CHAPTER

4

Physiology of Forage Plants

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Forage physiology describes the integrated function of forage plants, from biochemical pathways operating at the cellular level to plant growth and development in the field. The most fundamental biochemical pathways in plants are **photosynthesis** and **respiration**.

Photosynthesis captures solar energy and stores it in simple sugars, referred to as **photosynthate**. Photosynthesis is the first step in the food chain that supports all animals, including humans. Photosynthate can either be used immediately in metabolism, to provide the building blocks of plant tissues, or it can be stored for future use. Respiration releases the energy stored in photosynthate, allowing it to be used for cellular metabolism. This energy is needed to build proteins, lipids, and complex carbohydrates, to fuel the growth of roots and shoots, to absorb mineral nutrients from the soil, and to support the enzymatic reactions that maintain mature tissue.

The goal of this chapter is to demonstrate how underlying plant processes interact with management and the environment to determine the productivity, nutritive value, and persistence of forages and grasslands.

Forages and the Productivity of Agricultural Land

The majority of land available for agriculture is too steep, wet, dry, rocky, or vulnerable to wind or water erosion to be used for row crop production. Grazing of forage plants by ruminants can be the most beneficial use of this land, because perennial forage grasses and legumes provide permanent ground cover that harvests solar energy, slows wind, aids water infiltration, adds significant organic matter and nitrogen (N) to improve soil health, and can—through grazing—support the production of high-quality protein for human consumption. During grazing, most of the nutrients contained in the forages consumed by livestock are recycled to the soil as urine and dung. Thus perennial forages can preserve or improve the least favorable agricultural land while supporting food production by converting solar energy into feed for animals.

The Photosynthetic Process

Photosynthesis occurs in **chloroplasts**, which are located in the cells of all green tissues of plants, but especially in the leaves. Leaves have the optimal structure for maximizing the interception of light and the absorption of carbon dioxide (CO_2) that is converted into photosynthate. Chloroplasts are filled with an aqueous matrix, called the **stroma**, which contains a system of **thylakoid membranes** that are folded into stacks called **grana** (Fig. 4.1).

The thylakoid membranes are flattened tubes in which clusters of chlorophyll and carotenoid pigments are embedded next to enzyme complexes. In the initial photochemical reactions of photosynthesis, light increases the energy of the pigment clusters, causing the release of electrons that are captured by the enzyme complexes. Some of the chemical energy generated as these electrons are passed along an electron transport chain on the thylakoid membranes is sequestered inside the thylakoid membranes, and then released in a controlled process that is used to form adenosine triphosphate (**ATP**). The final step in the photochemical reactions of photosynthesis is the capture of the chlorophyll electrons and their energy in the reduction of nicotinamide adenine dinucleotide phosphate (**NADPH**).

Both ATP and NADPH are formed in the stroma of the chloroplast, where they are subsequently used to reduce CO_2 to sugars. Nitrate (NO_3^-) and sulfate (SO_4^{2-}) are also reduced in chloroplasts using ATP. However, N constitutes

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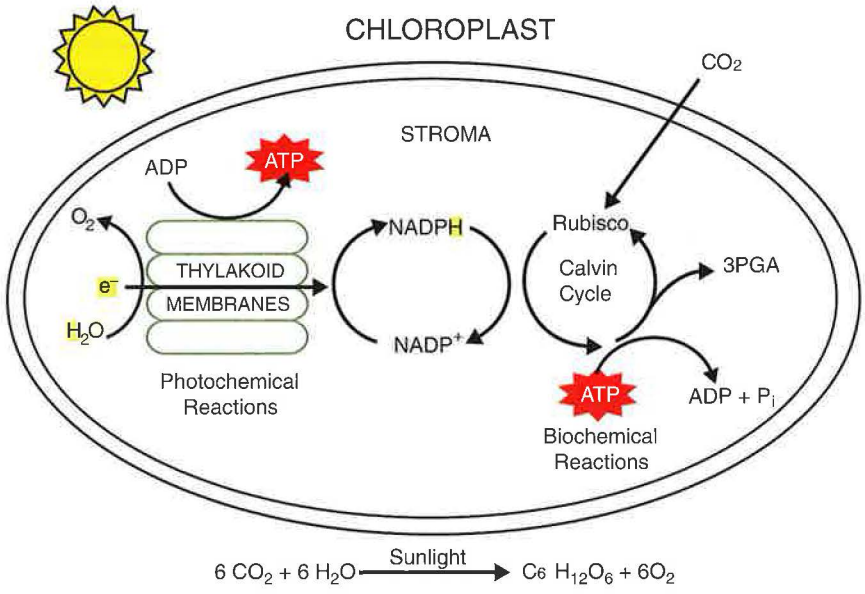

Fig. 4.1. In chloroplasts, the photochemical reactions of photosynthesis capture solar energy, while the biochemical reactions of photosynthesis use the energy for carbohydrate synthesis. (Adapted from MacAdam, 2009.)

just 2–3% of plant dry weight and sulfur (S) only 0.2%, whereas carbon (C) represents about 45% of the dry weight of plants.

Carbohydrates are formed in chloroplasts by adding CO_2 to the 5-C sugar ribulose-1,5-bisphosphate (RuBP) through the activity of the enzyme ribulose bisphosphate carboxylase/oxygenase (**rubisco**) (Fig. 4.1). Rubisco is an abundant enzyme, constituting about 40% of the soluble protein in the leaves of **cool-season** or **C_3 plants**. Functioning as a carboxylase, rubisco adds CO_2 to RuBP to form a highly unstable 6-C compound that immediately splits to form two molecules of the 3-C compound 3-phosphoglycerate (**3PGA**) in the Calvin cycle (Fig. 4.1). Because the first measured product of photosynthesis was a 3-C compound, plants that use only this basic photosynthetic pathway are referred to as C_3 plants.

During daylight hours, when the rate of photosynthesis is high, most of the new 3PGA is used to form glucose molecules that are linked together to form **starch**, which accumulates in the stroma. At night, the starch is broken down again to glucose and then to phosphorylated 3-C sugars (triose phosphates) that are exchanged for inorganic P (P_i) from the **cytosol** (Fig. 4.2). In the cytosol, two molecules of triose phosphate are combined to form glucose or fructose, both 6-C sugars. One fructose molecule and one glucose molecule are linked to form sucrose for transport through the **phloem** (Fig. 4.2).

Sucrose can be transported throughout the plant via the phloem to sites that require energy for respiration, growth, or storage. Cattle prefer tall fescue hay cut in the late afternoon to hay cut early in the morning (Fisher et al., 1999), in part because the gradual accumulation of sucrose and other **non-structural carbohydrates** in leaves during the day results in higher nutritional value and palatability.

Photorespiration

Rubisco also catalyzes the addition of molecular oxygen (O_2) to RuBP (Fig. 4.3). For every two molecules of O_2 that are added to a molecule of RuBP, two molecules of a 2-C compound, 2-phosphoglycolate, are formed, along with two molecules of 3PGA. This reaction is the "oxygenase" function of rubisco, because O_2 rather than CO_2 is a substrate of the reaction. Metabolism of the 2-phosphoglycolate creates one more 3PGA molecule, but also results in the loss of one CO_2 molecule from the plant.

During drought, reducing the loss of water from the leaves becomes a higher priority than absorbing CO_2 for photosynthesis, and when plants close their stomata to prevent dehydration, the CO_2 levels inside the leaves become depleted. This favors the reaction of rubisco with O_2 and the subsequent loss of CO_2, which is termed **photorespiration** and is most likely to occur in hot, dry weather.

O_2 is present at much higher concentrations in the atmosphere (21%) than is CO_2 (0.04%), but rubisco has a

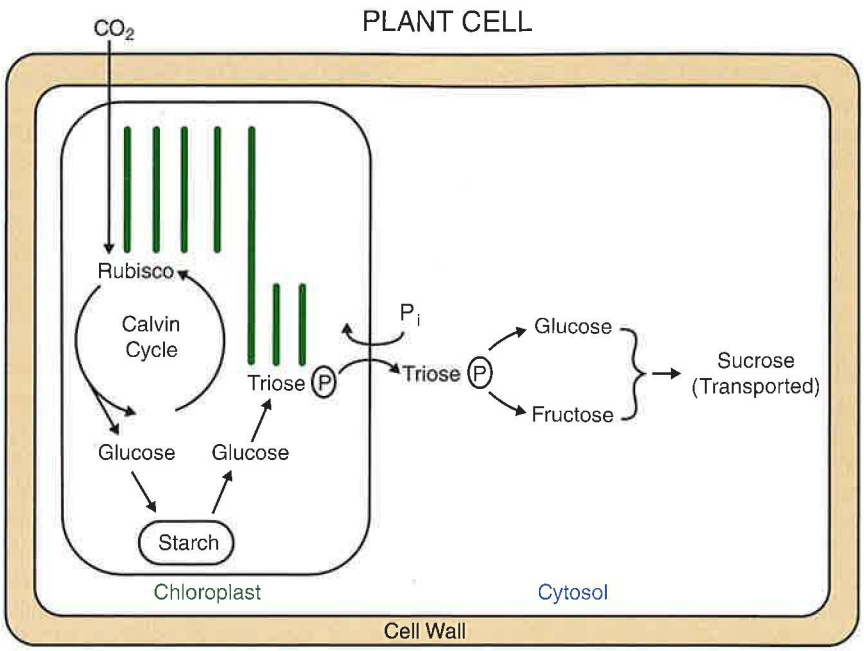

Fig. 4.2. Carbohydrates formed by photosynthesis are used to make sucrose, which is the form in which photosynthate is translocated via the phloem from sources to sinks. (Adapted from MacAdam, 2009.)

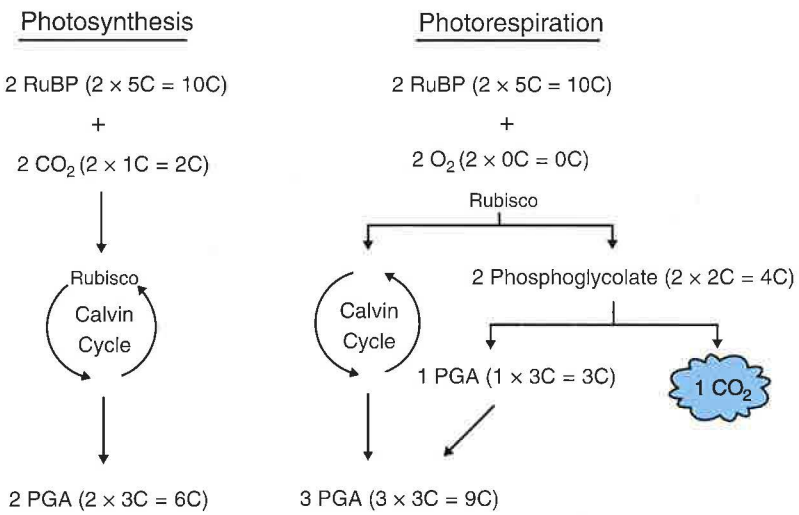

Fig. 4.3. Photorespiration occurs when the internal leaf concentration of CO_2 becomes so low that rubisco adds O_2 instead of CO_2 to RuBP. The result is the loss of one CO_2 molecule for every two O_2 molecules added to RuBP. (Adapted from MacAdam, 2009.)

higher affinity for CO_2 than for O_2 until the concentration of CO_2 near chloroplasts is greatly reduced. The enzyme rubisco is highly conserved, meaning that it has hardly changed since it first appeared millions of years ago, and attempts to reduce the oxygenase function of rubisco have been unsuccessful. Photorespiration effectively reduces C_3 photosynthesis by 10–50% or more depending on the temperature.

