

# **MAXIMIZING CHLOROPLAST FUNCTION FOR INTENSIVELY MANICURED ATHLETIC FIELDS**

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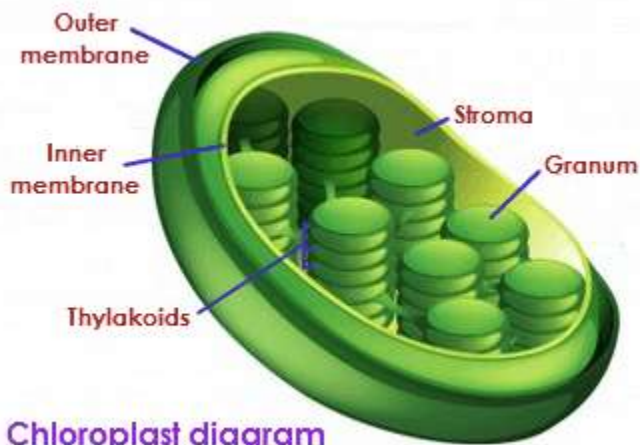
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## **Chloroplast Definition**

The word chloroplast is derived from the Greek word "chloros" meaning "green" and "plastēs" meaning "the one who forms". The chloroplasts are cellular organelles of green plants and some eukaryotic organisms. These organelles conduct photosynthesis. They absorb sunlight and convert it into sugar molecules and also produce free energy stored in the form of ATP and NADPH through photosynthesis. Chloroplasts are unique organelles and are said to have originated as endosymbiotic bacteria.

## Chloroplast

## Diagram

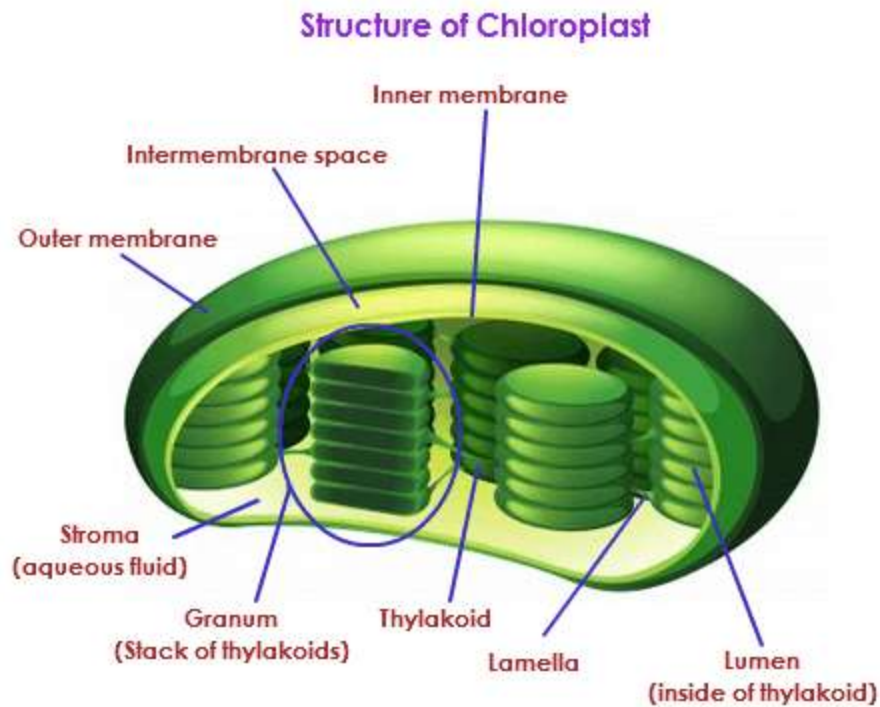


## Chloroplast Structure

Chloroplasts found in higher plants are generally biconvex or planoconvex shaped. In different plants chloroplasts have different shapes, they vary from spheroid, filamentous saucer-shaped, discoid or ovoid shaped.

