi, pers. comm.
* Irradiance is expressed in μmol $m^{-2}$ $s^{-1}$. Percentages of the photosynthetic carbon gains have been indicated in brackets; nd is not determined; in the original paper the species name is erroneously given as *Alocasia macrorrhiza.*

and respiration than does the sun species *Spinacia oleracea* (spinach), when the two species are compared under the same growth conditions (Table 8). The net daily carbon gain of the leaves (photosynthesis minus respiration) is rather similar for the two species, when expressed as a proportion of photosynthesis. Similarly, understory species of *Piper* (pepper) have lower respiration rates than species from shaded and exposed habitats, when both are grown in the same environment (Fredeen & Field 1991). Because rates of photosynthesis and respiration show parallel differences between sun and shade species (both lower in the shade species), differences in the carbon balance between sun and shade species probably reflect different patterns of biomass allocation rather than differences in photosynthesis and respiration.

Respiration rates tend to be higher in plants grown at higher light intensity. Acclimation to higher levels of irradiance involves up-regulation of genes involved in the metabolism of carbohydrates and in energy-requiring processes (**coarse control**). In the short term, respiration may respond to irradiance because this affects the availability of respiratory substrate (**control by supply**). Sudden exposure of shade plants to a high light intensity may require a change in activation state of the alternative oxidase, associated with accumulation of reactive oxygen species (**stress response**).

Acclimation of respiration is relatively fast (hours to days), when compared with that of photosynthesis (days to weeks). This is largely accounted for by the fact that some aspects of photosynthetic acclimation require the production of new leaves with a different structure, whereas acclimation of respiration requires only production of new proteins.

## 4.5 Temperature

Respiration increases as a function of temperature, with the magnitude of increase depending on the **temperature coefficient** ($Q_{10}$) of respiration. This temperature effect on respiration is characteristic of most heterothermic organisms and is a logical consequence of the temperature sensitivity of the enzymatically catalyzed reactions involved in respiration. The temperature stimulation of respiration also reflects the increased demand for energy to support the increased rates of biosynthesis, transport, and protein turnover that occur at high temperatures (Sect. 5.2).

Temperature-mediated changes in plant respiration are an important component of the biosphere's response to global climate change. The $Q_{10}$ is often modeled to be 2 (i.e., respiration doubles per 10°C rise in temperature). However, upon longer-term exposure to a different temperature, the initial temperature effect of a $Q_{10}$ of 2 may diminish, and the long-term $Q_{10}$ declines predictably with increasing temperature across diverse plant taxa and biomes (Fig. 17A). This is due to **thermal acclimation**, i.e., the adjustment of respiration rates to compensate for a change in temperature. The temperature dependence of $Q_{10}$ is linked to shifts in the control by maximum **enzyme activity** at low temperature and **substrate limitations** at high temperature (Fig. 17B). In the long term, acclimation of respiration to temperature is common, reducing the temperature sensitivity of respiration to changes in thermal environment. Temperature acclimation results in a tendency toward **homeostasis** of respiration, such that warm-acclimated (temperate, lowland) and cold-acclimated alpine or high-arctic plants display similar rates of respiration when measured at their

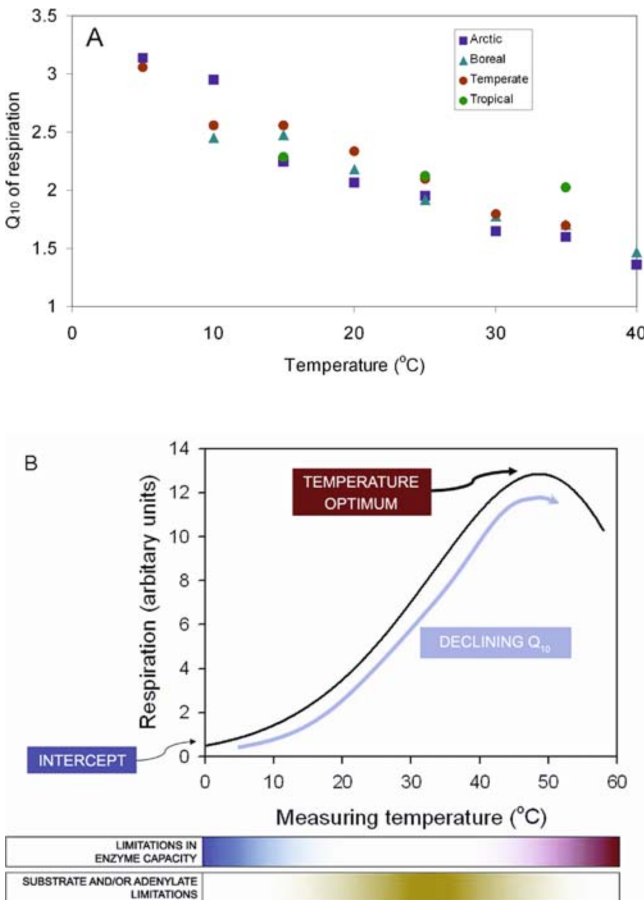


**FIGURE 17. Effects of temperature on plant respiration. (A) $Q_{10}$ of foliar respiration rates in relation to short-term measurement temperature. Symbols are the mean $Q_{10}$ of species of arctic, indicated in blue (49 species), boreal, indicated in green (24 species), temperate, indicated in brown (50 species), and tropical, indicated in orange (3 species). (B) Assuming a rate of respiration of 0.5 at 0°C (arbitrary units), respiration at other temperatures was predicted using the linear decline in $Q_{10}$ with increasing temperature (shown in A). Both the intercept (i.e., $R$ at 0°C) and the temperature optimum of respiration (i.e., temperature where respiration rates are maximal) are shown. The lower panels indicate the degree to which respiratory flux is likely limited by enzyme capacity vs. substrate supply and adenylates. The temperatures where respiratory flux is likely be limited by maximum catalytic enzyme activity (i.e., $V_{max}$) are indicated in blue (limitations in the cold) and red (limitations at supra-optimal temperatures). At moderate temperatures, respiratory flux is likely regulated by the availability of substrate and/or adenylates (i.e., the absolute concentration of ADP and the ratio of ATP:ADP) (after Atkin & Tjoelker 2003; copyright Elsevier Science, Ltd.).**

respective growth temperatures; however, complete homeostasis is uncommon. Acclimation can play an important role in weakening positive feedback through the warming-respiration-atmospheric $CO_2$ concentration connection (Atkin & Tjoelker 2003). **Acclimation** of leaf respiration to temperature is larger in conifers than in broad-leaved species (Tjoelker et al. 1999); other than that, there are no major systematic differences in the degree of acclimation among contrasting plant species (Loveys et al. 2003).

The mechanism of temperature acclimation of respiration is not yet fully understood. At low measurement temperatures (e.g., 5°C), respiratory flux is probably limited by the $V_{max}$ (Covey-Crump et al. 2002) (lower panels in Fig. 17) of the respiratory apparatus [i.e., glycolysis, the TCA cycle, and mitochondrial electron transport (Sects. 2.2 and 2.3)]. At moderately high temperatures (e.g., 25°C), respiratory flux is less limited by enzymatic capacity because of increases in the $V_{max}$ of enzymes in soluble and membrane-bound compartments; here, respiration is likely limited by substrate availability and/or adenylates. Increased leakiness of membranes at high temperatures may further contribute to substrate limitations. The net result of temperature-mediated shifts in control from **capacity** (at low temperatures) to **substrate** or **adenylate** limitation (at moderately high temperatures) (Fig. 17) is that a rise in measurement temperature has less impact on respiratory flux at moderate-high temperatures than it does in the cold. As a result, the calculated $Q_{10}$ is lower when calculated across a high measurement temperature range than at a range of low measurement temperatures. To firmly establish if respiratory enzyme capacity limits respiratory flux in the cold, data are needed on the maximum potential flux of the respiratory apparatus in intact tissues at low temperatures. These can be obtained via measurements of respiration in isolated mitochondria, in the presence of saturating substrates and ADP (Fig. 4). Mitochondrial rates can then be scaled up to the whole-plant level (Atkin & Tjoelker 2003). Thermal acclimation may require changes in the expression of genes that encode respiratory

enzymes or levels of substrates (Sect. 4.4). Acclimation of leaf respiration in field-grown *Eucalyptus pauciflora* (snow gum) occurs without changes in carbohydrate concentrations in leaves (Atkin et al. 2000). Temperature acclimation may also be associated with changes in leaf N concentration, which may affect photosynthesis (Sect. 6.1 of Chapter 2A on photosynthesis) and, consequently, the respiratory energy requirement for phloem loading (Sect. 5) (Tjoelker et al. 1999). Thermal acclimation in leaves of *Arabidopsis thaliana* (thale cress) is associated with an increase in rates of $O_2$ uptake per unit mitochondrial protein in mesophyll cells (Armstrong et al. 2006).

In addition to the acclimation potential of total respiration, acclimation may also change the partitioning of electrons between the cytochrome and alternative pathways as well as the activity of uncoupling proteins. Using roots of *Triticum aestivum* (wheat) and *Oryza sativa* (rice) cultivars with different degrees of respiratory homeostasis, shows that high-homeostasis cultivars maintain shoot and root growth at low temperature (Kurimoto et al. 2004a). Irrespective of a cultivar's capacity to maintain homeostasis, **cytochrome path capacity** of intact roots and isolated root mitochondria are larger for plants grown at low temperature, and the maximal activity of cytochrome oxidase show a similar trend. In contrast, **cyanide-resistant respiration** of intact roots and relative amounts of alternative oxidase protein in mitochondria isolated from those roots, are lower in high-homeostasis plants grown at low temperature. In the roots of low-homeostasis cultivars, relative amounts of alternative oxidase protein are higher at low growth temperature. Relative amounts of **uncoupling protein** show similar trends. Maintenance of growth rates in high-homeostasis plants grown at low temperature is obviously associated with both respiratory homeostasis and a high efficiency of respiratory ATP production (Kurimoto et al. 2004b).