C_4 Photosynthesis

In the leaves of C_3 plants, only the **mesophyll** cells have well-developed chloroplasts, but in **C_4 plants** both mesophyll and **bundle sheath** cells, which surround the vascular bundles, have chloroplasts (Fig. 4.4A). In C_4 plants, nearly all the rubisco is found in bundle sheath chloroplasts, while CO_2 is captured in mesophyll cells by the enzyme phosphoenolpyruvate carboxylase **(PEP carboxylase)** (Fig. 4.4B). Unlike rubisco, PEP carboxylase has no reaction with O_2, and adds CO_2 to the 3-C PEP to form the 4-C organic acid oxaloacetate—hence the name "C_4 plants"—which is used in turn to form the 4-C compounds malate or aspartate.

The 4-C compounds diffuse through **plasmodesmata** from the mesophyll into the bundle sheath cells, where CO_2 is released and is then absorbed by the chloroplasts. In the bundle sheath cells, CO_2 is released and reacts with rubisco in the Calvin cycle, just as in C_3 plants (Fig. 4.4B). In C_4 bundle sheath cells the release of CO_2 from the 4-C acid results in formation of the 3-C molecule pyruvate, which returns to the mesophyll cell to capture another molecule of CO_2.

The formation of PEP from pyruvate requires the equivalent of two molecules of ATP, which is an extra energy cost for C_4 photosynthesis. This means that C_4 photosynthesis is only more efficient than C_3 photosynthesis under the hot, dry conditions that lead to photorespiration in C_3 plants. Separating the capture of CO_2 in mesophyll cells from the Calvin cycle of photosynthesis in bundle sheath cells effectively eliminates photorespiration in C_4 plants by keeping the CO_2 concentration high enough at the reaction site of rubisco to compete with O_2.

The Role of Stomata

CO_2 moves by diffusion from the outside air into the leaf through stomata, and then becomes dissolved in the film of water that coats the mesophyll cells. Dissolved CO_2 diffuses through the cell walls and the cell membranes to reach the reaction site of rubisco in the stroma of the chloroplasts. As CO_2 is used for photosynthesis, it creates a concentration gradient—from high levels outside the leaf to low levels inside the chloroplast—that drives CO_2 uptake.

Stomata are pores in the epidermis of plants (Fig. 4.4) that open when the concentration of CO_2 is depleted through photosynthesis, which means that stomata normally open during the day and close at night. The concentration of water vapor inside the leaf tissue is constantly replenished by water from the roots, so when the stomata open to allow CO_2 to diffuse in, water vapor

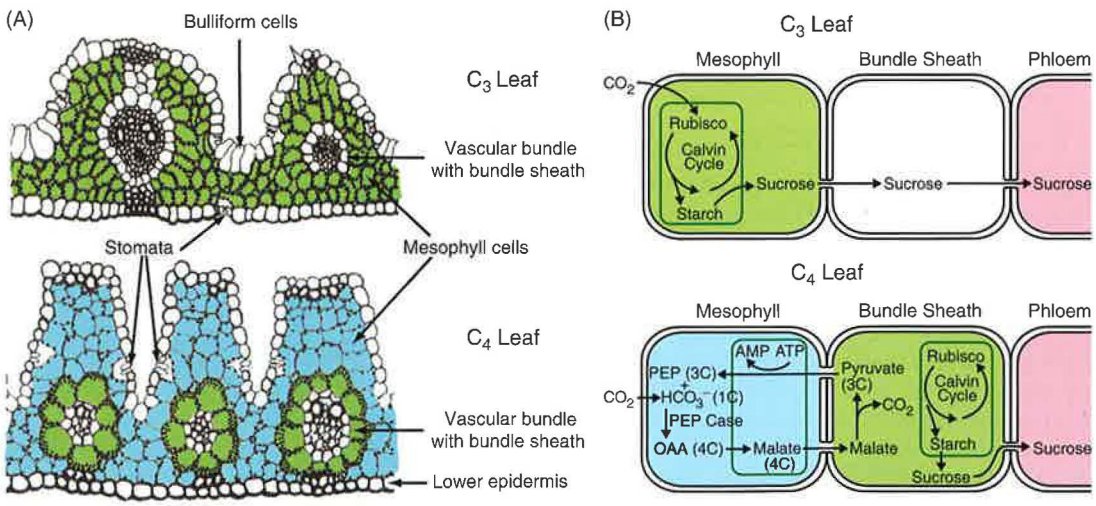

Fig. 4.4. The Calvin cycle of photosynthesis takes place in the mesophyll of C_3 leaves and in the bundle sheath cells of C_4 leaves (green cells, A and B), while the mesophyll of C_4 leaves captures CO_2 as 4-C compounds (blue cells, A and B) that are transported to bundle sheath cells. (Figure 4.4A: Drawings of tall fescue and cordgrass hybrid leaves adapted from Burr and Turner, 1933. Figure 4.4B: Adapted from MacAdam, 2009.)

diffuses out. This evaporation of water from the leaves is termed **transpiration**. However, when drought occurs, the stomata close during the day to reduce the transpiration of water and prevent wilting.

There are often high levels of solar radiation during drought that continue to drive photosynthesis. When the stomata of C_3 plants are partially or completely closed, ongoing photosynthesis uses the available CO_2, thus reducing the CO_2 concentration inside the stroma and causing photorespiration to occur. In contrast, the more efficient CO_2 uptake and transfer system of C_4 plants concentrates CO_2 in the bundle sheath cells even when the stomata are partially closed. The internal CO_2 concentration becomes lower in C_4 plants than in C_3 plants when the stomata are closed, creating a steeper CO_2 concentration gradient from the outside air to the stroma, and therefore increasing the rate of CO_2 uptake when C_4 plants open their stomata. This means that the photosynthetic efficiency of C_4 plants is greater than that of C_3 plants under hot, dry conditions.

Water use efficiency (WUE) is the amount of biomass produced per volume or weight of water used. Because CO_2 uptake and use are more efficient in C_4 plants, C_4 photosynthesis occurs with less water loss, so the WUE of C_4 plants is greater than that of C_3 plants. The C_4 grasses of the tall-grass prairie, such as switchgrass and big bluestem, root deeper than C_3 grasses. This adaptation allows these C_4 plants to avoid drought by extracting water from deeper in the soil profile during hot, dry periods, further improving their productivity during periods of hot, dry weather.

The CO_2 concentration in the atmosphere is expected to increase from the current level of 0.04% to 0.06% by the middle of this century (US Environmental Protection Agency, 2016) unless effective mitigation measures are implemented. As the CO_2 level rises, the concentration gradient from the outside to the inside of leaves will also increase, so the rate of diffusion of CO_2 into chloroplasts will increase. In locations where the climate does not become hotter and drier, the photosynthetic efficiency and productivity of C_3 plants could increase by 30% or more through reduction of photorespiration. The efficiency of C_4 plants will probably increase little if at all as the atmospheric CO_2 concentration rises, causing them to lose some of their ecological advantage.

Cacti and Life after Dark

Cacti are an important component of the vegetation of deserts and very arid rangelands, but how do they manage to grow and survive with a lower water supply than other plants? Cacti, pineapple, and several other arid plant species have developed a variation on capturing CO_2 for C_3 photosynthesis called crassulacean acid metabolism (CAM). The stomata of CAM plants open at night rather than during the day to minimize transpiration losses, and CO_2 is added to PEP by PEP carboxylase to form 4-C organic acids as in C_4 plants (Fig. 4.4B). These organic acids are stored in the vacuole at night. Then, during the day, when sunlight is available to drive photosynthesis, the stomata remain closed to conserve water. The 4-C acids, in the same way as in C_4 plants, release their CO_2 in chloroplasts, where it is captured by rubisco and used for C_3 photosynthesis. Pyruvate, the 3-C residual, is used to regenerate PEP that is ready to capture more CO_2 when the stomata open the following night. As in C_4 plants, there is an extra energy cost for converting pyruvate to PEP. The result is that these plants grow slowly but are extremely water efficient.

Radiation Effects

Light intensity influences the rate of the photochemical reactions of photosynthesis that occur on the thylakoid membranes to produce ATP and NADPH. Full sun is the maximum intensity of solar radiation outdoors near midday, which is approximately 2000 μmol photons m^{-2} s^{-1}. At low light intensities, as the interception of solar radiation increases, photosynthesis increases in a nearly linear manner, but C_3 photosynthesis begins to saturate at lower light intensities than in C_4 plants (Fig. 4.5A).

In C_3 plants, the rates of NADPH and ATP synthesis continue to increase as the light intensity increases, but the rate of CO_2 diffusion into the leaves for the biochemical reactions becomes limiting, so rubisco begins to react with O_2, causing increased photorespiration. In contrast, the photosynthesis of C_4 plants continues to increase with increasing radiation intensity, mainly because PEP carboxylase keeps CO_2 levels low in the mesophyll cells, creating a steeper CO_2 gradient from the outside to the inside of leaves, and thus enhancing the rate of CO_2 diffusion into the leaf.

The efficiency of CO_2 fixation at low radiation intensity is similar for C_3 and C_4 plants, but in full sun the photosynthetic rate of C_4 species may be nearly double that of C_3 species. Using cutting and grazing management to optimize radiation interception by C_3 and C_4 plants is important for production of pastures and hay crops.