They are vesicular and have a colorless center. Some chloroplasts are in shape of club, they have a thin middle zone and the ends are filled with chlorophyll. In algae a single huge chloroplast is seen that appears as a network, a spiral band or a stellate plate.

The size of the chloroplast also varies from species to species and it is constant for a given cell type. In higher plants, the average size of chloroplast is 4-6  $\mu$  in diameter and 1-3  $\mu$  in thickness.



The chloroplast are double membrane bound organelles and are the site of photosynthesis. The chloroplasts have a system of three membranes: the outer membrane, the inner membrane and the thylakoid system. The outer and the inner membrane of the chloroplast enclose a semi-gel-like fluid known as the stroma. This stroma makes up much of the volume of the chloroplast, the thylakoids system floats in the stroma.

**Outer membrane** - It is a semi-porous membrane and is permeable to small molecules and ions, which diffuses easily. The outer membrane is not permeable to larger proteins.

**Intermembrane Space** - It is usually a thin intermembrane space about 10-20 nanometers and it is present between the outer and the inner membrane of the chloroplast.

**Inner membrane** - The inner membrane of the chloroplast forms a border to the stroma. It regulates passage of materials in and out of the chloroplast. In addition of regulation activity, the fatty acids, lipids and carotenoids are synthesized in the inner chloroplast membrane.

**Stroma**

Stroma is an alkaline, aqueous fluid which is protein rich and is present within the inner membrane of the chloroplast. The space outside the thylakoid space is called the stroma. The chloroplast DNA, chloroplast ribosomes and the thylakoid system, starch granules and many proteins are found floating around the stroma.

### **Thylakoid System**

The thylakoid system is suspended in the stroma. The thylakoid system is a collection of membranous sacks called thylakoids. The chlorophyll is found in the thylakoids and is the site for the process of light reactions of photosynthesis to happen. The thylakoids are arranged in stacks known as grana. Each granum contains around 10-20 thylakoids.

Thylakoids are interconnected small sacks, the membranes of these thylakoids is the site for the light reactions of the photosynthesis to take place. The word 'thylakoid' is derived from the Greek word "thylakos" which means 'sack'.

Important protein complexes which carry out light reaction of photosynthesis are embedded in the membranes of the thylakoids. The Photosystem I and the Photosystem II are complexes that harvest light with chlorophyll and carotenoids, they absorb the light energy and use it to energize the electrons.

The molecules present in the thylakoid membrane use the electrons that are energized to pump hydrogen ions into the thylakoid space, this decreases the pH and becomes acidic in nature. A large protein complex known as the ATP synthase controls the concentration gradient of the hydrogen ions in the thylakoid space to generate ATP energy and the hydrogen ions flow back into the stroma.

Thylakoids are of two types - granal thylakoids and stromal thylakoids. Granal thylakoids are arranged in the grana are pancake shaped circular discs, which are about 300-600 nanometers in diameter. The stromal thylakoids are in contact with the stroma and are in the form of helicoid sheets.

The granal thylakoids contain only photosystem II protein complex, this allows them to stack tightly and form many granal layers with granal membrane. This structure increases stability and surface area for the capture of light.

The photosystem I and ATP synthase protein complexes are present in the stroma. These protein complexes act as spacers between the sheets of stromal thylakoids.

## Chloroplast Function

Functions of chloroplast:

- In turf plants, all the cells participate in plant immune response as they lack specialized immune cells. The chloroplasts with the nucleus and cell membrane and ER are the key organelles of pathogen defense.
- The most important function of chloroplast is to make food by the process of photosynthesis. Food is prepared in the form of sugars. During the process of photosynthesis sugar and oxygen are made using light energy, water, and carbon dioxide.
- Light reactions take place on the membranes of the thylakoids.
- Chloroplasts, like the mitochondria use the potential energy of the H<sup>+</sup> ions or the hydrogen ion gradient to generate energy in the form of ATP.
- The dark reactions also known as the Calvin cycle take place in the stroma of chloroplast.
- Production of NADPH<sub>2</sub> molecules and oxygen as a result of photolysis of water.