Needles or leaves of cold-hardened plants that maintain relatively low rates of respiration when exposed to higher temperatures maintain higher concentrations of soluble sugars which confers greater **frost tolerance**. During a 5°C warmer-than-average winter in north-eastern Sweden, *Vaccinium myrtillus* (bilberry) may suffer lethal injuries due to the progressive respiratory loss of **cryoprotective sugars** from their leaves. Initial leaf carbohydrate reserves last 4 months only if tissue water content remains high due to frequent misty and rainy days; when dehydrated, the leaves' cold tolerance increases (Ögren 1996). Climate warming may impact significantly on cold hardiness of some northern European woody plants such as *Picea abies* (Norway spruce), *Pinus sylvestris* (Scots pine), and *Pinus contorta* (lodgepole pine). In lodgepole pine seedlings, needle sugar concentrations may decrease by 15% which makes them more sensitive to frost. If the seedlings contain unusually large carbohydrate reserves, as found for Scots pine, these may buffer respiratory expenditure of sugars, and thus avoid frost damage. A strong, linear relationship exists between levels of cold hardiness and sugars (Ögren 2001).

## 4.6 Low pH and High Aluminum Concentrations

Root respiration rate increases as the **pH** in the rhizosphere decreases to a level below that at which growth is no longer possible (Fig. 18). Net $H^+$ release from roots by $H^+$-ATPase activity is a prerequisite for continued root growth and limits root growth at very low pH values (Schubert et al. 1990). One way of coping with excess $H^+$ uptake at a low pH is to increase active $H^+$ pumping by plasma-membrane ATPases. This increases the **demand for respiratory energy** (Fig. 18). Increased respiration rates can, therefore, allow plants to maintain root growth at noncritical low pH values, by increasing the supply of ATP for $H^+$ pumping by plasma-membrane ATPases.

At very low pH values, root growth, net $H^+$ release, and respiration rates decline (relative to rates at pH 7.0). The increased entry of $H^+$ into the roots under these circumstances appears to be responsible for these effects (Yan et al. 1992). Such increased uptake of $H^+$ tends to disturb cytosolic pH and ultimately root growth. The decrease in root respiration at very low pH might, therefore, result from the decreased respiratory demand for growth.

A low pH may also increase respiration due to the increased solubility of **aluminum** (Sect. 3.1 of Chapter 6 on mineral nutrition). Respiration of intact roots increases in response to aluminum in both aluminum-resistant and sensitive cultivars of *Triticum aestivum* (wheat) (Collier et al. 1993). Root growth and respiration decline at much higher aluminum concentrations in the resistant than in sensitive cultivars (of *Sorghum bicolor* (sorghum) Tan & Keltjens 1990a,b).

The increase in respiration of intact roots suggests that root functioning in the presence of aluminum imposes a demand for additional respiratory energy. These increased costs have little to do with the mechanism explaining resistance (i.e., excretion of chelating carboxylates) because such excretion

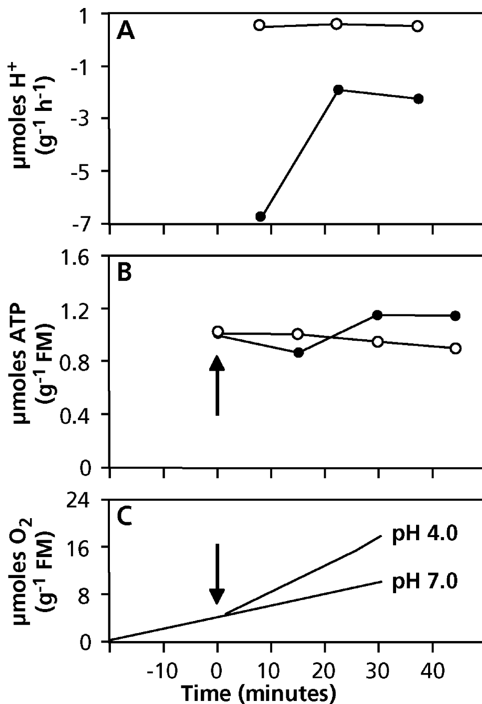


**FIGURE 18. Effect of the pH in the rhizosphere on (A) net $H^+$ release, (B) ATP concentration, and (C) respiration of *Zea mays* (maize) roots. Seedlings were grown at pH 7.0, and either kept at pH 7.0 (*open symbols*) or exposed to a pH of 4.0 (*filled symbols*) at the time indicated by the arrow. Note that the slopes in A and C give the *rate* of $H^+$ release and respiration (after Yan et al. 1992). Copyright American Society of Plant Biologists.**

does not occur to any major extent in the sensitive cultivar (Sect. 3.1 of Chapter 6 on mineral nutrition).

## 4.7 Partial Pressures of $CO_2$

$CO_2$ concentrations in air pockets in soil are up to 30-fold higher than those in the atmosphere. Although respiration rates are highest in superficial layers of soil where root biomass is concentrated, the $CO_2$ concentration increases with increasing profile depth, due to the restricted diffusion of gases in soil pores (Richter & Markewitz 1995).

The $CO_2$ concentration in the soil may increase substantially upon **flooding** of the soil. Values of 2.4 and 4.2 mmol $CO_2$ mol$^{-1}$ (0.24 and 0.42%, respectively) occur in flooded soils supporting the growth of desert succulents, as opposed to 0.54 and 1.1 mmol mol$^{-1}$ in the same soils, when well-drained (Nobel & Palta 1989). Good & Patrick (1987) found $CO_2$ concentrations of 5.6 and 3.8% in silt loam, supporting the growth of *Fraxinus pennsylvanica* (green ash) and *Quercus nigra* (water oak), respectively. Do such high $CO_2$ concentrations affect root respiration?

Root respiration is reversibly inhibited by 5 mmol $CO_2$ mol$^{-1}$ in two cacti [*Opuntia ficus-indica* (prickly pear) and *Ferocactus acanthodes* (compass barrel cactus)] (Nobel & Palta 1989). Full inhibition occurs at 20 mmol $CO_2$ mol$^{-1}$ (2%) which is irreversible if lasting for 4 hours. Root respiration of *Pseudotsuga menziessii* (Douglas fir) and *Acer saccharum* (sugar maple) is also inhibited at soil $CO_2$ levels in a range normally found in soil (Qi et al. 1994, Burton 1997), whereas no such inhibition occurs for a range of other species (e.g., Bouma et al. 1997, Scheurwater et al. 1998). Because respiration is only affected by **$CO_2$**, and *not* by **bicarbonate** (Palet et al. 1992), the pH of the root environment will greatly affect experimental results (Fig. 51 in Chapter 2A on photosynthesis).

How can we account for effects of very high $CO_2$ concentration on respiration? The effects of soil $CO_2$ concentrations on root respiration is probably *indirect*, due to inhibition of energy-requiring processes. There may also be *direct* effects of a high concentration of $CO_2$ on respiration (i.e., inhibition of **cytochrome oxidase**) (Sect. 3.6). Other mitochondrial enzymes are also affected by high concentrations of inorganic carbon (Gonzàlez-Meler et al. 1996, Bruhn et al. 2007). Malic enzyme, which oxidizes malate to form pyruvate and $CO_2$, is rather strongly inhibited by $HCO_3^-$ in a range that may well account for inhibition of respiration by $CO_2$ as found for some tissues (Chapman & Hatch 1977, Neuberger & Douce 1980). Some of the effects in vitro for several mitochondrial enzymes, however, only appear at $CO_2$ concentrations that are much higher than expected to occur in intact roots.

The information in the literature is still too scanty to draw the robust conclusion that $CO_2$ levels that normally occur in well drained soil have a *direct* inhibitory effect on root respiration (Lambers et al. 2002). After much discussion on inhibition of **leaf respiration** by elevated atmospheric $CO_2$ concentrations due to **global change**, there is now wide consensus that these are mostly artifacts of the methodology (Jahnke & Krewitt 2002, Davey et al. 2004). However, there are *indirect* effects of long-term exposure of plants to elevated [$CO_2$]. These effects are due to changes in, e.g., allocation, plant growth rate, chemical composition of the biomass, rather than accounted for by *direct* effects (Tjoelker et al. 1999, Griffin et al. 2001, Davey et al. 2004). Across all studies, mass-based leaf dark respiration is reduced by 18%, while area-based leaf respiration