Temperature Effects

Whereas the direct effect of light on photosynthesis is on the photochemical reactions that lead to the formation of ATP and NADPH, the effect of temperature on photosynthesis is a function of the biochemical reactions that occur in the stroma. The rate of change with a 10°C increase in temperature is called the Q_{10} temperature coefficient. Rates of biological enzyme reactions, including photosynthesis and respiration, typically double with a 10°C (18°F) increase in temperature. Most C_3 grasses and legumes can fix CO_2 at temperatures near freezing, and the net

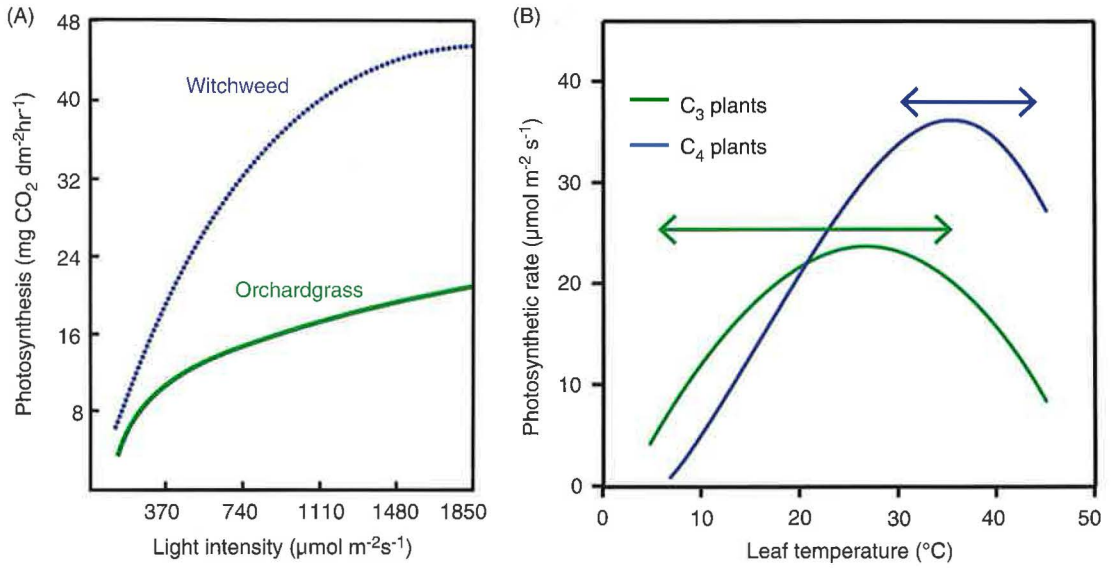

Fig. 4.5. A. In low light, the rate of photosynthesis increases linearly with increasing light intensity, but the greater efficiency of delivery of CO_2 to rubisco in C_4 plants (e.g., witchweed) compared with C_3 plants (e.g., orchardgrass) results in higher rates of C_4 photosynthesis in full sun. B. The photosynthesis of C_3 plants is more efficient at low temperatures than is that of C_4 plants, but at temperatures above 30°C, C_3 photosynthesis is reduced by photorespiration, which is effectively eliminated in C_4 plants. (Fig. 4.5A adapted from Singh et al., 1974. Fig. 4.5B adapted from Yamori et al., 2014, reproduced with permission of Springer.)

photosynthetic rate (photosynthesis minus respiration and photorespiration) of C_3 plants reaches a maximum at temperatures in the range 20–25°C (68–77°F) (Fig. 4.5B). Photosynthesis is reduced at temperatures above 30°C (86°F) because the solubility of CO_2 in the cytosol decreases more with increasing temperature than does the solubility of O_2. The result is that as the ratio of CO_2 to O_2 in the stroma decreases, photorespiration is favored, and the net photosynthesis rate in C_3 plants decreases rapidly. In contrast, the photosynthesis rate of C_4 plants is low at 10°C (50°F) because C_4 plants have relatively low concentrations of rubisco and other C_3 photosynthetic enzymes. Levels of these Calvin cycle enzymes are sufficient at higher temperatures (under which conditions their activity is higher) since the CO_2 is captured by the C_4 pathway (Fig. 4.5B). C_4 photosynthesis increases to a maximum at 35–40°C (95–104°F), and then decreases as proteins become destabilized by excess heat. The higher temperature optimum for C_4 plants is mainly due to control of photorespiration.

Leaf Anatomy and Forage Quality

Plant cells that are metabolically active, such as mesophyll cells, are thin-walled, contain abundant sugars and proteins, and are rapidly degraded by rumen microbes. Leaves of grasses with C_3 photosynthesis, such as tall fescue, have several layers of chloroplast-lined mesophyll cells that radiate from widely spaced veins (Fig. 4.4A). In contrast, leaves of C_4 grasses such as bermudagrass have closely spaced veins often surrounded by only one layer of mesophyll cells, because mesophyll cells function only to capture CO_2 and transport it to bundle sheath cells around the veins in C_4 plants.

The major veins of all grasses contain **xylem** elements with reinforced walls for water transport, clusters of phloem sieve tubes, and companion cells. There are also bundles of thick-walled fiber cells located at the epidermis above and below veins, and these sometimes extend from the upper to the lower epidermis to provide strength and rigidity. One or more minor veins are interspersed with each major vein; these may only contain phloem, to facilitate the transport of photosynthate from the leaves to the other organs of the plant. Minor veins are positioned closer together in C_4 grass leaves because the efficiency of photosynthesis is high, requiring fewer mesophyll cells per unit of photosynthate.

The thick walls of fiber cells are composed largely of cellulose, which is a carbohydrate, so fiber cells are relatively low in protein. Cellulose strengthens the fiber cell walls, and the deposition of lignin in fiber cell walls makes them

rigid, so veins function as both the circulatory system and the skeleton of leaves. Lignin is also deposited in response to diseases.

Lignin strongly resists microbial degradation, resulting in slow digestion in the rumen, where the structural carbohydrates from cell walls are digested by microbes. The proportion of lignified xylem elements and fiber cells, which constitute the fiber fraction of the forages used as animal feed, is higher in C_4 than in C_3 grasses because the proportion of veins to mesophyll cells is higher in C_4 plants. The rate of fiber digestion in the rumen decreases as the concentration of lignin increases, and the fiber from C_4 grasses has a higher lignin concentration than the fiber from C_3 grasses (Akin, 1989).

The PEP carboxylase that is present in C_4 leaves is a smaller molecule than rubisco, and in C_4 leaves rubisco is sequestered in the bundle sheath cells where it is used very efficiently. Therefore less rubisco, which is a major protein, is needed in C_4 leaves than in C_3 leaves to achieve the same or higher rates of photosynthesis. This, combined with the greater number of veins and the associated increased proportion of fiber, results in a lower protein concentration in C_4 grasses than in C_3 grasses.

Due to their lower protein content, C_4 grasses can produce more biomass per unit of fertilizer N, making their use of nitrogen more efficient, but the lower protein concentration reduces the nutritive value of C_4 grasses for ruminants. Moreover, rumen degradation of C_3 grasses is generally faster and more complete than that of C_4 grasses.

Shaping Up a Grass Leaf

Leaf blades of grasses consist of different cell types that determine leaf size, shape, and morphology. Grass blades are vertical during emergence. After emergence, the angle of the blade and sheath increases. Parallel veins are apparent as ridges along grass leaves, and consist of bundles of fiber cells grouped above and below major veins to form the vascular bundle. This forms a girder-like structure to control the arching of the leaf blade and its final orientation to the sun. The final angle affects the number of leaves that can productively intercept light, and therefore determines the critical **leaf area index (LAI)**.

When drought stressed, many grass leaves reduce their surface area by rolling the blade. When water stressed, the large thin-walled bulliform cells located between veins (Fig. 4.4) lose turgor and shrink in diameter, causing the leaf blade to roll inward forming a long, thin cylinder with the upper surface on the inside. Reducing the leaf area that is exposed to light and heat reduces water loss through the stomata. If the plant is re-watered before the leaf is damaged, the bulliform cells will restore turgidity and the leaf will unroll.

The long slender leaves of grasses, supported by parallel veins that are continuous from base to tip, have a higher fiber content than the small round or oval leaflets that comprise legume leaves, which are supported by a midrib and a network of smaller veins. Since legumes all have C_3 photosynthesis and can synthesize nitrogen through associations with rhizobia bacteria, they also typically contain higher levels of protein than grasses. Although legume stems can develop high levels of fiber, forage legumes generally have significantly greater protein content, digestibility, and intake than forage grasses.

Translocation of Carbohydrates

Plant organs that provide resources to other tissues in the same plant are termed "sources", and those that receive the resources are termed "sinks." Sucrose from a source cell, such as a mesophyll cell, is loaded into the phloem for long-distance transport, termed translocation (Fig. 4.2 and 4.4B). Sucrose from the phloem is unloaded at a "sink", such as a developing seed or a root, where it is used for respiration, growth, or storage. When plants are defoliated by cutting or grazing, the process is reversed and storage organs such as roots and residual stubble become sources and growing shoots become sinks.

While they are still growing, the young leaves of shoots are sinks that import resources from older leaves. As their surface area increases, leaves begin to produce photosynthate in excess of their own needs, and they then export this excess to even younger leaves at the shoot apex, or to nearby tillers or branches developing from axillary buds. As each leaf matures, its exported photosynthate is used for root growth or storage while newer leaves support growth at the shoot apex which initiates new leaves. Grass leaves attached at nodes located at the base of the stem support the growth of axillary buds as they form new tillers, rhizomes, or stolons, as well as the growth of new roots.

New growth at the top of the canopy shades the lower, older leaves, eventually causing them to senesce (i.e., gradually die). **Senescence** is an active process during which nonstructural carbohydrates and proteins are broken down to form sucrose and amino acids, which are then translocated to storage or to younger growing tissues. Mobile mineral nutrients such as N, P, K, and Mg are also translocated from older to younger leaves to support the growth of the youngest leaves at the top of the canopy, where they can provide the greatest benefit to the plant.

The strength of a sink for photosynthate is a function of sink size, growth rate, and distance from the source. Grasses develop fibrous root systems with minimal storage capacity, and thus are more dependent on current photosynthate. When a grass stand is harvested or grazed, leaf area is decreased and root growth stops almost completely and does not recover until the canopy has partially regrown (see Chapter 2). Without a taproot, grasses are dependent

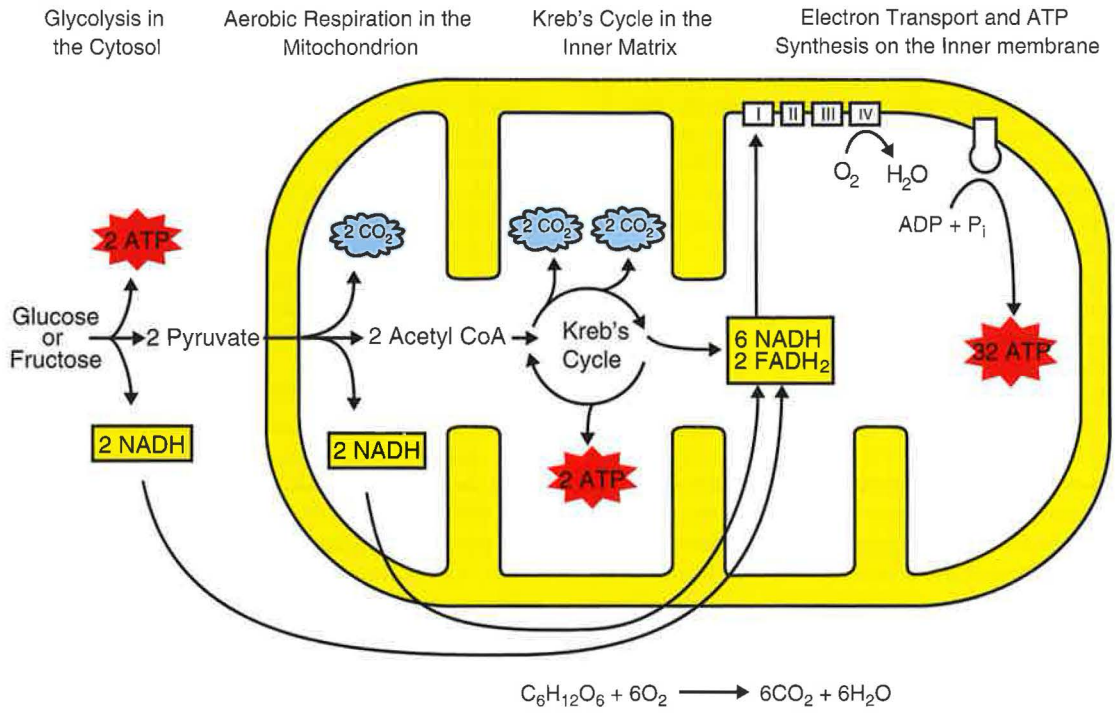

Fig. 4.6. Respiration of glucose or fructose begins with glycolysis outside the mitochondria forming two molecules of pyruvate and two molecules of ATP. In mitochondria, each pyruvate is used to form a molecule of acetylCoA that enters the Kreb's cycle, with the loss of all carbon as CO_2. In electron transport, NADH and $FADH_2$ are used to form ATP, with a total theoretical yield of 32 molecules of ATP from each glucose or fructose molecule. (Adapted from MacAdam, 2009.)

on the leaf area remaining after harvest to supply photosynthate for initial leaf and root regrowth. This makes stubble height an important consideration, especially in the management of pastures.