### The chloroplast genome

Like [mitochondria](#), chloroplasts contain their own genetic system, reflecting their evolutionary origins from photosynthetic bacteria. The genomes of chloroplasts are similar to those of mitochondria in that they consist of circular [DNA](#) molecules present in multiple copies

per organelle. However, [chloroplast](#) genomes are larger and more complex than those of mitochondria, containing approximately 120 genes.

The [chloroplast](#) genomes of several plants have been completely sequenced, leading to the identification of many of the genes contained in the organelle DNAs. These chloroplast genes encode both RNAs and [proteins](#) involved in [gene](#) expression, as well as a variety of proteins that function in [photosynthesis](#). Both the ribosomal and transfer RNAs used for [translation](#) of chloroplast mRNAs are encoded by the organelle genome. These include four rRNAs (23S, 16S, 5S, and 4.5S) and 30 tRNA species. In contrast to the smaller number of tRNAs encoded by the mitochondrial genome, the chloroplast tRNAs are sufficient to translate all the mRNA codons according to the universal [genetic code](#). In addition to these [RNA](#) components of the translation system, the chloroplast genome encodes about 20 ribosomal proteins, which represent approximately a third of the proteins of chloroplast [ribosomes](#). Some subunits of [RNA polymerase](#) are also encoded by chloroplasts, although additional RNA polymerase subunits and other factors needed for chloroplast gene expression are encoded in the [nucleus](#).

### **Import and sorting of chloroplast proteins**

Protein import into chloroplasts generally resembles mitochondrial protein import. Proteins are targeted for import into chloroplasts by N-terminal sequences of 30 to 100 amino acids, called transit peptides, which direct protein translocation across the two membranes of the [chloroplast](#) envelope and are then removed by proteolytic cleavage. As in [mitochondria](#), molecular chaperones on both the cytosolic and stromal sides of the envelope are required for protein import, which requires energy in the form of ATP. In contrast to the pre-sequences of mitochondrial import, however, transit peptides are not positively charged and the translocation of [polypeptide](#) chains into chloroplasts does not require an electric potential across the membrane.

Protein import into the chloroplast stroma: Proteins are targeted for import into chloroplasts by a transit peptide at their amino terminus. The transit peptide directs polypeptide translocation through the Toc complex in the chloroplast outer membrane. Proteins incorporated into the thylakoid lumen are transported to their destination in two steps. They are first imported into the stroma, as already described, and are then targeted for translocation across the [thylakoid membrane](#) by a second [hydrophobic signal sequence](#), which is exposed following cleavage of the transit peptide. The hydrophobic signal sequence directs translocation of the [polypeptide](#) across the thylakoid membrane and is finally removed by a second proteolytic cleavage within the lumen.

### **Photosystems I and II**

Within the thylakoid membranes of the chloroplast, are two photosystems. Photosystem I optimally absorbs photons of a wavelength of 700 nm. Photosystem II optimally absorbs photons of a wavelength

of 680 nm. The numbers indicate the order in which the photosystems were discovered, not the order of electron transfer. Under normal conditions electrons flow from PSII through cytochrome bf (a membrane bound protein analogous to Complex III of the mitochondrial electron transport chain) to PSI. Photosystem II uses light energy to oxidize two molecules of water into one molecule of molecular oxygen. The 4 electrons removed from the water molecules are transferred by an electron transport chain to ultimately reduce  $2\text{NADP}^+$  to  $2\text{NADPH}$ . During the electron transport process a proton gradient is generated across the thylakoid membrane. This proton motive force is then used to drive the synthesis of ATP. This process requires PSI, PSII, cytochrome bf, ferredoxin-NADP<sup>+</sup> reductase and chloroplast ATP synthase.