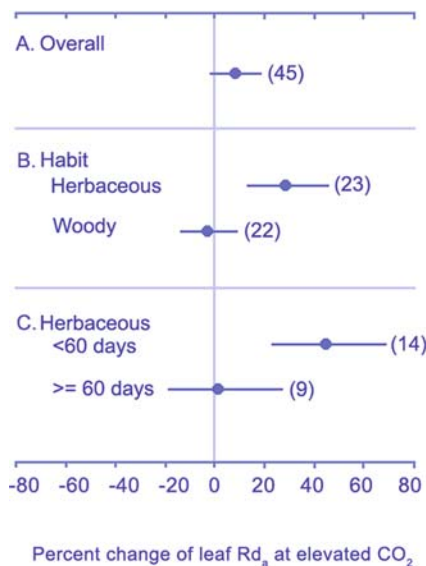


**FIGURE 19. Long-term effects of elevated atmospheric $CO_2$ on leaf dark respiration expressed on a leaf area basis ($R_{da}$) across 45 independent observations. Effects of growth habit (herbaceous vs. woody species) on leaf $R_{da}$ response and time (length of $CO_2$ exposure) on herbaceous species leaf $R_{da}$ response to elevated $CO_2$ are also shown. Mean values ± 95% confidence interval; the number of observations is shown in brackets (Wang & Curtis 2002).**

is marginally increased (8%), under elevated atmospheric $CO_2$ concentrations. Area-based leaf respiration of **herbaceous** species increases by 28%, but is unaffected in **woody** species (Fig. 19). Mass-based reductions in leaf respiration tend to increase with prolonged exposure to elevated [$CO_2$]. In cladodes of *Opuntia ficus-indica* (prickly pear), reductions in respiration are associated with a decrease in **mitochondrial number** and cytochrome path activity, and an increase in activity of the alternative path (Gomez-Casanovas et al. 2007). A **meta-analysis** of published results suggests that the amount of carbon use in leaf dark respiration will increase in a higher-[$CO_2$] environment, because of higher area-based leaf respiration rates and a proportionally greater leaf biomass increase than reductions in mass-based leaf respiration (Wang & Curtis 2002).

## 4.8 Effects of Plant Pathogens

Pathogen attack on roots or leaves causes an increase in respiration, but the pattern of this respiratory response may differ between sensitive and resistant varieties of plants. For example, **nematode** infection of roots of a susceptible variety of *Solanum lycopersicum* (tomato) causes root respiration first to increase, but then to return to the level of uninfested plants. By contrast, the resistant variety shows no initial change in root respiration in response to nematode attack, but after 8 days the respiration rate exceeds that of control plants (Zacheo & Molinari 1987).

Just as with tomato roots, leaves of a susceptible variety of *Hordeum distichum* (barley) show a large increase in respiration when infected with the **fungus** causing powdery mildew. This is expected, as both fungus and host have high demands for energy (the fungus for growth, the host for defense). In the case of barley, most of the respiration is accounted for by host respiration (Farrar & Ryans 1987).

Both **mRNA levels** that encode the alternative oxidase and the amount of **alternative oxidase protein** strongly increase in leaves of *Arabidopsis thaliana* (thale cress) that are infiltrated with the leaf-spotting bacterium *Pseudomonas syringae* (Simons et al. 1999). What could be the functional significance for an increase of this pathway? Pathogenic fungi may produce **ethylene** and enhance the concentration of **salicylic acid** and **reactive oxygen species** in the plant (Overmyer et al. 2003). These compounds may trigger the increased activity of the alternative path. In ripening fruits ethylene enhances alternative respiration; salicylic acid induces the large increase in respiration in the spadix of thermogenic *Arum* species (Sect. 3.1) and in vegetative organs of nonthermogenic plants; reactive oxygen species trigger expression of the alternative oxidase in a range of species (Purvis & Shewfelt 1993, Considine et al. 2002). Quite likely, the enhanced synthesis of defense-related compounds (phytoalexins and other phenolics; Sect. 3 of Chapter 9C on effects of microbial pathogens) requires a large production of NADPH in the **oxidative pentose phosphate** pathway (Fig. 2) (Shaw & Samborski 1957). This pathway, unlike glycolysis (Fig. 3), is not regulated by the demand for metabolic energy. Products of the oxidative pentose phosphate pathway can enter glycolysis, bypassing the steps controlled by energy demand. Additional NADPH can be produced by cytosolic **NADP-malic enzyme**, which oxidizes malate, producing pyruvate and $CO_2$. This enzyme is induced upon addition of "elicitors" (i.e., chemical components of a microorganism that induces the synthesis of defense compounds in plant cells) (Sect. 3 of Chapter 9C on effects of microbial pathogens) (Schaaf et al. 1995). The increased activity of the oxidative pentose pathway and of NADP-malic enzyme probably leads to

the delivery of a large amount of pyruvate and malate to the mitochondria, without there being a large need for ATP. As a result, the cytochrome path becomes saturated with electrons, the alternative oxidase is activated (Sect. 2.6.2), and much of the electrons are transported via the alternative pathway (Sect. 3.3) (Simons & Lambers 1999).

### 4.9 Leaf Dark Respiration as Affected by Photosynthesis

Both photosynthesis and mitochondrial respiration ("dark" respiration, as opposed to photorespiration) produce ATP and NAD(P)H to meet demands for plant growth and maintenance. The light reaction in photosynthesis provides ATP and NAD(P)H for biosynthesis in a leaf cell during illumination, but mitochondrial respiration in the light is necessary for biosynthetic reactions in the cytosol, such as sucrose synthesis (Krömer 1995). Respiratory activity in the light can be considered part of the photosynthetic process, because it is needed to regulate the redox state of the stroma in the chloroplast during photosynthesis (Foyer & Noctor 2000) and to maintain the cytosolic ATP pool (Krömer 1995). The rate of mitochondrial respiration during photosynthesis is therefore determined by the need for this process to provide energy and carbon skeletons in the light. Light inhibits leaf "dark" respiration, but the extent of inhibition depends on species and environmental conditions. In leaves of *Eucalyptus pauciflora* (snow gum), respiration is inhibited most at very low light intensities and moderate temperatures, and considerably less at higher irradiance. The irradiance necessary to maximally inhibit R at 6 to 10°C is lower than that at 15 to 30°C (Atkin et al. 2000). In leaves of *Xanthium strumarium* (common cocklebur) respiration is inhibited at both ambient and elevated $CO_2$ concentrations, but to a lesser degree for plants grown at elevated (17–24%) than for those grown at ambient (29–35%) $CO_2$ concentrations, presumably because elevated $CO_2$-grown plants have a higher demand fo energy and carbon skeletons (Wang et al. 2001). Variations in light inhibition of leaf respiration can have a substantial impact on the proportion of carbon fixed in photosynthesis that is respired.

The metabolic origin of the $CO_2$ production in leaf "dark" respiration during photosynthesis can be analyzed by feeding $^{13}C$-enriched glucose or pyruvate to intact leaves. Using metabolites that are $^{13}C$-enriched in different positions, reveals that in leaves of *Phaseolus vulgaris* (common bean) the activity of the TCA cycle is reduced by 95% in the light; pyruvate dehydrogenase activity, however, is much less reduced (27%). Glucose molecules are scarcely metabolized to liberate $CO_2$ in the light, because glycolysis is down-regulated. Instead, glucose is mainly used for sucrose synthesis. Several metabolic processes (glycolysis, TCA cycle) are down-regulated, leading to a light-dependent inhibition of mitochondrial respiration (Tcherkez et al. 2005).

## 5. The Role of Respiration in Plant Carbon Balance

### 5.1 Carbon Balance

Approximately half of all the photosynthates produced per day are respired in the same period, the exact fraction depending on species and environmental conditions (Table 1). Globally rising temperatures tend to increase the proportion of carbon gained in photosynthesis that is subsequently used in respiration (Atkin et al. 2007). The level of irradiance and the photoperiod appear to affect the carbon balance of acclimated plants to a relatively small extent, but factors such as inadequate nutrient supply and water stress may greatly increase the proportion of photosynthates used in respiration. This is accounted for by a much stronger effect of nutrients on biomass allocation, when compared to that of irradiance and photoperiod (Chapter 7 on growth and allocation). Root temperature is also likely to affect plant carbon balance because this has a major effect on biomass allocation (Sect. 5.2.2 of Chapter 7 on growth and allocation).

#### *5.1.1 Root Respiration*

Root respiration accounts for approximately 10–50% of the total carbon assimilated each day in photosynthesis (Table 1) and is a major proportion of the plant's carbon budget (Fig. 20). This percentage is much higher in slow-growing than in fast-growing plants. This is true for a comparison of species that vary in their **potential growth rate** (Poorter et al. 1991) and for plants of the same species that vary in growth rate, due to variation in the **nutrient supply** (Van der Werf et al. 1992a). Root temperatures that enhance biomass allocation to roots (Sect. 5.2.2 of Chapter 7 on growth and allocation) probably also increase the proportion of carbon required for root respiration. When slow growth is due to exposure to low light levels, however, no greater respiratory burden is incurred (Sect. 4.4). To some extent the proportionally greater carbon use in slow-growing plants is accounted for by their

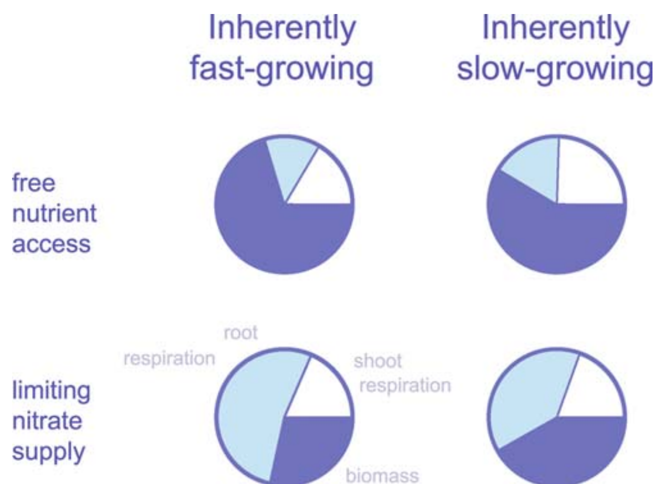


**FIGURE 20. The fraction of all carbohydrates produced in photosynthesis per day that is consumed in respiration as dependent on species and the nitrogen supply. Measurements were made on inherently fast-growing (pies on the left) and slow-growing (pies on the right) grass species grown with free nutrient availability (pies at the top) and at a N supply that allowed a relative growth rate of approximately 40 mg $g^{-1}$ $day^{-1}$ (pies at the bottom). The percentages at each pie indicate the carbon gain in photosynthesis per unit plant mass, relative to that of the fast-growing species grown with free access to nutrients. The black section of the pie refers to carbon invested in growth; the other two sections refer to carbon used in shoot respiration (white sector), and with root respiration depicted as the loose section of the pie (Van der Werf et al. 1992a). Copyright SPB Academic Publishing.**

relatively low carbon gain per unit plant mass (Sect. 3 of Chapter 7 on growth and allocation) (Poorter et al. 1995). This does not explain the entire difference, however; variation in respiratory efficiency and/or respiratory costs for processes like ion transport may play an additional role (Sect. 5.2.3).