Leaf growth is affected by even mild water stress, which appears to be a protective mechanism that allows the plant to slow leaf expansion and thus additional transpiration while maintaining photosynthesis (see Chapter 5). A reduction in the rate of growth of leaves and stems during stress allows more photosynthate to be partitioned to the roots, supporting increased growth in root length during drought, and effectively changing the balance between potential water uptake and water loss. Conversely, when adequate water and nutrients are available, shading reduces photosynthesis but not leaf expansion, so less photosynthate is allocated to the roots for growth.

Aerobic Respiration

Although aerobic respiration in plants is sometimes referred to as "dark respiration" to distinguish it from photorespiration, aerobic (O_2-requiring) respiration occurs continuously in the **mitochondria** of all living cells (Fig. 4.6). The summary equation for aerobic respiration, in which CO_2 is respired and the energy stored in the chemical bonds of glucose molecules is used to form nicotinamide adenine dinucleotide (**NADH**) and ATP, is essentially the reverse of the summary equation for photosynthesis (Fig. 4.1). The structure of NADH only differs from the structure of NADPH formed in photosynthesis by a single phosphate group (P_i). The molecules that enable photosynthesis and cellular metabolism to occur, namely ATP and NADPH, cannot be transported from cell to cell, so sucrose is translocated instead, to provide a substrate for respiration that in turn provides distant cells with ATP and NADH.

Non-structural carbohydrates such as starch, fructan, and sucrose are reduced to their subunits, consisting of glucose and fructose molecules, before they are respired. The initial phase of respiration is glycolysis, which occurs in the cytosol. Glycolysis can supply a small amount of energy in the absence of oxygen, and becomes the major source of energy in oxygen-deprived tissue, a state termed **hypoxia**, such as occurs in the roots of waterlogged plants. Glycolysis releases just two molecules of ATP per glucose, and

yields two molecules of the 3-C compound pyruvate. If oxygen is unavailable, pyruvate is metabolized to generate the raw material (NAD^+) needed for glycolysis to continue, resulting in the production of lactic acid or ethanol, both of which contain unused energy. Glycolysis is much less efficient than aerobic respiration, and the byproducts can accumulate to levels that are toxic to plants. This inefficient respiration is one of the reasons why flooding and ice encasement can injure plants (see Chapter 5).

Oxygen is needed in order for pyruvate to be used in the next phases of aerobic respiration, which take place in the mitochondria. This is why aeration is needed in solution culture (hydroponics), and why plants that are tolerant of flooding can form a continuous network of intercellular air channels (aerenchyma) connecting the leaves, stem, and roots to carry oxygen, which allows them to avoid depending solely on glycolysis.

Mitochondria, like chloroplasts, have an outer and an inner membrane that enclose an inner aqueous enzyme-rich matrix analogous to the stroma (Fig. 4.6). Instead of an inner thylakoid membrane system, the inner membrane of mitochondria has an enlarged surface area that folds and protrudes into the matrix, where it functions in electron transport and ATP synthesis, in a similar way to the thylakoid membranes.

Pyruvate is taken up from the cytosol by mitochondria, and it passes through both the outer and inner membrane and into the matrix. As it is processed, the three carbon atoms of pyruvate are oxidized in succession and released as CO_2 molecules, at the same time using the released energy to form NADH and a similar molecule, $FADH_2$. These molecules have "reducing power" and are used to form ATP.

Electron transport, which is the last phase of aerobic respiration, takes place on the inner membrane and in the matrix of the mitochondria. NADH and $FADH_2$ donate their high-energy electrons to one of a series of protein complexes (I–IV) that are successively reduced and oxidized to form ATP. The process is similar to the synthesis of ATP in photosynthesis, except that NADH donates electrons at the start of electron transport, rather than being created at the end. As the last step the electrons from NADH or $FADH_2$ are donated to oxygen, reducing it to water (H_2O). Approximately 32 ATP molecules can in theory be formed for every glucose molecule that is completely respired in the cell.

Aerobic respiration is central to the biochemistry of plants, not only because it generates the energy needed for the metabolism that allows cells to function, but because the intermediate compounds formed during the process can leave the mitochondria to be used in other biochemical pathways. For instance, α-ketoglutarate is the "carbon skeleton" to which an amino ($-NH_2$) group is added to form glutamate, which is one of the 20 amino acids and one that is particularly important in the synthesis of the transported forms of fixed N in legumes.

Respiration uses 30–80% or more of new photosynthate each day, depending on the temperature and other environmental factors. Plant yield is essentially the difference between photosynthesis and respiration, so improving the efficiency of respiration could improve yield. The energy generated by aerobic respiration can conceptually be separated into that used for the synthesis of new tissues, termed **growth respiration**, and that used to repair and ensure the proper functioning of mature, non-growing tissues, termed **maintenance respiration**. Respiration of mature, stored seed or of a fully developed leaf where no growth is taking place is purely maintenance respiration, whereas respiration in a young growing root tip, leaf base, or developing seed is largely growth respiration.

Growth Respiration

The rate of growth respiration is directly linked to growth rate. A portion of the sugar transported to regions of cell division (meristems) and cell expansion is respired in order to provide the energy needed to assemble other carbohydrates into cell walls. In addition to building cell walls, respiratory intermediates are used to synthesize lipids, nucleic acids, and proteins. Growth respiration usually adds weight to the plant and occurs where cells are dividing or elongating, or in cells that are synthesizing secondary cell walls and lignin as they mature.

The respiratory cost of the synthesis of new tissue depends on tissue composition. About 1.21 g of glucose is needed, both as a building material and as the substrate for growth respiration, to synthesize 1 g of cellulose (cell wall) or 1 g of starch (Penning de Vries et al., 1983). Protein synthesis is more costly due to the need to take up and reduce NO_3^-, requiring about 2.48 g of glucose to synthesize 1 g of protein or nucleic acid. About 3 g of glucose are needed to synthesize 1 g of lipid, and 2.12 g of glucose to synthesize 1 g of lignin.

Organic acids, which are used in photosynthesis, respiration, and stomatal guard cell regulation, are more oxidized than glucose, which means that their synthesis is more energetically favorable. Therefore only about 0.91 g of glucose is required to synthesize 1 g of organic acids. Root uptake of mineral ions such as K^+, Mg^{2+}, calcium (Ca^{2+}), and phosphate ($H_2PO_4^-$) only costs about 0.05 g of glucose per gram of mineral. By weight, good-quality dried forages are composed of 50–80% carbohydrates, 10–25% proteins, 2–5% lipids, and 6–12% minerals (Schwab et al., 2006).

In a study of young barley leaves, respiration was measured as oxygen (O_2) uptake in regions of cell division and elongation (Fig. 4.7; Thompson et al., 1998). While respiration per cell (open blue squares) was constant from the youngest tissue at the base of the leaf to differentiating tissue toward the leaf tip, respiration per unit of protein (closed blue squares) was greatest in the youngest, fastest-growing tissue at the leaf base, where the cells were smallest.

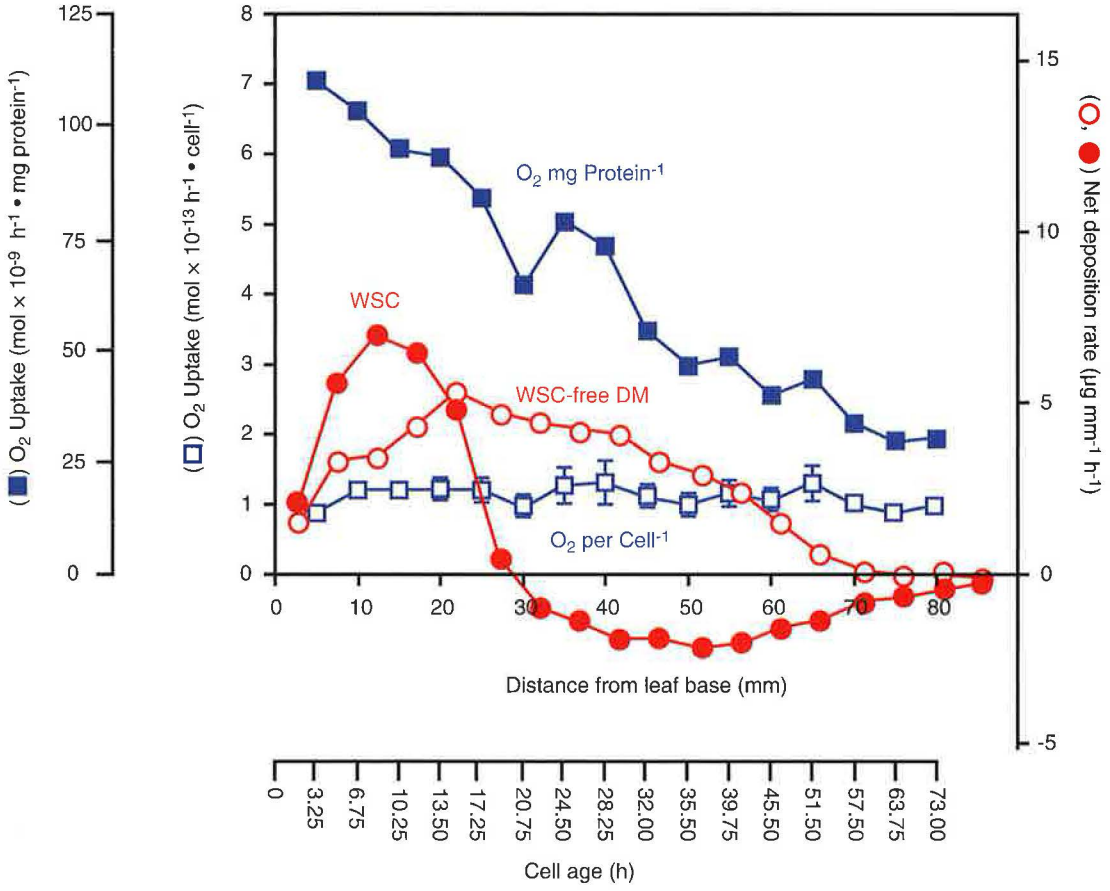

Fig. 4.7. The length of growth regions (cell division, elongation, and differentiation) at the base of grass leaves is similar in barley and tall fescue leaves. Growth respiration is highest in the region of cell division (2–10 mm), at the base of the leaf, while the deposition of water-soluble carbohydrates (WSC) peaks in the elongation zone (2–25 mm), where rapid cell wall synthesis is needed to support cell growth. A net loss of WSC in differentiating leaf tissue (25–80 mm) is used for the accumulation of structural dry matter (DM). (Adapted from Thompson et al., 1998, and Allard and Nelson, 1991. Reproduced with permission of the American Society of Physiologists and CCC Republication.)