**I. Photosystem II** Photosystem II transfers electrons from water to plastoquinone and in the process generates a pH gradient. Plastoquinone (PQ) carries the electrons from PSII to the cytochrome bf complex. Plastoquinone is an analog of Coenzyme Q. The only differences are the methyl groups replacing the methoxy groups of Q and a variable isoprenoid tail. Plastoquinone can function as a one or two electron acceptor and donor. When it is fully reduced to PQH<sub>2</sub> it is called plastoquinol. Like CoQ, PQ is a lipophilic mobile electron carrier carrying electrons from PSII to cytochrome bf. Photosystem II is homologous to the purple bacterial photoreaction center we talked about previously. PSII is an integral membrane protein. The core of this membrane protein is formed by two subunits D1 and D2. These two subunits span the membrane and are homologous to subunits L and M of the bacterial photosystem. Of course PSII is more complicated than its prokaryotic counterpart. PSII contains a lot more subunits and additional chlorophylls to achieve a lot higher efficiency than bacterial systems. The overall reaction of PSII is shown below.

$$2\text{PQ} + 2\text{H}_2\text{O} \rightarrow \text{O}_2 + 2\text{PQH}_2$$

$\text{O} \text{H}_3\text{C} \text{H}_3\text{C} \text{CH}_3 \text{C} \text{H}_2 \text{C} \text{H} \text{C} \text{C} \text{H}_2 \text{CH}_3 \text{H} \text{n} = 6-10$  Plastoquinone  $\text{OH} \text{OH} \text{H}_3\text{C} \text{H}_3\text{C} \text{CH}_3 \text{C} \text{H}_2 \text{C} \text{H} \text{C} \text{C} \text{H}_2 \text{CH}_3$   
 $\text{H} \text{n}$  Plastoquinol  $2\text{e}^- - 2\text{H}^+$  +

The ribbon diagram of the crystal structure of PSII is shown below. The D1 subunit is shown in red and is homologous to the L subunit of the bacterial photosystem. The D2 subunit is shown in blue and is homologous to the M subunit of the bacterial photosystem. The structure of a bound cytochrome molecule is shown in yellow. The chlorophyll molecules are shown in green. The manganese center is shown in purple. Just as in the bacterial photosystem, there is a special pair of chlorophylls in PSII bound by D1 and D2 that are in close proximity of each other. This special pair is analogous to the special pair of bacteriochlorophylls in the bacterial photosystem. The PSII special pair consists of 2 chlorophyll a molecules that absorb light at an optimal wavelength of 680 nm. This special pair of chlorophylls is called P680. On excitation—either by the absorption of a photon or exciton transfer—P680\* rapidly transfers an electron to a nearby pheophytin a. Pheophytin a is a chlorophyll a molecule with the Magnesium replaced by two protons. The electron is then transferred to a tightly bound plastoquinone at the QA site. The electron is then transferred to an exchangeable plastoquinone

located at the QB site of the D2 subunit. The arrival of a second electron to the QB site with the uptake of two protons from the stroma produces plastoquinol, PQH<sub>2</sub>. When the electron is rapidly transferred from P680\* to pheophytin a, a positive charge is formed on the special pair, P680<sup>+</sup>. P680<sup>+</sup> is an incredibly strong oxidant which

$$\begin{array}{cccccccccccccccccccc}
\text{N} & \text{N} & \text{H} & \text{N} & \text{H} & \text{N} & \text{H}_3\text{C} & \text{H}_3\text{C} & \text{HC} & \text{CH}_2 & \text{CH}_3 & \text{CH}_2 & \text{H}_3\text{C} & \text{O} & \text{H} & \text{O} & \text{H}_3\text{C} & \text{O} & \text{O} & \text{P} & \text{O} \\
\text{Pheophytin a} & \text{N} & \text{N} & \text{N} & \text{N} & \text{H}_3\text{C} & \text{H}_3\text{C} & \text{HC} & \text{CH}_2 & \text{CH}_3 & \text{CH}_2 & \text{H}_3\text{C} & \text{O} & \text{H} & \text{O} & \text{H}_3\text{C} & \text{O} & \text{O} & \text{P} & \text{O} & \text{Chlorophyll a} & \text{Mg}
\end{array}$$