Root respiration provides the driving force for root growth and maintenance and for ion absorption and transport into the xylem. The percentage of total assimilates that are used in root respiration tends to decrease as plants age. Such a decrease may be due to a decrease in the demand for respiratory energy, when the energy required for root growth and ion uptake decreases with increasing age. Furthermore, the root mass ratio tends to decrease with increasing age, thus decreasing the respiratory burden of roots.

The fraction of carbohydrates used in root respiration, including the respiration of symbionts, if present, is affected by both abiotic and biotic environmental factors (Table 1). Root respiration is higher in the presence of an **$N_2$-fixing symbiont** than when nonnodulated roots are supplied with $NO_3^-$ as a N source. This reflects the greater energy requirement for N-assimilation during $N_2$-fixation compared with $NO_3^-$-assimilation (Sect. 3 of Chapter 9A on symbiotic associations). The fraction of carbohydrates used in root respiration is also greater in the presence of a symbiotic **mycorrhizal fungus** than in nonsymbiotic plants (Table 1).

The *proportion* of the carbohydrates translocated to roots that is used in respiration, rather than root biomass accumulation, increases with plant age. This is primarily due to the increasing role of maintenance respiration, as root growth slows down and as the *quantity* of assimilates translocated to roots declines (Sect. 5.2). Low nutrient supply also increases the proportion of carbohydrates respired in the roots. At a high supply of nutrients, plants respire approximately 40% of the carbon imported into the roots. This fraction increases to 60% at very low nutrient supply (Van der Werf et al. 1992a). This increase is largely accounted for by a relatively high carbon requirement for maintenance processes compared with that in growth processes. An additional factor is the proportionally low requirement for root growth (relative to maintenance) under these low-nutrient conditions. Finally, specific costs for maintenance or ion uptake might increase when nutrients are in short supply (Van der Werf et al. 1994) (Sect. 5.2).

### *5.1.2 Respiration of Other Plant Parts*

Leaf respiration provides some of the metabolic energy for leaf growth and maintenance, for ion

transport from the xylem and export of solutes to the phloem. Leaf respiration, expressed as a fraction of the carbon gain in photosynthesis, however, varies much less than root respiration, because photosynthesis, leaf respiration and biomass allocation are affected similarly by changes in nutrient supply. This differs from the situation for roots, where a major cause of the large variation found for root respiration (Table 1) is the effect of nutrient supply and genotype on **biomass allocation** to roots.

Rates of photosynthesis and leaf respiration often vary in a similar manner with changes in environment (e.g., N supply and growth irradiance) (Reich et al. 1998). This may be explained by greater respiratory costs of export of photosynthates from leaves which vary with the carbon gained in photosynthesis. There may also be greater maintenance costs in leaves with high rates of photosynthesis and high protein concentrations. Specific costs for major energy-requiring processes (e.g., for transport of assimilates from the mesophyll to the sieve tubes) may also vary among species and environmental conditions (Cannell & Thornley 2000).

The respiration of other plant parts (e.g., fruits) is largely accounted for by their growth rate and the respiratory costs per unit of growth. The maintenance component also plays a role. In green fruits, a substantial proportion of this energetic requirement may be met by photosynthesis in the fruit (De Jong & Walton 1989, Blanke & Whiley 1995). Respiration of the flowers of *Citrus paradisi* (grapefruit) shows a distinct peak about 42 days after emergence. This peak occurs after a peak in respiration that is associated with growth of the flower. A major part of the respiration of the grapefruit flowers is probably accounted for by the alternative path (Bustan & Goldschmidt 1998, Considine et al. 2001).

## 5.2 Respiration Associated with Growth, Maintenance, and Ion Uptake

The rate of respiration depends on three major energy-requiring processes: **maintenance** of biomass, **growth,** and (ion) **transport**, as summarized in the following overall equation:

$$r = r_m + c_g \text{ RGR} + c_t \text{ TR} \quad (2)$$

where $r$ is the rate of respiration (normally expressed as nmol $O_2$ or $CO_2$ $g^{-1}$ $s^{-1}$, but to comply with the units in which RGR is expressed, we use here μmol $g^{-1}$ $day^{-1}$); $r_m$ is the rate of respiration to produce ATP for the maintenance of biomass; $c_g$ (mmol $O_2$ or $CO_2$ $g^{-1}$) is the respiration to produce ATP for the synthesis of cell material; RGR is the relative growth rate of the roots (mg $g^{-1}$ $day^{-1}$); $c_t$ (mol $O_2$ or $CO_2$ $mol^{-1}$) is the rate of respiration required to support TR, the transport rate (μmol $g^{-1}$ $day^{-1}$). In roots TR, equals the net ion uptake rate and the rate of xylem loading; in photosynthesizing leaves TR equals the rate of export of the products of photosynthesis (from mesophyll to sieve tubes). Although respiration can be measured as either $O_2$ uptake or as $CO_2$ release, the measurements do not yield exactly the same values. First, RQ may not equal 1.0 (Sect. 2.1); second, the rate of $CO_2$ release varies with the rate of $NO_3^-$ reduction, whereas rates of $O_2$ consumption do not. For this reason **$O_2$ consumption** is preferred as a basis to compare plants when we are interested in **respiratory efficiency**, whereas **$CO_2$ release** is preferred when comparing the **carbon budgets** of different plants.

By examining these three requirements for respiratory energy, we can estimate how the ATP produced in respiration is used for major plant functions. This equation assumes a tight correlation between the rate of respiration and the rates of major energy-requiring processes; there is no implicit assumption that respiration controls the rate of the energy-requiring processes, or vice versa.

### 5.2.1 Maintenance Respiration

Once biomass is produced, energy must be expended for repair and maintenance. Estimates of the costs of maintaining biomass range from 35 to 80% of the photosynthates produced per day (Amthor 2000), higher values pertaining to plants that grow very slowly (Lambers et al. 2002) and lower values to shade-adapted species (Noguchi et al. 2001b). The energy demands of the individual maintenance processes in vivo are not well known and reliable estimates of individual maintenance costs are scarce. A major part of the maintenance energy costs is supposed to be associated with **protein turnover** and with the maintenance of **solute gradients** across membranes. These costs of maintenance have been estimated from basic biochemical principles (Penning de Vries 1975, Amthor 2000, Bouma 2005).

In higher plants approximately 2–5% of all the proteins are replaced daily, with extreme estimates being as high as 20% (Van der Werf et al. 1992b, Bouma et al. 1994). It is quite likely that **protein turnover** rates vary among plant organs, species and with growth conditions, but the data are too scanty to make firm statements. The cost of

synthesizing proteins from amino acids is estimated at 4.7–7.9 ATP, and possibly double that, per peptide bond, or approximately 0.26 (possibly 0.52) g glucose $g^{-1}$ protein (Amthor 2000). Approximately 75% of amino acids from degraded proteins are recycled (Davies 1979). The remaining 25% must be synthesized from basic carbon skeletons, at a cost of 0.43 g glucose $g^{-1}$ protein. The total cost of protein turnover is about 28–53 mg glucose $g^{-1}$ $day^{-1}$, or 3–5% of dry mass per day. Similar calculations for lipids suggest that membrane turnover constitutes a much lower energy requirement, approximately 1.7 mg glucose $g^{-1}$ $day^{-1}$, or 0.2% of dry mass per day. Based on an experimentally determined protein half-life of 5 days, the respiratory energy requirement to sustain protein turnover is approximately 1 mmol ATP $g^{-1}$ (dry mass) $day^{-1}$ [i.e., 7% of the total respiratory energy produced in roots of *Dactylis glomerata* (cocksfoot)]. Expressed as a fraction of the total maintenance requirement as derived from a multiple regression analysis (Sect. 5.2) [i.e., 2.7 mmol ATP $g^{-1}$ (dry mass) $day^{-1}$ for *Carex* (sedge) species], the maintenance requirement for protein turnover is quite substantial (Van der Werf et al. 1992b).

Maintenance of **solute gradients** is also an important maintenance process. Some estimates suggest that the cost of maintaining solute gradients are up to 30% of the respiratory costs involved in ion uptake, or approximately 20% of the total respiratory costs of young roots (Bouma & De Visser 1993).