As the cells elongated and then differentiated, the protein concentration in successive leaf segments decreased, as did the respiration rate.

The growth of elongating tall fescue leaves occurs over a similar distance, so data for the deposition of water-soluble carbohydrates (WSC; closed red circles) and the accumulation of other macromolecules in leaf tissue (WSC-free dry matter; open red circles) have been overlaid on the same graph (Fig. 4.7; Allard and Nelson, 1991). Combining these spatially similar grass leaf data allows us to see that the highest rate of deposition of photosynthate (WSC) occurs in the elongation zone, where cell wall growth must keep up with cell expansion. In more mature tissue, WSC in newly elongated cells is used for the synthesis of secondary cell walls in differentiating xylem and fiber cells.

It has been determined that leaf blades of smooth bromegrass that are comprised of 16% protein, 6.5% mineral, 2.5% lipid, 4% organic acids, and 71% cell wall (96% carbohydrate and 4% lignin) require about 1.39 g of glucose for the synthesis of 1 g of leaf tissue dry weight. When N for the growth of new leaves is recycled from senescing lower (older) leaves (grass tillers usually only support three mature leaves at one time), the amino ($-NH_2$) group of protein is already formed, so biosynthesis of new protein costs only 1.62 g of glucose per gram. This reduces total

synthesis costs to 1.26 g of glucose per gram of new leaf. Leaves of legumes such as alfalfa have a higher protein content and are therefore more costly to synthesize than grass leaves. Similarly, due to the higher protein concentration in leaves, more glucose is required to form leaves than stems, and more glucose is needed to form stems than roots, which are low in protein.

Most forages are herbaceous perennials that persist from year to year but maintain only their below-ground shoot and root biomass over the winter in cold-temperate climates. This allows them to adapt new growth to the prevailing nutrient and climatic conditions during each growing season. Growth temperature influences the composition of the tissue formed, which in turn affects growth respiration. For example, at temperatures above the optimum for leaf development, cell wall growth is slowed and a higher proportion of the cell wall is lignin, leading to an increase in the proportion of leaf protein relative to cell wall. In this case, the respiratory efficiency of growth is reduced because both lignin and protein are more expensive to synthesize than cellulose.

Maintenance Respiration

Once formed, all living tissue needs to be maintained and repaired. Maintenance respiration includes that needed for synthesis of proteins and lipids to replace those that have broken down with time or to alter the rate of metabolic activity, and maintenance of ion concentrations, such as K^+, which is needed to stabilize cellular proteins. The rate of maintenance respiration is strongly affected by temperature, being very low at 0°C (32°F), but increasing as the temperature rises (Fig. 4.8). Of the 30–80% of photosynthate that is used for aerobic respiration each day, most is used for maintenance respiration of the large amount of mature tissue.

Some plant species that are well adapted to northern regions do not survive in the South because of excessive maintenance respiration. Lower night-time temperatures in the North, in dry climates or at high altitudes, decrease maintenance respiration costs. These same conditions may cause sufficient chilling injury to the membranes of perennial C_4 plants to restrict their adaptation in these locations (see Chapter 5).

Rates of maintenance respiration are closely related to the age of plant tissue and the metabolic activity of an organ. Many enzymes degrade rapidly, some within a few hours, especially at high temperature, requiring maintenance respiration for continued resynthesis. Maintenance respiration begins as a small component of total respiration in young seedlings, and then becomes a higher proportion of overall respiration as more tissue stops growing and matures. Senescence can require the breakdown (catabolism) of macromolecules of older tissue and the transport of their subunits or of nutrient ions to newer growth or storage. This temporarily increases maintenance respiration, but respiration declines again as the protein content of the senesced tissue decreases.

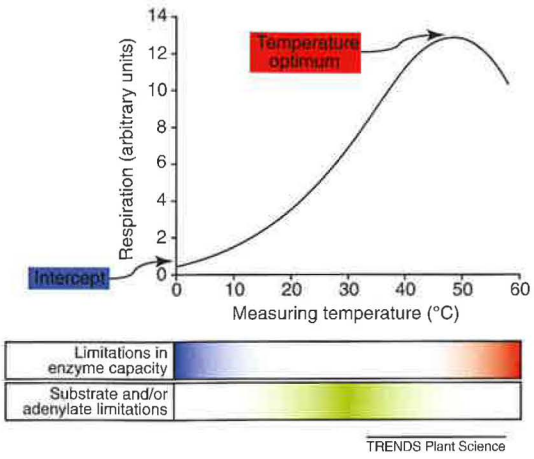

Fig. 4.8. Maintenance respiration of mature tissues peaks at approximately 45°C. At very low (violet) and very high (red) temperatures, respiration is limited by the rate of enzyme activity. From about 10°C to 45°C respiration is limited by the supply of carbohydrates (substrate) or ADP (adenylate). (Adapted from Atkin and Tjoelker, 2003. Reproduced with permission of Elsevier.)

While growth respiration is required for plant productivity, it would be beneficial to reduce maintenance respiration. Wilson (1982) successfully selected for low aerobic respiration rate in mature leaf blades of perennial ryegrass, which led to decreased maintenance respiration and an increase in forage yield. The carbohydrate conserved was available for growth, and was expressed as an increase in production of new tillers.

Inorganic Nutrient Uptake

Mineral nutrient ions in the soil solution, such as K^+, Mg^{2+}, Ca^{2+}, $H_2PO_4^-$, SO_4^{2-}, and NO_3^-, come into contact with roots passively via water uptake and move to the plasma (cellular) membrane of root cells without restriction. The internal anatomy of plant roots forces these mineral nutrients to move across the plasma membrane and into cells, often requiring the energy of ATP for active uptake, before they are transported into the xylem and pulled into shoots in the transpiration stream.

The requirement for active uptake allows the plant to selectively accumulate some nutrients (K^+, $H_2PO_4^-$, and NO_3^-) to much higher concentrations than those at which they occur in the soil solution, and to effectively exclude less desirable elements, such as sodium (Na^+). This selectivity is critical, but the use of ATP is a respiratory cost.

Table 4.1. Forage legume nodule symbionts showing specificity for nodulation of a host

Genus	Species	Biovar[a]	Host genus	Host common name
Mesorhizobium	*haukuii*		*Astragalus*	Milkvetch
Rhizobium	*leguminosarum*	*trifolii*	*Trifolium*	Clovers
Rhizobium	*leguminosarum*	*viciae*	*Vicia*	Vetches, peas
Mesorhizobium	*loti*		*Lotus*	Trefoil
Ensifer	*meliloti*		*Medicago, Melilotus*	Alfalfa, sweetclovers

Source: Mousavi et al., 2015. Reproduced with permission of Elsevier.
[a]Biovars are groupings within a species.

As plants mature, the rate of mineral uptake is slowed, but the maintenance respiration needed to retain nutrient ion concentrations tends to rise to as much as 50% of the total aerobic respiration of roots. The rates are higher for forages with taproots that store carbohydrates and proteins.

Nitrogen Uptake from the Soil

In warm (> 10°C or 50°F) soils with sufficient O_2 available for soil microbes, ammonium (NH_4^+) is rapidly converted to NO_3^-. Plants readily take up NO_3^- and store the excess in the **vacuoles** of cells. However, before the NO_3^- can be used in the plant, it must be reduced to NH_4^+, which requires NADPH from photosynthesis or NADH from respiration. In some plants, absorbed NO_3^- will be reduced to NH_4^+ and used to form nitrogen compounds in the roots. In others, especially after fertilization, when NO_3^- is abundant in the soil solution, the excess NO_3^- moves to the shoot in the xylem stream and is accumulated in the stem and leaves, where it can eventually be reduced. Excessive amounts of transiently stored NO_3^- in plants are potentially harmful to animals because NO_3^- is reduced to nitrite (NO_2^-) by microbes in the rumen. Nitrite that is absorbed into the blood interferes with the ability of hemoglobin to transport O_2 in the blood (see Chapter 16).

Nitrogen Assimilation

In legumes, the nitrogen required for plant growth can be taken up from the soil as ammonium (NH_4^+) or nitrate (NO_3^-). When these ions are too deficient to support growth, atmospheric dinitrogen (N_2) is fixed into ammonia (NH_3) in a symbiotic relationship with soil bacteria from the genera *Ensifer*, *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, or *Sinorhizobium* (all of which are referred to as **rhizobia**).

Symbiotic Nitrogen Fixation

A **symbiotic** (mutually beneficial) relationship exists between many legume species and rhizobia, in which the rhizobia receive carbohydrates and other nutrients from the plant that are then used to reduce N_2 from the air to usable forms of nitrogen for the plant, such as amides and transported amino acids. If they are not enclosed in a root nodule, rhizobia do not fix N, because low-O_2 conditions are required for the N-fixing bacterial enzyme nitrogenase to function, and the pores of a well-drained soil contain atmospheric O_2.

Rhizobia are attracted to the roots of legumes by chemical signals specific to the legume species, and infection occurs only when there is a correct match between a rhizobium species and a legume species (Table 4.1). In response to a chemical interchange between a legume root and compatible rhizobia, cells in the cortex of the root multiply to create a nodule primordium in anticipation of infection.

Rhizobia enter the young root through root hairs, which are outgrowths of the cell walls of epidermal cells (Fig. 4.9). Rhizobia attach to the root hair cell wall, and the root hair may curl to enclose them as the rhizobia degrade the cell wall of the root hair. An infection thread, which is an ingrowth of the root hair plasma membrane, encloses the dividing bacteria and grows through the root tissue into the

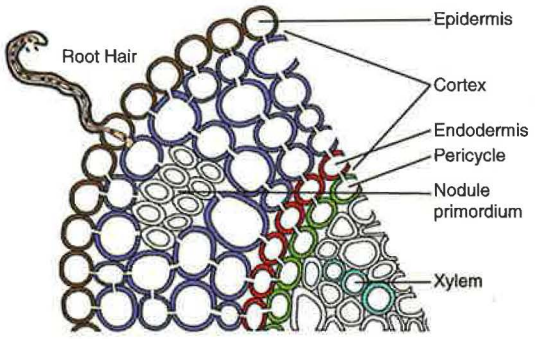

Fig. 4.9. Legume root cross-section illustrating the invasion of a root hair by soil-living rhizobia. An ingrowth of the plasma membrane, the infection thread, encloses rhizobia during growth through successive cell layers to the developing nodule primordium. (Adapted from MacAdam, 2009.)

cortex until it reaches the newly divided root cells that comprise the developing nodule. The bacteria, still enclosed in remnants of the plasma membrane, multiply further in the nodule cells. In this low-O_2 environment they alter their form to become N-fixing **bacteroids**. Nodule primordia are initiated in the cortex of the root, adjacent to lobes of the xylem, and branches of xylem and phloem develop along with the nodule for translocation of water and nutrients.