extracts electrons from water molecules bound at the manganese center. The structure of this manganese center includes 4 Manganese ions, a calcium ion, a chloride ion, and a tyrosine radical. Manganese is the core of this redox center because it has four stable oxidation states (Mn<sup>2+</sup>, Mn<sup>3+</sup>, Mn<sup>4+</sup> and Mn<sup>5+</sup>) and coordinates tightly to oxygen containing species. Each time the P680 is excited and an electron is kicked out, the positively charged special pair extracts an electron from the manganese center. 2H<sub>2</sub>O → O<sub>2</sub> + 4e<sup>-</sup>. 4 electrons must be transferred to 2 molecules of plastoquinone in order to oxidize H<sub>2</sub>O to molecular oxygen. This requires 4 photochemical steps. The Manganese center is oxidized one electron at a time, until two molecules of H<sub>2</sub>O are linked to form O<sub>2</sub> which is then released from the center. A tyrosine residue not shown participates in the proton electron transfers. The structures are designated S<sub>0</sub> through S<sub>4</sub> to indicate the number of electrons removed. The oscillation in O<sub>2</sub> evolution dampens over repeated flashes and converges to an average value. We know the manganese center exists in five different oxidation states numbered for S<sub>0</sub> to S<sub>4</sub> as shown above. One electron and a proton are removed during each photochemical step. When S<sub>4</sub> is attained, an O<sub>2</sub> molecule is released and two new molecules of water bind. The reason the third pulse of light produces O<sub>2</sub> is because the resting state of the PSII in the chloroplast is S<sub>1</sub> not S<sub>0</sub>. PSII spans the thylakoid membrane. The site of plastoquinone reduction is on the stroma side of the membrane. The manganese complex is on the thylakoid lumen side of the membrane. For every four electrons harvested from H<sub>2</sub>O, 2 molecules of PQH<sub>2</sub> are formed extracting four protons from the stroma. The four protons formed during the oxidation of water are released into the thylakoid lumen. This distribution of protons across the thylakoid membrane generate a pH gradient with a low pH in the lumen and a high pH in the stroma.

II. Cytochrome b<sub>f</sub>

The plastoquinol formed by PSII contributes its electrons through an electron transport chain that terminates at PSI. The intermediary electron transfer complex between PSII and PSI is cytochrome b<sub>f</sub> also known as cytochrome b<sub>6</sub>f. In this electron transfer complex electrons are passed one at a time from plastoquinol to plastocyanin (Pc) a copper protein of the thylakoid lumen. The reaction is shown below:

$$\text{PQH}_2 + 2\text{Pc}(\text{Cu}^{2+}) \rightarrow 2\text{Pc}(\text{Cu}^+) + 2\text{H}^+$$

The protons are released into the thylakoid lumen. Plastocyanin is a water soluble electron carrier found in the thylakoid lumen of chloroplasts. It contains a single Copper atom coordinated to two histidine residues and a cysteine residue in a distorted tetrahedron. The molecule is intensely blue in the cupric form. This mobile electron carrier carries electrons from



cytochrome *bf* to PSI. The electron transfer of cytochrome *bf* is very similar to the electron transfer catalyzed by Complex III of the mitochondrion. The subunit components of cytochrome *bf* are homologous to the subunits of Complex III, cytochrome *c* reductase. The cytochrome *bf* contains two *b*-type heme cytochromes, a Rieske protein-type Fe-S protein, and a *c*-type cytochrome similar to cytochrome *c*<sub>1</sub>. This enzyme transfers electrons from plastoquinol through the same Q cycle as Complex III. The net result is two protons are picked up from the stroma side of the thylakoid membrane and 4 protons are released into the lumen contributing to the pH gradient.