Other processes (e.g., cytoplasmic streaming and turnover of other cellular constituents) are generally assumed to have a relatively small cost. Based on these many (largely unproven) assumptions, the total estimated maintenance respiration is approximately 30–60 mg glucose $g^{-1}$ $day^{-1}$ (3 to 6% of dry mass $day^{-1}$). Measured values of maintenance respiration (8–60 mg glucose $g^{-1}$ $day^{-1}$) suggest that these rough estimates are reasonable.

These experimental values for maintenance respiration suggest that protein turnover and the maintenance of solute gradients are by far the largest costs of maintenance in plant tissues. If true, then this conclusion has important implications for plant carbon balance because it suggests that any factor that increases protein concentration or turnover or the leakiness of membranes will increase maintenance respiration.

The positive correlation of respiration rate with N concentration (Reich et al. 2006) is consistent with the prediction that maintenance respiration depends on protein concentration. Thus, leaves that have a high N investment in Rubisco and other photosynthetic enzymes have a correspondingly high maintenance respiration. Whether this is a general phenomena remains to be investigated (Van der Werf et al. 1992b). Higher respiration rates might also reflect greater costs for the loading of photosynthates in the phloem, which is an ATP-requiring process (Sect. 3.3 of Chapter 2C on long-distance transport). Whatever the explanation for the higher leaf respiration rates, they do contribute to their higher light-compensation point (Sect. 3.2.1 of Chapter 2A on photosynthesis) and, therefore, place a higher limit on the irradiance level at which these leaves can maintain a positive carbon balance. Thus, there is a **trade-off** between high metabolic activity (requiring high protein concentrations and rapid loading of the phloem) and the associated increase in cost of maintenance and transport.

The stimulation of maintenance respiration by temperature is a logical consequence of the increased leakage and of protein turnover that occurs at high temperature (Rachmilevitch et al. 2006). This provides a conceptual framework for studies that seek to explain why different tissues and species differ in their $Q_{10}$ of respiration. Perhaps this reflects differences in membrane properties upon prolonged exposure to higher temperatures or in thermal stability of proteins, with corresponding differences in protein turnover (Criddle et al. 1994). It might also reflect a difference in contribution of the cytochrome and the alternative pathways.

Increased maintenance respiration is often assumed to be the cause of declines in forest productivity in late succession (e.g., Waring & Schlesinger 1985). Maintenance respiration remains relatively constant through succession, however, while growth respiration declines (Fig. 21). The more likely cause of

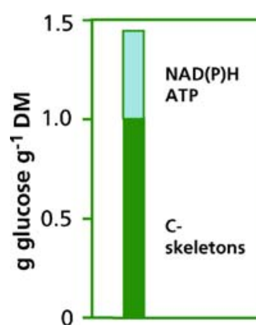


**Figure 21. Construction costs of leaf biomass. Most of the glucose required for biomass production ends up in the carbon compounds in the biomass. Because the average carbon compound biomass is more reduced than the carbohydrates from which it is produced, some glucose is required to produce NADPH. Some of the glucose is required to produce ATP, that drives many energy-requiring biosynthetic reactions in the cell. The data are for an "average" leaf.**

reduced growth in old forest stands is a reduced carbon gain caused by loss of leaf area and loss of photosynthetic capacity associated with reduced hydraulic conductance and in some cases with reduced nutrient availability (Ryan et al. 1997).

### *5.2.2 Growth Respiration*

Production of biomass (**biosynthesis**) requires the input of carbohydrates, partly to generate ATP and NAD(P)H for biosynthetic reactions and partly to provide the carbon skeletons present in biomass (Fig. 22; Table 9). Plant tissue is, in general, more reduced than the carbohydrates from which it is produced, and the cost of biosynthesis from primary substrates must therefore include the carbohydrates necessary to supply reducing power, for example for the reduction of $NO_3^-$. If a more reduced source of N is absorbed instead (e.g., $NH_4^+$ or amino acids) (Sect. 2.2 of Chapter 6 on mineral nutrition), then biosynthetic costs are less. When a tissue senesces, most of the chemical constituents are lost to the plant, but some are resorbed and can be used in the production of new tissues. The **final cost** of producing a tissue is the **initial cost** minus **resorption** (Fig. 23).

In photosynthetically active leaves, some of the metabolic energy (ATP and NAD(P)H) may come directly from photosynthesis. In heterotrophic tissues such as roots, and in leaves in the dark, respiration provides the required energy. The amount of respiratory energy that is required for biosynthesis can be calculated from the composition of the biomass in several ways, as discussed in this section.

First, costs for biosynthesis can be derived from detailed information on the **biochemical composition**, combined with biochemical data on the costs of synthesis of all the major compounds: protein, total nonstructural carbohydrates (i.e., sucrose, starch, fructans), total structural carbohydrates (i.e., cellulose, hemicellulose), lignin, lipid, organic acids, minerals. This can be extended to include various other compounds, e.g., soluble amino acids, nucleic acids, tannins, lipophilic defense compounds, alkaloids, but these are mostly ignored and generally combined with the major ones. Taking glucose as the standard substrate for biosynthesis, one can estimate the amount of glucose required to provide the carbon skeletons, reducing equivalents and ATP for the biosynthesis of plant compounds in tissues (Table 9; Poorter & Villar 1997).

Note that the amount of product produced per unit carbon substrate (production value, PV′) varies nearly threefold among chemical constituents (Table 10), with lipid and lignin being "most expensive" (i.e., requiring greatest glucose investment per gram of product), and organic acids "least expensive". Compounds like proteins and lipids are very costly in terms of ATP required for their biosynthesis, whereas carbohydrates and cellulose are not. There are both expensive and cheap ways to produce structure in plants (lignin and cellulose/hemicellulose, respectively) and to store energy (lipid and sugars/starch, respectively) (Chapin 1989). Plants generally use energetically cheap structural components (cellulose/hemicellulose) and energy stores (sugars and starch). By contrast, mobile animals and small seeds, where mass is an important issue, often use lipids as their energy store. Immobile animals, like plants, use carbohydrate (glycogen) as their primary energy store. Knowing the costs and concentrations of the major compounds

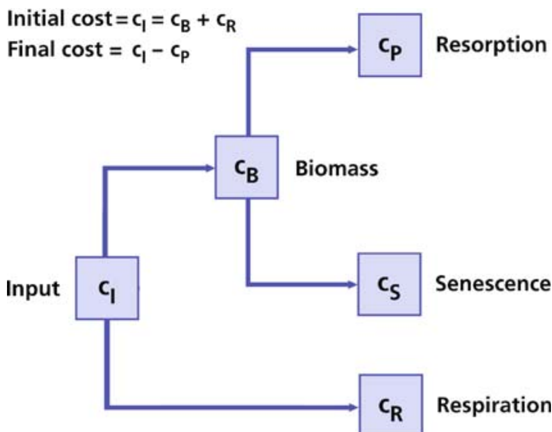


FIGURE 22. Fate of carbon that is initially invested ($C_I$) in synthesizing a structure. Some of the carbon is retained in the biomass ($C_B$), the remainder is required for respiration ($C_R$). Of the carbon in the biomass ($C_B$), most is lost or respired when a plant part is shed ($C_S$) but some is resorbed ($C_P$) for subsequent use (after Chapin 1989).

TABLE 9. Values for characterizing the conversion of substrates to products during biosynthesis, excluding costs of substrate uptake from the environment.*

| Compound | PV′ | ORF′ | CPF′ | RQ′ | HRF | ERF |
|---|---|---|---|---|---|---|
| Amino acids with $NH_4^+$ | 700 | 169 | 5772 | 34 | –11.2 | –1.4 |
| Amino acids with $NO_3^-$ | 700 | 169 | 5772 | 34 | 26.7 | 39.0 |
| Protein with $NH_4^+$ | 604 | 163 | 5727 | 35 | –12.9 | 34.9 |
| Protein with $NO_3^-$ | 604 | 163 | 5727 | 35 | 31.4 | 82.0 |
| Carbohydrates | 853 | 0 | 1295 | – | –3.6 | 12.2 |
| Lipids | 351 | 0 | 10705 | – | –10.1 | 51.0 |
| Lignin | 483 | 1388 | 5545 | 4 | –4.3 | 18.7 |
| Organic acids | 1104 | 0 | –1136 | – | 16.9 | –4.5 |

*Source*: De Visser et al. (1992).

* Production Value, PV′: mg of the end product per g of substrate required for carbon skeletons and energy production, without taking into account the fate of excess or shortage of NAD(P)H and ATP (the term Production Value, PV, is used when PV′ is corrected for this component); Oxygen Requirement Factor, ORF′: μmol of $O_2$ consumed per gram of substrate required for carbon skeletons and energy production, without taking into account the fate of excess or shortage of NAD(P)H and ATP; Carbon dioxide Production Factor, CPF′: μmol of $CO_2$ produced per g of substrate required for carbon skeletons and energy production, without taking into account the fate of excess or shortage of NAD(P)H and ATP (the term Carbon dioxide Production Factor, CPF, is used when CPF′ is corrected for this component); RQ′ is the ratio of CPF′ and ORF′; Hydrogen Requirement Factor, HRF: moles of NAD(P)H required (–) or produced (+) per gram of end product; Energy Requirement Factor, ERF: moles of ATP required (–) or produced (+) per gram of end product (Penning de Vries et al. 1974). More recent findings, for example on the importance of targeting sequences of proteins which are required to "direct" the synthesized proteins to a specific compartment in the cell, indicate that the costs for protein synthesis are likely to be substantially, possibly even double the value as presented in this table.