Nitrogenase, the nitrogen-fixing enzyme, is synthesized by bacteroids but is irreversibly inactivated by O_2. However, it requires considerable amounts of ATP, which has to be generated in nodule cells by aerobic respiration. To supply sufficient O_2 for respiration while at the same time protecting nitrogenase from O_2, **leghemoglobin,** an O_2 carrier, is synthesized jointly by bacteroids and the plant. It captures O_2 entering nodule cells before the O_2 reaches the nitrogenase complex, and then releases it as needed for aerobic respiration to drive nitrogen fixation. Like hemoglobin in blood, leghemoglobin with captured O_2 imparts a pink pigmentation to healthy nodules.

The N_2 gas used in fixation diffuses from the air through the soil and into the nodule to reach the active site of the nitrogenase enzyme. Each N_2 molecule is reduced stepwise using 16 ATP molecules from aerobic respiration. In the first step, N≡N is reduced to HN=NH. In the second step, HN=NH is reduced to $H_2N–NH_2$, and in the third step $H_2N–NH_2$ is reduced to two molecules of ammonia (NH_3). In legumes of temperate origin, the ammonium (NH_4^+) ion is added to glutamate to form glutamine and/or asparagine for transport, and in legumes of tropical origin, glutamine is further converted to ureides for transport. If legumes are fertilized with inorganic nitrogen, such as NO_3^-, they will take it up via their roots and utilize it as readily as do grasses, and nitrogen fixation in the nodules will decrease.

The inoculation process takes up to 4 weeks to result in nodulation and N fixation, so young seedlings are dependent on seed reserves and soil nitrogen. Low to moderate amounts of fertilizer nitrogen do not interfere with nodule formation, and as soil nitrogen becomes depleted, active N_2 fixation will occur. However, nitrogen fertilization will also benefit weeds that can compete with legume seedlings. It is rarely economical to fertilize established forage legumes or legume–grass mixtures with nitrogen.

Is Biological Nitrogen Fixation Better or Cheaper?

When nitrogen (N) fertilizer is produced commercially, ammonia (NH_3) is formed by reacting N gas (N_2) with methane (CH_4) under conditions of high temperature and high pressure. The NH_3 that is formed is readily converted into other N fertilizers, such as calcium ammonium nitrate. When incorporated into the soil, the ammonium (NH_4^+) released is converted to nitrate (NO_3^-) ions by soil microbes, so NO_3^- is the form commonly encountered by plant roots. Producing N fertilizer from N_2 and CH_4 costs about 3 g of glucose per gram of N (International Fertilizer Industry Association, 1998). The cost to the plant of uptake and assimilation of NO_3^- into usable organic N (–NH_2; the amino group) is about 8 g of glucose per gram of N (Gutschick, 1981), giving a total energy cost of 11 g of glucose per gram of N used. The cost for legume fixation of N_2 to –NH_2 is about 12 g of glucose per gram of N (Gutschick, 1981), mainly from higher rates of root respiration in nodulated legumes. However, 5.5 g of CO_2 equivalent greenhouse gas emissions occur for every gram of N applied as fertilizer, due to manufacture, transportation, machinery use during application, and direct loss of N_2O from the fertilized soil (International Fertilizer Industry Association, 1998), even when carbon sequestration from elevated crop production has been deducted (Kim and Dale, 2008).

Legumes support symbiotic N_2 fixation for their own benefit, providing from 30% to 95% of total plant N requirements; the rest of the N used by legumes comes from the soil. Often grasses growing next to legumes are greener, which suggests better N availability than for grasses growing further away. There is some evidence for direct N transfer between intermingled grass and legume roots, but nodule turnover may be most important in species such as birdsfoot trefoil. That species has determinate nodules that are sloughed after a harvest or a killing frost in the autumn, and must be reinitiated as part of regrowth. Other species, such as alfalfa and white clover, have indeterminate nodules that show a reduction in activity when plants are cut or grazed, and recover as the plants regrow.

Nodules are not considered to be nitrogen storage organs, and nodule mass is too small compared with root organic matter to account for a significant portion of N mineralization following harvest. Nitrogen transfer can occur indirectly from leaf drop or death of the legume plant followed by microbial mineralization of organic matter. However, the most significant and effective redistribution of fixed N occurs as a result of animals grazing legumes and depositing urine and dung within the same pasture.

Organic Food Reserves

When photosynthesis exceeds growth and maintenance respiration, legumes and grasses store carbohydrates in readily available forms in various plant parts. The principal storage organ may be the root (as in alfalfa), the stolons (as in white clover), the rhizomes (as in smooth bromegrass), or the leaf and stem bases (as in orchardgrass and other bunchgrasses). Plants also accumulate storage

proteins, and the accumulation of these carbohydrate and protein ("organic") reserves is coordinated.

Compounds that are synthesized as storage forms of carbohydrates and proteins are used to support respiration and growth when leaf area is insufficient to support growth in early spring or after cutting or grazing. At these times, the reserves are reconverted to sucrose and amino acids and are translocated to meristematic sites to support growth. They are also used to develop heat and cold resistance, to support respiration and metabolism during periods of **dormancy**, and to provide carbohydrates and N required for flower and seed formation. These organic reserves are a readily accessible buffer to support critical metabolic needs such as the initial development of plant photosynthetic capacity.

Starch and Fructan Accumulators

Starch, a polymer of glucose, is the primary non-structural carbohydrate stored in roots, rhizomes, and stolons of legumes and C_4 grasses such as big bluestem, switchgrass, and bermudagrass. Legumes and all grasses also store starch in seed, and transiently accumulate starch in chloroplasts during photosynthesis. Amyloplasts, which are similar to chloroplasts but do not develop thylakoid membranes or the enzymes needed for photosynthesis, are found in storage tissue of these species and can accumulate starch as insoluble granules.

In contrast with C_4 grasses, C_3 grasses such as orchardgrass, perennial ryegrass, and tall fescue accumulate **fructan**, a polymer of fructose, in their vegetative tissues. Fructans (also referred to as "fructosans") are chains of fructose molecules that vary in length and have a single glucose molecule at one end. Fructans are water-soluble and accumulate in the cell vacuole. Enzymes for fructan metabolism function at lower temperatures than those for starch metabolism, and fructan storage may have contributed to the adaptation of C_3 grasses to cool-temperate climates.

Seasonal Cycles

The initial growth of shoots, tillers, and fibrous roots in perennial forages is rapid in the spring, and occurs at the expense of storage (Fig. 4.10a). With sufficient leaf growth, excess photosynthate accumulates in storage organs until the first cutting or grazing. Within each cycle of harvest and regrowth, the leaf area for photosynthesis is initially low, so stored carbohydrates are drawn from storage organs such as the roots of perennial legumes, to support this new shoot growth (Fig. 4.10b). In autumn, in preparation for overwintering in temperate climates, growth slows as well-adapted species and overwintering storage organs such as basal stubble of grasses and the rhizomes, stolons, and taproots of legumes become strong sinks for photosynthate storage (Fig. 4.10c).

Seasonal trends of carbohydrate storage in roots help to explain why birdsfoot trefoil can be cut or grazed

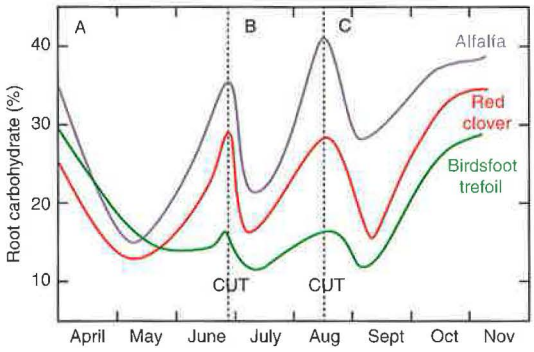

Fig. 4.10. Changes in the content of root storage carbohydrates in field-grown alfalfa, red clover, and birdsfoot trefoil. A. Root carbohydrate levels are high in early spring for all three species, and then decrease as the roots serve as a source to support initial shoot growth. In late spring, root carbohydrate reserves are restored in alfalfa and red clover before the first harvest. B. In birdsfoot trefoil, root carbohydrates are not restored regardless of management during the summer. C. In autumn, as all forage legumes become dormant, root carbohydrates are restored from photosynthesis by the leaves that develop in late summer, if no further harvests are taken. (Adapted from Smith, 1962, with permission of *Crop Science*.)

frequently, but not closely. The carbohydrates that are stored over winter in birdsfoot trefoil roots are used to support spring growth, but photosynthate is not used to restore the roots' carbohydrates after flowering in birdsfoot trefoil as it is in alfalfa and red clover (Fig. 4.10). Instead, birdsfoot trefoil continues to show active growth, even during seed fill and storage. Unlike alfalfa or red clover cut at bloom stage, there is no pool of stored carbohydrates in the taproot of birdsfoot trefoil. Therefore each time birdsfoot trefoil is cut or grazed, a tall stubble with leaves and axillary buds is needed to support shoot regrowth.

As in grasses, new stems that form from axillary buds on the stubble of birdsfoot trefoil will have functioning leaves to furnish the carbohydrates needed for continued regrowth. The similarity of desirable stubble heights and flexible cutting frequencies make birdsfoot trefoil an excellent companion for many C_3 bunchgrasses in mixtures. Storage remains at a low level in birdsfoot trefoil until growth slows down in autumn, when photosynthate is partitioned from the shoots to storage in the roots (Fig. 4.10).

For persistence of birdsfoot trefoil, it is absolutely critical that grazing or cutting ceases from early autumn until complete dormancy, usually marked by the first killing frost. This is the only period when birdsfoot trefoil stores carbohydrates and proteins for spring regrowth.

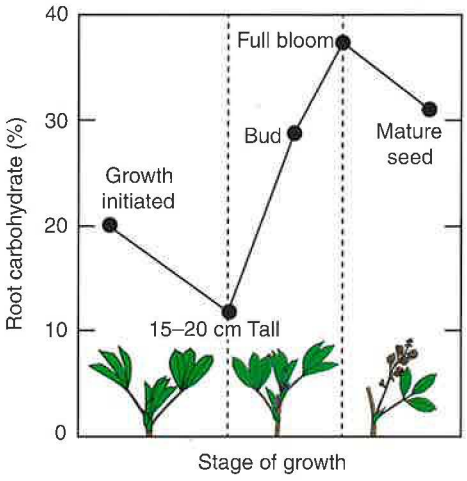

Fig. 4.11. Alfalfa root carbohydrate storage decreases from the initiation of spring growth or summer regrowth until sufficient leaf area has developed to produce excess photosynthate. Root storage increases with continuing shoot development and increased photosynthetic capacity through the bud (shown) and bloom stages. Seed fill and the initiation of new shoots from the crown combined with the maintenance of older leaves deplete storage carbohydrates because the requirements of seed storage are added to maintenance respiration and senescence. (Wisconsin field data from Graber et al., 1927. Alfalfa stage drawings from Fick and Mueller, 1989.)

In alfalfa roots, depletion of organic reserves occurs during the early vegetative phase of each growth cycle, until the shoots are 6–8 in. tall, when there is enough leaf area to produce photosynthate to fully support respiration and continuing shoot growth (Fig. 4.11). When alfalfa shoots exceed 6–8 in., photosynthate is produced in excess of the needs for ongoing respiration and growth in shoots, and is translocated to the crown and roots for storage (Fig. 4.11).

Accumulation in the roots continues as the shoot grows and flower buds form, reaching the highest level of storage near full bloom if the plants have not already been harvested (Fig. 4.11). In seed fields, some carbohydrate is used from alfalfa roots between full bloom and mature seed stages because the leaves are aging, seed are developing rapidly, and new shoots are being initiated from axillary buds on the crown, which means that total respiration of uncut plants will be high (Fig. 4.11).

High temperature may increase growth rate in well-adapted plant species, or simply increase maintenance respiration more than photosynthesis and hasten maturity. In either case, less storage is likely to occur in mid-summer. Conversely, low temperatures, particularly at night when photosynthesis has stopped, reduce shoot growth and respiration more than they reduce photosynthesis, so carbohydrate storage is enhanced by low temperature, low N supply, and moderate drought stress.

Nitrogen Reserves

In a classic study of storage carbohydrates, Graber et al. (1927) noted significant changes in the N compounds stored in alfalfa taproots during regrowth in spring or following harvest. Due to their low concentration, N reserves were thought to be less important than carbohydrate reserves. However, it is now understood that young regrowth is very high in protein, especially in the form of enzymes, and therefore it is high in N compared with other tissues. N_2 fixation in the nodules of legumes declines dramatically after harvest, to the point where the rate of N_2 fixation cannot meet the N needs of new shoots. During this period, even if inorganic nitrogen (i.e., NO_3^- or NH_4^+) is available in the soil, amino acids from plant-N storage sources are critical for the initiation of new shoot tissues.

In a study of perennial ryegrass, 27% of the N in new leaves was found to be remobilized from N stored in stubble and roots in the 20 days following harvest (Fig. 4.12); however, remobilized N was the *sole source* of N to support regrowth during the first 4 days following harvest. Even when uptake of inorganic N from the rooting medium occurred, it was slow until the leaves were able to supply the roots with new photosynthate (Volenec et al., 1996).

The rate of leaf elongation in grasses such as tall fescue is generally not carbohydrate limited, but is often limited by N availability. In grasses, N stimulates cell division, which is fundamental to leaf growth. Detailed analyses of tall fescue leaves demonstrated that N fertilization increased the rate of cell division as well as the number of dividing cells. However, N had little effect on the rate of cell elongation and final cell size (MacAdam et al., 1989), indicating that N regulates the number of cells, while the elongation of new cells uses large amounts of carbohydrate for respiration and cell wall synthesis (Fig. 4.7). Clearly, both N reserves and carbohydrate reserves are important for regrowth.

Greater N storage in the form of protein in roots increases the stress tolerance of forage legumes. Proteins accumulate in taproots as plants harden for winter (see Chapter 5), with the greatest accumulation occurring in alfalfa and the least in red clover. This stored N is used for growth in spring and after cutting. Volenec et al. (1996) found that, in alfalfa, 39% of N in the first 24 days of regrowth came from N storage in roots and crowns, while the balance was supplied by uptake (Fig. 4.12). In alfalfa, red clover, and yellow sweetclover, greater protein accumulation in autumn was associated with increased winterhardiness. Potassium content in legumes increases with protein content, so the value of protein accumulation for winterhardiness may explain the increase in alfalfa

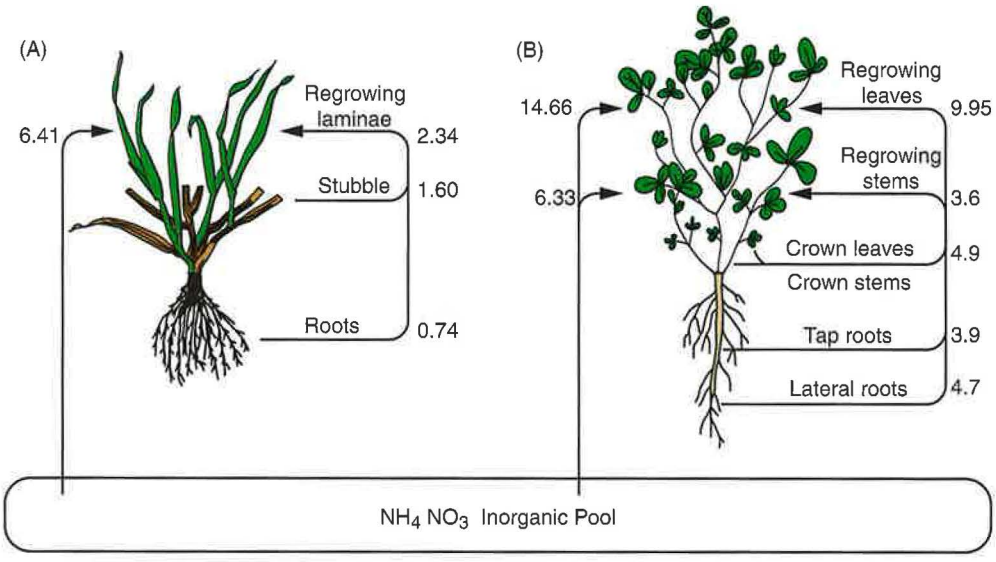

Fig. 4.12. Organic nitrogen, expressed as milligrams of N per plant, was reallocated from storage in roots and stubble during the first 20 and 24 days of perennial ryegrass and alfalfa regrowth, respectively. Organic N withdrawn from storage sources (right arrows) equals deposition in new leaves and stems (N sinks). Perennial ryegrass (A) relied solely on organic N reserves for the first 6 days of regrowth, and alfalfa (B) mainly relied on reallocation for the first 10 days of regrowth. The remaining 73% (perennial ryegrass) or 61% (alfalfa) of N used during this regrowth period was provided as inorganic N from the rooting medium. (Redrawn from Volenec et al., 1996, with permission of John Wiley & Sons.)

winterhardiness with increasing rates of K^+ fertilization in this species.

Proteins and NO_3^- are the forms in which N is most often accumulated in the storage organs of herbaceous plants. Rubisco is not just the enzyme that adds CO_2 to RuBP to "fix" carbon in photosynthesis—it is effectively the most important N storage protein in leaves. Rubisco and other leaf proteins gradually degrade as the leaves mature, releasing N in the form of amino acids for transport to the meristems for use in the synthesis of new leaf tissues needed to intercept sunlight at the top of the canopy.

Managing the Canopy

To maximize net photosynthesis and therefore productivity, the canopy of leaves in a pasture or hayfield should be managed so as to maximize light interception over the course of the growing season. Defoliation by cutting or grazing reduces leaf area, which must be regenerated in order to intercept radiation. After defoliation the period of rapid increase in forage shoot dry matter continues until the leaves in the canopy intercept about 95% of the sunlight.

The leaf area index (LAI) is the ratio of the leaf area to the land area that it covers, and the critical leaf area index is the LAI at 95% light interception. For plants with leaves that are oriented horizontally, such as white and red clover, the critical LAI is 3–5; for alfalfa, with tall stems and smaller leaflets, it is 5–6; and for grasses with vertically oriented leaf blades, such as orchardgrass and perennial ryegrass, it is 7–10.

After 95% light interception has been achieved, shading will cause the lower leaves to senesce, so while new leaves continue to be added at the top of the canopy, older leaves are shaded and will die, so there is little net increase in usable forage mass. To maximize the productivity of a stand, unless there are other management goals to consider, dry matter should be harvested as soon as the canopy has achieved 95% light interception. The leaf area or reserves of carbohydrates and proteins remaining after harvest will determine the length of the lag phase before rapid regrowth occurs and the time to the next harvest.

Canopy structure

Leaf angle markedly affects light penetration into a grass or legume canopy (Fig. 4.13). The leaves of grasses are narrow and emerge more or less vertically at the top of the canopy. This results in a favorable leaf arrangement for light penetration and a high critical LAI, and places the leaves with the greatest requirement for photosynthate at the top where light interception is unimpeded.

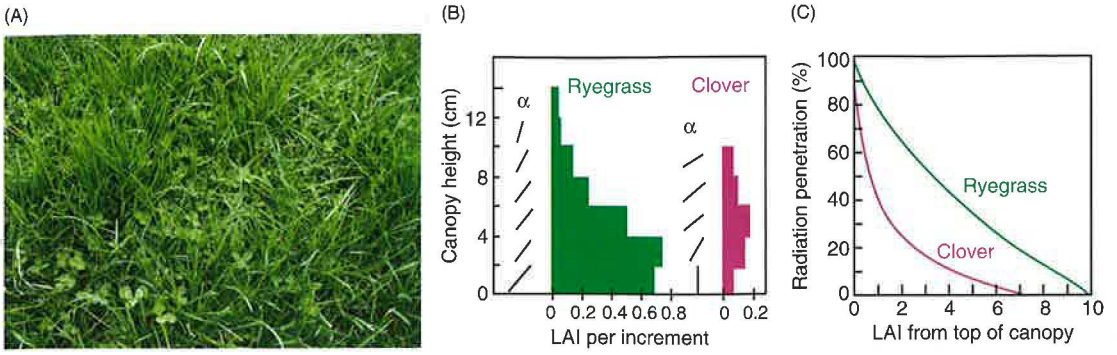

Fig. 4.13. In a perennial ryegrass–white clover canopy (A), the grass leaf area is concentrated at the bottom of the canopy (B), and leaf angles (α) become more upright from the bottom to the top of the leaf canopy to aid light penetration. White clover leaf area is concentrated near the middle of the canopy, and leaf angles become more horizontal from the bottom to the top. Light is distributed effectively throughout the dense grass canopy (C), only becoming reduced to a penetration of 25% at an LAI of 6, whereas light penetration in white clover drops to 25% at an LAI of only 2. (Adapted from Loomis and Williams, 1969, with permission of ACSESS.)

In contrast, the leaflets of white clover are folded early in development, and are then moved by petiole extension to the top of the canopy, where the blade unfolds to be displayed almost horizontally. In this flat-leafed arrangement, most radiation is intercepted by the young leaf blades in the upper part of the canopy, with less light getting through to the lower leaves, resulting in a low critical LAI. This effectively shades weeds, but it also causes older leaves with shorter petioles to senesce and die.