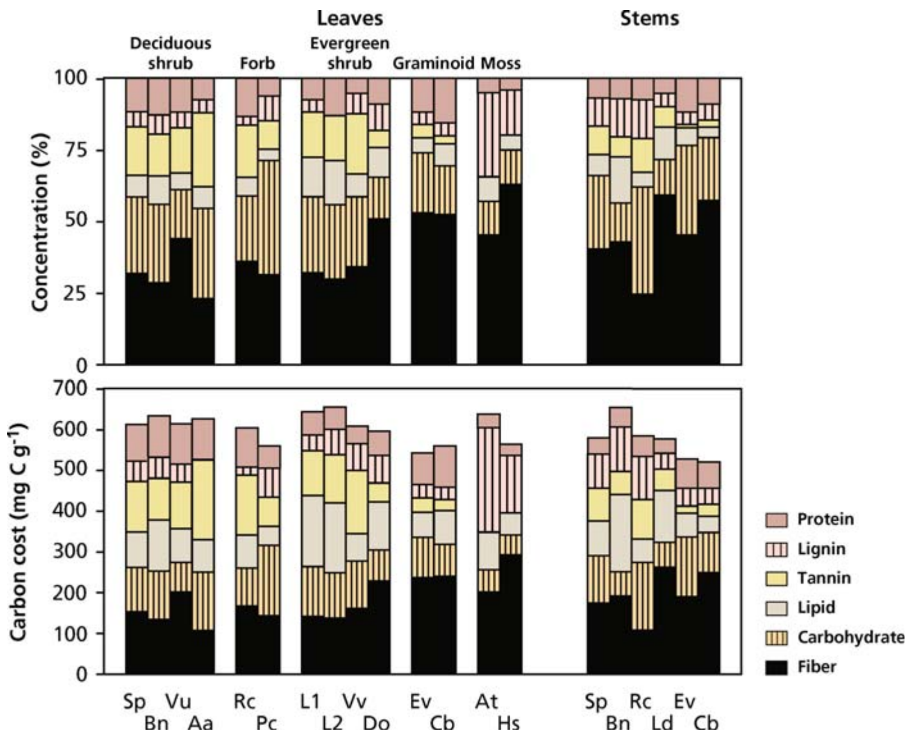


FIGURE 23. The chemical composition and carbon cost of producing leaves and stems of 13 species of tundra plants. Species shown are *Salix pulchra* (willow, Sp), *Betula nana* (dwarf birch, Bn), *Vaccinium uliginosum* (blueberry, Vu), *Arctostaphylos alpina* (bearberry, Aa), *Rubus chamaemorus* (cloudberry, Rc), *Pedicularis capitata* (wooly lousewort, Pc), *Ledum decumbens* (Labrador tea, Ld, including 1-year old, L1, and 2-year old, L2, leaves), *Vaccinium vitis-idaea* (low-bush cranberry, Vv), *Dryas octopetala* (mountain avens, Do), *Eriophorum vaginatum* (tussock cottongrass, Ev), *Carex bigelowii* (Bigelow sedge, Cb), *Aulocomnium turgidum* (turgid aulacomnium moss, At), and *Hylocomium splendens* (feathermoss, Hs) (after Chapin 1989).

TABLE 10. An example of a simplified calculation of the variables characterizing biosynthesis of biomass from glucose, nitrate and minerals.

| Compound | Concentration in biomass required(mg $g^{-1}$ dry mass) | Glucose for synthesis | $O_2$ required for synthesis (μmol) | $CO_2$ production during synthesis (mmol) | NAD(P)H required for synthesis (mmol) | ATP required for synthesis (mmol) |
|---|---|---|---|---|---|---|
| N-compounds | 230 | 371 | 65 | 2100 | 7.14 | 17.83 |
| Carbohydrates | 565 | 662 | 0 | 857 | −2.03 | 6.92 |
| Lipids | 25 | 71 | 0 | 807 | 0.25 | 1.27 |
| Lignin | 80 | 166 | 230 | 918 | −0.34 | 1.50 |
| Organic acids | 50 | 45 | 0 | −52 | −0.84 | −0.23 |
| Minerals | 50 | 0 | 0 | 0 | 0 | 0 |
| Total | 1000 | 1315 | 295 | 4630 | 3.68 | 27.29 |

*Source*: Penning de Vries et al. (1974).

in plant biomass, we can calculate the costs for a gram of biomass. As for individual compounds, these costs can be expressed in terms of glucose, $O_2$ requirement, $CO_2$ release, requirement for reducing power and ATP (Table 10).

The major assumption underlying the approach based on the biochemical composition of the biomass is that glucose is the sole substrate for all ATP, reductant, and carbon skeletons. When some of these resources are derived directly from photosynthesis, costs may be lower. Costs may be higher when the alternative path, rather than the cytochrome path plays a predominant role in respiration. If we restrict this approach to nonphotosynthetic tissues in which the contribution of the cytochrome and alternative respiratory pathway is known, then there is still a source of error, if these tissues import compounds other than glucose, for example amino acids, as a substrate for biosynthesis.

A second method for estimating the construction cost is based on information on the **elemental composition** of tissues: C, H, O, N, and S (McDermitt & Loomis 1981). The constructions costs that are not covered by this equation include costs of mineral uptake and transport of various compounds in the plant, costs for providing ATP for biosynthetic reactions, and reductant required to reduce molecular oxygen in some biosynthetic reactions. This method is less laborious than the first method, which requires detailed chemical analysis; however, it is based on the observations of the first method (i.e., that expensive compounds are generally more reduced than glucose, whereas cheap compounds are more oxidized) (Poorter 1994). Although this method, based on elemental analysis of plant biomass, may seem a crude approach, the approach is surprisingly effective. First, this is because two thirds of the construction costs are costs to provide carbon skeletons rather than for respiration. Second, most of the carbon that does not end up in the carbon skeletons of biomass is required to reduce carbon skeletons, and not for the production of ATP. So, even in the absence of detailed information on respiratory pathways, construction costs can be estimated rather accurately. In fact, the second method can be simplified even further, taking into account only the **carbon and ash content** of biomass and ignoring minor constituents that have only a small effect on the production value (Vertregt & Penning de Vries 1987).

The level of reduction of plant biomass is approximately linearly related to its **heat of combustion** as well as its costs of construction (McDermitt & Loomis 1981). For example, lipids are highly reduced compounds and have a high heat of combustion. A third method, therefore, uses this approximation to arrive at costs for providing carbon skeletons and reductant for biosynthesis (Williams et al. 1987).

Given the three-fold range in the cost of producing different organic constituents in plants and the large range in concentrations of these constituents among plant parts and species [often 2–10-fold (Fig. 24)], we might expect large differences in costs of synthesizing tissues of differing chemical composition. A given tissue, however, tends to have *either* a high concentration of proteins and tannins (allowing high metabolic activity and chemical defense of these tissues) *or* a high concentration of lignin and lipophilic secondary metabolites (Chapin 1989). The negative correlation between the concentrations of these two groups of

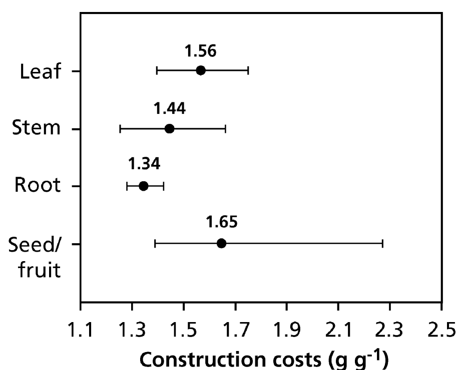


**Figure 24. Range of construction costs for a survey of leaves (*n* = 123), stems (*n* = 38), roots (*n* = 35), and fruits/ seeds (*n* = 31). Values are means and 10th and 90th percentiles (Poorter 1994). Copyright SPB Academic Publishing.**

expensive constituents is seen in the comparison of leaves *vs.* stems or in the comparison of leaves of rapidly growing species (e.g., forbs) and slowly growing species (evergreen shrubs) (Fig. 24). The net result of this trade-off between expensive components allowing rapid metabolic activity (proteins) vs. those allowing persistence (lignin and lipophilic defensive compounds) is that the cost of all plant species and plant parts are remarkably similar: approximately 1.5 g glucose per gram of biomass (Figs. 23 and 24). Another important correlation that explains the similarity of construction costs across species and tissues is that tissues of fast-growing species that have high protein concentrations (an expensive constituent) also have high concentrations of minerals (cheap constituents) (Poorter 1994, Villar et al. 2006). This explains why extremely simple relationships are excellent predictors of costs of synthesis. The similarity of cost of synthesis across species, plant parts and environments (Chapin 1989, Villar et al. 2006) differs from early conclusions that emphasized the high costs associated with lipids and lignin in evergreen leaves (Miller & Stoner 1979).