Forage dry matter will not be used efficiently for animal production unless it is grazed or cut as soon as the critical LAI is reached. White clover has prostrate stolons and is therefore tolerant of close grazing, but is not as shade-tolerant as legumes such as red clover, which has an upright stem and a higher critical LAI.

There is relatively little carbohydrate storage in the fibrous root system of grasses. In bunchgrasses, the highest concentration of carbohydrates is in the leaf sheaths and stem bases (the **pseudostem**) in the vegetative stages, and in the lower stem in the reproductive stages. Therefore it is critical to gauge stubble height in order to retain sufficient storage carbohydrates and basal leaf area for the support of regrowth.

If the leaf blades but not the leaf sheaths of a grass tiller are removed by moderate grazing, the effect on regrowth rate and on root biomass is minimal because the basal storage sites were not removed (Fig. 4.14). However, if severe grazing or close cutting remove a significant portion of the sheath area or pseudostem of grass tillers, a significant proportion of the stored carbohydrates and proteins will be removed, leaving less reserves to support root maintenance and shoot regrowth. A reduction in storage reserves results

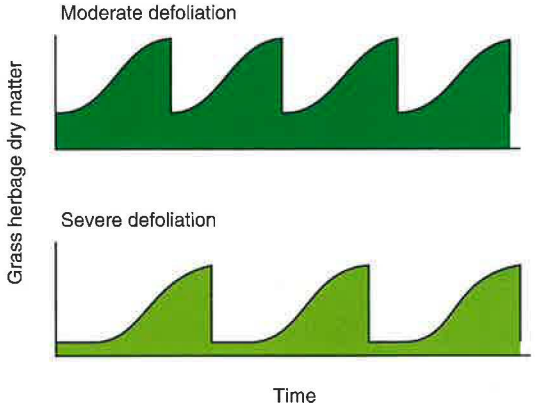

Fig. 4.14. Under well-managed defoliation of grasses (top panel), sufficient stubble remains to supply stored carbohydrates for initial regrowth, so the lag phase for dry matter accumulation following grazing or cutting is minimal. With severe defoliation (bottom panel), when leaf and stem base storage tissues have been removed, grass regrowth is slow to begin, extending the lag phase and reducing the seasonal productivity of the stand. In both cases, defoliation has occurred each time the critical LAI (95% light interception) was reached. (Adapted from Walton, 1983, with permission of Pearson Education.)

in slower regrowth, which provides a greater opportunity for weeds to germinate and compete with the grass for light, moisture, and nutrients (Fig. 4.14).

Adaptation of forage species to close grazing depends on their growth habit and the availability of storage organs such as rhizomes and stolons. Several C_3 grass species were compared in Michigan. Kentucky bluegrass, which is low-growing and rhizomatous, was least injured by close and continuous clipping, followed in order by the taller, rhizomatous grasses quackgrass and smooth bromegrass, which were followed by timothy and orchardgrass, both of which are upright bunchgrasses with storage in the base of the stem (Harrison and Hodgson, 1939). In North Carolina, with regard to C_4 species, dallisgrass, a tall bunch-type warm-season grass, was injured more by close cutting than were carpetgrass and bermudagrass, which are both stoloniferous and have canopies with many leaves near the soil surface (Lovvorn, 1945).

Continuous vs. Rotational Stocking

Cutting or grazing of forage plants that have a reduced capacity to support shoot and root regrowth, either from the remaining leaves or from storage, undermines the long-term competitiveness of desirable plants. This is why the unrestricted grazing that occurs in a continuously stocked pasture will alter the plant species composition, while animal production will probably be reduced gradually. The storage carbohydrates of the most frequently and closely grazed forage species will become exhausted, while the least desirable and therefore least grazed species with unrestricted growth and propagation will become dominant. Rotational stocking can slow down the change in botanical composition of pastures, both by reducing selectivity, so that stubble remains to support the regrowth of all forage species, and by guaranteeing an adequate rest period for the most desirable plants to restore their root and shoot food reserves.

Location of Meristems

Managing a canopy involves more than managing light interception for photosynthesis, and accumulation of reserve carbohydrates and N compounds in storage organs. Perennial forage plants need active meristems to provide new growth or regrowth after cutting or grazing. Both legumes and grasses have a shoot apex at the top of each stem, but the stems of most grasses are not elongated until the reproductive growth stage, when the seedhead (inflorescence) appears (see Chapter 2).

The most critical management stage for some grasses occurs during reproductive growth, when internode elongation elevates the inflorescence to a height at which it can be removed by cutting or grazing. When a shoot apex becomes an inflorescence, it will no longer produce leaves, so regrowth must come from tillers initiated from axillary buds. The lower canopy is often shaded, and elongation of the stem will have caused a transient redirection of photosynthate that suppresses tillering in some species, such as timothy and smooth bromegrass.

If axillary buds at the base of grass stems have not broken dormancy and developed into tillers by harvest, there will be little photosynthetic tissue in the stubble and a delay in the development of new tillers. The low LAI reduces competitiveness or creates an excessive lag phase for regrowth. Seed yield depends on the number of reproductive tillers, but regrowth depends on vegetative tillers.

Stem base carbohydrate levels in timothy are minimal when the grass is cut at the boot or stem elongation (SE) stage (Fig. 4.15). There are few axillary tillers present at the stem elongation stage, since they form close to anthesis (inflorescence emergence), after stem growth is complete. Thus cutting timothy at the boot stage can delay regrowth by 2 weeks or more. The reproductive (mother) tiller is no longer producing new leaves, and the few vegetative tillers

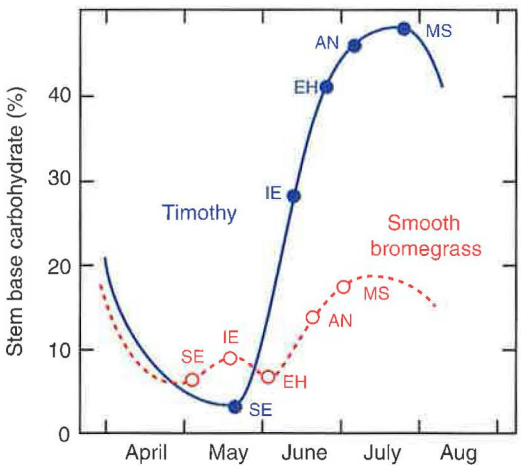

Fig. 4.15. Total non-structural carbohydrates in the stem bases of two C_3 grasses, timothy and smooth bromegrass, at successive stages of development in the field in Wisconsin. SE, beginning of stem elongation; IE, inflorescence emergence (or boot stage); EH, early heading; AN, early anthesis; MS, mature seed. Although the pattern of carbohydrate storage is similar, timothy reaches each growth stage later in the season than smooth bromegrass. As with root carbohydrate storage for legumes (Fig. 4.10), these grasses are most vulnerable to mismanagement when their carbohydrate storage is lowest, and will regrow and persist best if harvesting is managed with an understanding of the carbohydrate storage pattern. (Adapted from Smith et al., 1986, with permission of Kendall Hunt Publishing Company.)

that have broken dormancy have minimal energy reserves, and can be shaded by basal leaves or associated species.

Cutting or grazing *before* the initiation of stem elongation removes only leaf blades, leaving the shoot apex intact, which means that the stem can continue to elongate to produce an inflorescence. Delaying cutting until *after* anthesis will allow time for the axillary tillers to begin growth, and the regrowth rate will be improved.

Like alfalfa, timothy and smooth bromegrass are both good forages for hay, but they often fail to persist in mixtures with alfalfa when managed for high alfalfa hay quality. The reproductive stems of these grasses are often partially elongated when alfalfa is ready to harvest at the early flower stage, but the growth of new grass tillers is suppressed. In smooth bromegrass, significant storage of carbohydrate in the stem base occurs after inflorescence emergence. Although the crude protein concentration of forages declines with maturation, to improve the persistence of timothy or smooth bromegrass in mixtures with alfalfa, harvest should be delayed until anthesis of these grasses.

Orchardgrass recovers rapidly when cut at almost any growth stage, even when it is cut at the boot stage of stem elongation as part of an alfalfa mixture. The flowering shoots with active intercalary meristems for stem elongation appear to exert less apical dominance than those of timothy or smooth bromegrass, so new basal tillers are produced throughout the spring period. Therefore shoots at different stages of development are present at any given time. When harvested, new leaves develop rapidly on the axillary tillers, so photosynthesis of the canopy is only temporarily interrupted, making orchardgrass a potentially more persistent grass companion for alfalfa.

Summary

Plants need energy, meristems, and water for growth. Energy drives the assembly of carbohydrates, nitrogen (from the soil or the atmosphere), and minerals to support respiration and synthesis of new tissue in meristems. Water transports substances, cools the plant, and expands the cells. Depending on the species, plants grow within a range of temperatures, from 0°C (32°F) to above 38°C (100°F). Physiology influences growth rates, flowering, seed production, and resistance to or tolerance of abiotic and biotic stresses. Forage quality depends on rates of leaf and stem growth, including cell wall synthesis and the accumulation of chemical compounds that affect animal performance.

A knowledge of physiological processes forms the basis for understanding how genetics and management affect the rates and efficiency of critical growth processes for climate adaptation. For example, due to their enzymes, cool-season grass and legume species have lower photosynthesis rates than warm-season grasses at temperatures above about 27°C (80°F), but higher rates at 4°C (40°F). Perennial forage legumes store starch and proteins in their roots to support regrowth after harvest, whereas perennial cool-season grasses store carbohydrate and proteins in their stem bases and depend more on leaf area for photosynthesis during regrowth. Stomata in the leaves help the plants to control water stress.

Questions

1. What are the factors that influence the photosynthetic rate of C_4 grasses and allow it to continue to increase as the temperature rises beyond the optimal range for C_3 grasses?
2. What are the characteristics of forage nutritive value that cause cool-season grasses to be of higher quality than warm-season grasses?
3. What causes the total non-structural carbohydrate content of leaves to change between late afternoon and the following morning?
4. Will warm-season or cool-season grasses be more negatively affected by increasing biosphere CO_2 levels? Which will adapt better in a location where maximum summer temperatures and drought also increase?
5. Describe three situations where uninformed management of perennial forage crop carbohydrate reserves can undermine persistence. What are the recommended practices?
6. What is the principal storage organ for organic reserves in (a) alfalfa, (b) white clover, and (c) smooth bromegrass?
7. How does plant storage and plant use of carbohydrates in the roots of alfalfa differ from that in the roots of birdsfoot trefoil during the growing season? How should the cutting management of these two forages differ so as to optimize persistence based on mid-summer patterns of carbohydrate storage?
8. What is the difference between grazing to the proper height and overgrazing of a grass such as tall fescue in terms of plant carbohydrate storage, and what is the effect of each on the initial regrowth rate?
9. What is the critical LAI of white clover and of tall fescue? Why are these values different? How would this difference affect the potential accumulation of usable biomass in a monoculture pasture of each species?
10. Where is the meristem for a stem or branch located, relative to the rest of the branch? Provide an example in which the initial rate of regrowth is affected by the location of the reproductive stage of growth at cutting.

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