Small seeds are an exception to the generalization that all plant biomass has a similar cost of synthesis, because seed lipids are primarily an energy store (rather than an antiherbivore defense) and are positively associated with protein concentration (Fig. 25), leading to a high carbon cost. The similarity among species and tissues in carbon cost of synthesis has the practical consequence that biomass is an excellent predictor of carbon cost. One possible ecological explanation for this pattern is that carbon is such a valuable resource that natural selection has led to the same minimal carbon cost for the construction of most plant parts. An alternative, and more probable, explanation is that the negative correlations among ex-pensive constituents and the positive correlation between protein and minerals have a basic physiological significance that, by coincidence, leads to a similar carbon cost of synthesis in most structures. For example, lignin and protein concentrations may be negatively correlated because young expanding cells have a high protein concentration, but cell expansion would be prevented by lignin, or that heavy lignification might render cell walls less permeable to water and solutes which would be disadvantageous in tissues with high metabolic activity (as gauged by high protein concentration). In general, currently available data suggest that costs of synthesis differ much less within (10–20%) and among (25%) ecosystems than do other causes of variation in carbon balance, such as respiration and allocation (Chapin 1989, Villar et al. 2006).

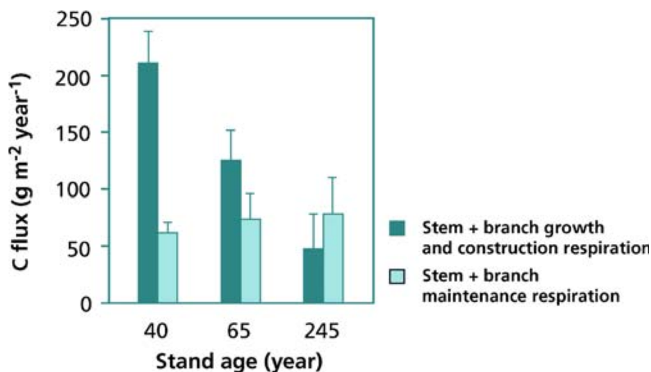


**Figure 25. Annual carbon use for stem and branch growth (growth costs) and for stem and branch maintenance respiration in a lodgepole pine (*Pinus contorta*) successional sequence. Error bars show 95% confidence intervals (Ryan & Waring 1992). Copyright Ecological Society of America.**

### *5.2.3 Respiration Associated with Ion Transport*

Ion transport across membranes may occur via ion channels, if transport is down an electrochemical potential gradient, or via ion carriers, which allow transport against an electrochemical potential gradient (Sect. 2.2.2 of Chapter 6 on mineral nutrition). Because cation **transport from the rhizosphere** into the symplast mostly occurs down an electrochemical potential gradient, cation channels are often involved in this transport. This requires respiratory energy to extrude protons into the apoplast and create an **electrochemical potential gradient**. Transport of anions from the rhizosphere into the symplast almost invariably occurs against an electrochemical gradient and hence requires respiratory energy, mostly because such anion transport is coupled to proton re-entry into the cells (Sect. 2.2.2 of Chapter 6 on mineral nutrition).

The situation is exactly the opposite for the transport of ions from the symplast to the xylem (**xylem loading**). Anions might enter the xylem via channels, as this transport is mostly down an electrochemical gradient; however, we know little about such a mechanism (De Boer & Wegner 1997). The transport of most cations is against an electrochemical gradient, and hence the transport of cations to the xylem depends directly on metabolic energy. Release of anions into the xylem may be passive, but it still depends on the presence of an electrochemical potential gradient, which is maintained by the expenditure of metabolic energy. On the other hand, resorption of anions must be active (involving carriers) whereas that of cations may occur via channels (Wegner & Raschke 1994, De Boer & Wegner 1997).

When $NO_3^-$ is the major source of N, this will be the major anion absorbed, because only 10% and 1%, respectively, as much P and S compared with N are required to produce biomass (Fig. 33 in Sect. 4.1.1 of Chapter 6 on mineral nutrition). Uptake of amino acids will also be against an electrochemical potential gradient and hence require a proton-cotransport mechanism similar to that described for $NO_3^-$. Like the uptake of $NO_3^-$ and amino acids, P uptake also occurs via a proton symport mechanism (Sect. 2.2.2 of Chapter 6 on mineral nutrition). When P availability is low, however, P acquisition may require exudation of carboxylates (Sect. 2.2.5 of Chapter 6 on mineral nutrition) which will incur additional carbon expenditure. Similarly, P acquisition through a symbiotic association with mycorrhizal fungi requires additional carbon (Sect. 2.6 of Chapter 9A on symbiotic associations).

As long as there is an electrochemical potential gradient, which is a prerequisite for the uptake of anions, cations can enter the symplast passively. In fact, plants may well need mechanisms to excrete cations that have entered the symplast passively, to avoid excessive uptake of some cation (e.g., $Na^+$) (Sect. 3.4.2 of Chapter 6 on mineral nutrition). When $NH_4^+$ is the predominant N source for the plant, such as in acid soils where rates of nitrification are low, this can enter the symplast via a cation channel. Rapid uptake of $NH_4^+$, however, must be balanced by excretion of $H^+$, so as to maintain a negative membrane potential. Hence, $NH_4^+$ uptake also occurs with expenditure of respiratory energy.

When $NO_3^-$ is the predominant N-source, rather than $NH_4^+$ or amino acids, there are additional costs for its reduction. These show up with carbon costs and $CO_2$ release, but not in $O_2$ uptake (Table 9), because some of the NADH generated in respiration is used for the reduction of $NO_3^-$ rather than for the reduction of $O_2$. As a result, the RQ strongly depends on the source of N ($NH_4^+$ or $NO_3^-$; Table 2) and on the rate of $NO_3^-$ reduction. Costs associated with $NO_3^-$ acquisition are less when the reduction of $NO_3^-$ occurs in leaves exposed to relatively high light intensities, as opposed to reduction in the roots, because the reducing power generated in the light reactions exceeds that needed for the reduction of $CO_2$ in the Calvin cycle under these conditions (Sect. 3.2.1 of Chapter 2A on photosynthesis).

Given that N is a major component of plant biomass, most of the respiratory energy associated with nutrient acquisition in plants with free access to nutrients will be required for the uptake of this nutrient.

### *5.2.4 Experimental Evidence*

Measurements made with roots provide an opportunity to test the concepts of maintenance respiration, growth respiration, and respiration associated with transport. We assume that the rate of respiration for maintenance of root biomass is linearly related to the root biomass to be maintained. Second, we assume that the rate of respiration for ion transport is proportional to the amount of ions taken up, whereas that for root growth is proportional to the relative growth rate of the roots, provided the chemical composition of the root biomass does not change in a manner that affects the

specific costs of biomass synthesis; superimposed is the maintenance respiration. Third, we assume that the contribution of the alternative path to total respiration is constant. Based on these assumptions, which are largely untested, the rate of ATP production per gram of roots and per day can be related to the relative growth rate of the roots and the rate of anion uptake by the roots. We can improve the approach by assessing the contribution of the **alternative path** (Box 2B.1), and correct for any changes during plant development (Florez-Sarasa et al. 2007). The costs of the three processes can then be estimated by **multiple regression analysis**, presented graphically in a three–dimensional plot (Fig. 26, left). If a plant's relative growth rate and rate of anion uptake are very closely correlated, which is common, then a multiple regression analysis cannot separate the costs of growth from those of ion uptake (Fig. 26, right).

Using the analysis as depicted in Fig. 26A, respiratory costs for growth, maintenance, and ion uptake have been obtained for a limited number of species (Table 11A). Quite often, the correlation between relative growth rate and nutrient uptake is so tight, that a linear regression analysis, as depicted in Fig. 26B, is the only approach possible (Table 11B). There is quite a large variation in experimental values among species. This may reflect real differences between species; however, the variation may also indicate that the statistical analysis "explained" part of respiration by ion uptake in one experiment and by maintenance in another. For example, a costly process like ion leakage from roots, followed by re-uptake, may show up in the slope or in the y-intercept in the graph, and suggest large costs for ion uptake or for maintenance, respectively. At the highest rates of growth and ion uptake (young plants, fast-growing species) these data suggest that respiration for growth and ion uptake together account for about 60% of root respiration, and that maintenance respiration is relatively small. With increasing age, when growth and ion uptake slow down, maintenance respiration accounts for an increasing proportion of total respiration (over 85%).

The specific costs for *Carex* (sedge) species (Table 11A) were used to calculate the rate of root respiration of 24 other herbaceous species of differing potential growth rate whose rates of growth and ion uptake were known. These calculations greatly over-estimate the rate of root respiration of fast-growing species, when compared with measured values (Fig. 27). This suggests that

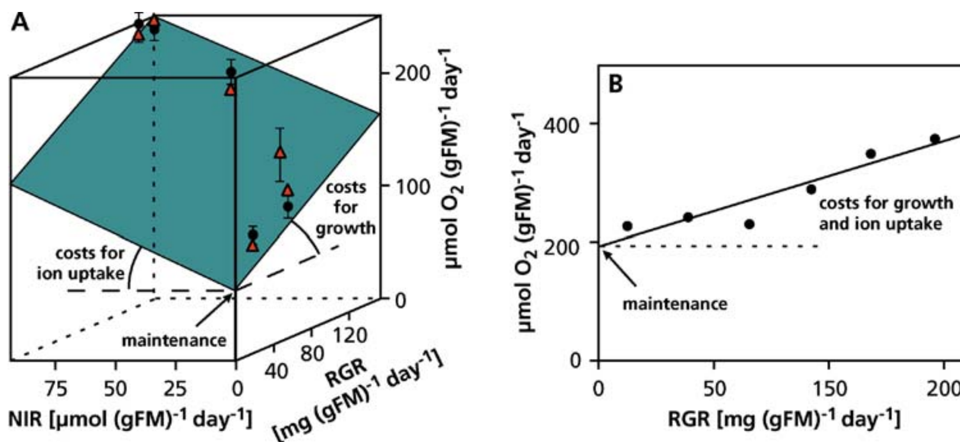


FIGURE 26. (A) Rate of $O_2$ consumption per unit fresh mass (FM) in roots as related to both the relative growth rate (RGR) of the roots and their net rate of anion uptake (NIR). (B) Rate of $O_2$ consumption per unit fresh mass in roots as related to the relative growth rate of the roots. The plane in (A) and line in (B) give the predicted mean rate of $O_2$ consumption. The intercept of the plane in (A) and the line in (B) with the *y*-axis gives the rate of $O_2$ consumption in the roots which is required for maintenance. The slope of the projection of the line on the y–z plane gives the $O_2$ consumption required to produce one gram of biomass. When projected on the *x*–*y* plane, the slope gives the specific respiratory costs for ion transport. In (B) the slope gives costs for growth including ion uptake (after Lambers et al. 2002).

Table 11. (A) Specific respiratory energy costs for the maintenance of root biomass, for root growth and for ion uptake. (B) Specific respiratory energy costs for the maintenance of root biomass and for root growth including costs for ion uptake.

| | *Carex* species | *Solanum tuberosum* | *Zea mays* | |
|---|---|---|---|---|
| A. | | | | |
| Growth, mmol $O_2$ (g dry mass)$^{-1}$ | 6.3 | 10.9 | 9.9 | |
| Maintenance, nmol $O_2$ (g dry mass)$^{-1}$ s$^{-1}$ | 5.7 | 4.0 | 12.5 | |
| Anion uptake, mol $O_2$ (mol ions)$^{-1}$ | 1.0 | 1.2 | 0.53 | |
| B. | | | | |
| | *Dactylis glomerata* | *Festuca ovina* | *Quercus suber* | *Triticum aestivum* |
| Growth + ion uptake, mmol $O_2$ (g dry mass)$^{-1}$ | 11 | 19 | 12 | 18 |
| Maintenance, nmol (g dry mass)$^{-1}$ s$^{-1}$ | 26 | 21 | 6 | 22 |

*Sources*: (A) The values were obtained using a multiple regression analysis, as explained in Figure 25A [Van der Werf et al. 1988: average values for *Carex acutiformis* (pond sedge) and *Carex diandra* (lesser panicled sedge); Bouma et al. 1996: *Solanum tuberosum* (white potato); Veen 1980: *Zea mays* (corn)]. (B) The values were obtained using a linear regression analysis, as explained in Figure 25B [Scheurwater et al. 1998: *Dactylis glomerata* (cocksfoot) and *Festuca ovina* (sheep's fescue); Mata et al. 1996: *Quercus suber* (cork oak); Van den Boogaard, as cited in Lambers et al. 2002: *Triticum aestivum* (wheat)].

either the efficiency of respiration is greater (e.g., relatively more cytochrome path and less alternative path activity) in fast-growing species, or that the specific costs for growth, maintenance or ion uptake are lower for fast-growing species. Is there any evidence to support either hypothesis?

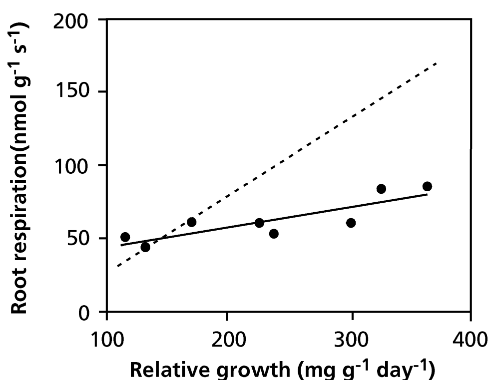


Figure 27. The rate of root respiration of fast-growing and slow-growing herbaceous $C_3$ species. The broken line gives the calculated respiration rate, assuming that specific costs for growth, maintenance, and ion uptake are the same as those given in Table 11 and identical for all investigated species (Poorter et al. 1991). Copyright Physiologia Plantarum.

Roots of fast-growing grass species exhibit higher rates of alternative path activity than slow-growing grasses (Millenaar et al. 2001), and hence there is no evidence for a more efficient respiration in roots of fast-growing species. Specific respiratory costs for root growth are somewhat higher for fast-growing species (Fig. 28A), and maintenance costs, if anything, are higher, rather than lower, for roots of fast-growing species, possibly be due to their higher protein concentrations and associated turnover costs (Scheurwater et al. 1998, 2000). If neither a low respiratory efficiency nor higher costs for growth or maintenance can account for unexpectedly fast respiration rates of slow-growing plants, then the discrepancy between the expected and measured rates of root respiration (Fig. 27) must be based on higher **specific costs** for ion uptake in the inherently slow-growing species (Fig. 28B). These higher specific costs when plants are grown with free access to $NO_3^-$ are accounted for by a large **efflux of $NO_3^-$** (Sect. 2.2.2 of Chapter 6 on mineral nutrition; Scheurwater et al. 1999). It should be noted that many slow-growing species naturally grow in a low-$NO_3^-$

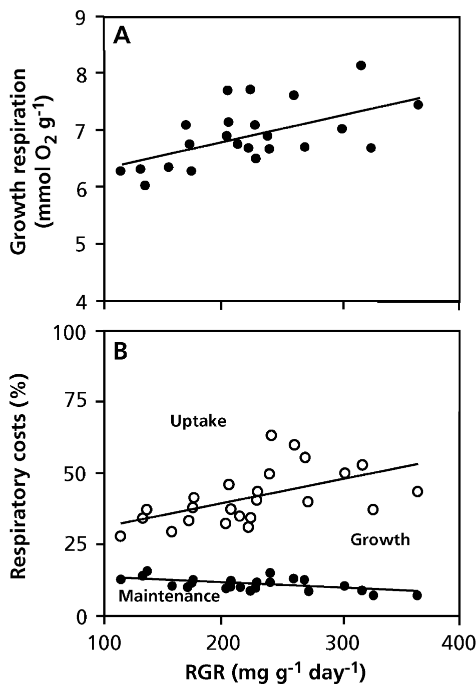


**FIGURE 28. Characteristics of root respiration of inherently fast- and slow-growing herbaceous species, grown at free nutrient availability. (A) Respiratory cost for growth, as derived from an analysis of the roots' chemical composition and known cost for the synthesis of the various plant compounds. (B) Assuming similar respiratory efficiencies and maintenance costs for all species and using the costs for growth as given in (A), the specific costs for ion uptake were calculated. It is suggested that these costs are substantially higher for slow-growing herbaceous species than for fast-growing ones (Poorter et al. 1991). Copyright Physiologia Plantarum.**

environment, and hence would rarely be exposed to the experimental conditions as used referred to here. The question that remains to be addressed is whether $NO_3^-$ efflux also plays a role when $NO_3^-$ availability limits plant growth. Given that root respiration rates are also unexpectedly high for plants grown at a severely limiting $NO_3^-$ supply (Fig. 14), this is certainly a likely possibility.

The rate of root respiration of plants grown with a limiting nutrient supply is lower than that of plants grown with free access to nutrients, but not nearly as low as expected from their low rates of growth and nutrient acquisition (Sect. 4.3). This again suggests increased specific costs, possibly for ion uptake. Further experimental evidence is needed to address this important question concerning the carbon balance and growth of slow-growing plants.

In summary, experimental data suggest that the concept of respiration associated with growth, maintenance, and ion uptake is a valuable tool in understanding the carbon balance of plants and that the partitioning of respiration among these functions may differ substantially with environment and the type of plant species.

## 6. Plant Respiration: Why Should It Concern Us from an Ecological Point of View?

A large number of measurements have been made on the gas exchange (i.e., rates of photosynthesis, respiration, and transpiration) of different plants growing under contrasting conditions. Those measurements have yielded fascinating experimental results, some of which have been discussed in Chapter 2A on photosynthesis. There is often the (implicit) assumption, however, that rates of photosynthesis provide us with vital information on plant growth and productivity. Certainly, photosynthesis is essential for most of the gain in plant biomass; however, can we really derive essential information on growth rate and yield from measurements on photosynthesis alone?

Rates of photosynthesis per unit leaf area are poorly correlated with rates of growth, let alone final yield (Evans 1980). One of the reasons that have emerged in this chapter on plant respiration is that the fraction of all carbohydrates that are gained in photosynthesis and subsequently used in respiration varies considerably. First, slow-growing genotypes require relatively more of their photoassimilates for respiration. Secondly, many environmental variables affect respiration more than photosynthesis. This is true both because respiration rate is sensitive to environment and because the size of nonphotosynthetic plant parts, relative to that of the photosynthetically active leaves depends on the environment, as discussed in Chapter 7 on growth and allocation. Clearly, an important message from this chapter on plant respiration should be that measurements of leaf photosynthesis by themselves cannot provide us with sound information on a plant's growth rate or productivity.

A second message worth emphasizing here is that respiration and the use of respiratory energy [NAD(P)H, ATP] are not as tightly linked as long believed. Respiration may proceed via pathways that do not yield the respiratory products needed

for growth, but produces heat instead. These components of respiration can be substantial, at least in some plants under some conditions. In specific tissues the production of heat may be of use to the plant, but the ecophysiological significance of it in other tissues is different.

A challenge for the future will be to explore to what extent respiration scales with other plants traits, as has been done for photosynthesis (Sect. 6 of Chapter 2A). There is clear evidence that specific respiration rates scale with tissue N concentration, just like photosynthesis does, but we have yet to explore scaling patterns with a range of other traits.

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