III. Photosystem I. The final stage of the light reactions is catalyzed by PSI. This protein has two main components forming its core, *psaA* and *psaB*. These two subunits are quite a bit larger than the core components of PSII and the bacterial photosystem. Nonetheless, the subunits are all homologous. The *psaA* and *psaB* subunits are shown in yellow with the regions homologous to the core of PSII shown in red and blue. Chlorophyll molecules are shown in green and the 3 4Fe-4S clusters are also shown in green. A special pair of chlorophyll *a* molecules lies at the center of the structure which absorbs light maximally at 700 nm. This special pair is denoted P700. Upon excitation—either by direct absorption of a photon or exciton transfer—P700\* transfers an electron through a chlorophyll and a bound quinone (QA) to a set of 4Fe-4S clusters. From these clusters the electron is transferred to ferredoxin (Fd) a water soluble mobile electron carrier located in the stroma which contains a 2Fe-2S cluster coordinated to 4 cysteine residues. The electron transfer produces a positive charge on the special pair which is neutralized by the transfer of an electron from a reduced plastocyanin. The overall reaction is shown below.  $Pc(Cu^+) + Fd_{ox} \rightarrow Pc(Cu^{2+}) + Fd_{red}$  The structure of ferredoxin is shown to the left. Ferredoxin contains a 2Fe-2S cluster which accepts electrons from PSI and carries them to ferredoxin-NADP<sup>+</sup> reductase.  $Pc(Cu^{2+}) + e^- \rightarrow Pc(Cu^+)$   $E_o' = +0.37\text{ V}$   $Fd_{ox} + e^- \rightarrow Fd_{red}$   $E_o' = -0.45\text{ V}$  The electron acceptor in the overall reaction shown above is the oxidized ferredoxin, the electron donor is the reduced plastocyanin. From the reduction potentials listed above, the change in reduction potential is:  $\Delta E_o' = -0.45 - 0.37 = -0.82\text{ V}$  which corresponds to a  $\Delta G_o' = 79.1\text{ kJ/mol}$ , very endergonic. This uphill electron transfer is driven by absorption of a 700-nm photon of light which has an energy of 171 kJ/mol. The electron transport pathway between PSII and PSI is called the Z-scheme because the redox diagram looks like a sideways letter Z.

IV. Ferredoxin-NADP<sup>+</sup> Reductase Ferredoxin is a strong reductant but can only function in one electron reductions. NADP<sup>+</sup> can only accept 2 electrons in the form of a hydride. Clearly we need an intermediary to facilitate the electron transfer. The transfer of electrons from reduced ferredoxin to NADP<sup>+</sup> is catalyzed by ferredoxin-NADP<sup>+</sup> reductase which is a flavoprotein. This complex contains a tightly bound FAD which accepts the electrons one at a time from ferredoxin. The FADH<sub>2</sub> then transfers a hydride to NADP<sup>+</sup> to form NADPH. This reaction takes place on the stromal side of the thylakoid membrane. The uptake of a proton by NADP<sup>+</sup>

further contributes to the pH gradient across the thylakoid membrane. V. Chloroplast ATP Synthase The transport of electrons from water to NADP<sup>+</sup> generated a pH gradient across the thylakoid membrane. This proton motive force is used to drive the synthesis of ATP. The synthesis of ATP in the chloroplast is nearly identical with ATP synthesis in the mitochondria. The pH gradient generated between the stroma and the thylakoid lumen is possible because the thylakoid membrane is impermeable to protons. When the chloroplast is illuminated the thylakoid lumen becomes markedly acidic, pH ≈ 4. The pH of the stroma is around 7.5. The light induced pH gradient is about 3.5 pH units. The transmembrane electrical potential is not a significant factor in the proton motive force in the chloroplast because the thylakoid membrane is permeable to Cl<sup>-</sup> and Mg<sup>2+</sup>. Because of this permeability, the thylakoid lumen remains electrically neutral while the pH gradient is generated. A pH gradient of 3.5 pH units thus corresponds to a proton motive force of -20 kJ/mol. The ATP synthase of the chloroplast is called the CF<sub>1</sub>-CF<sub>0</sub> complex where C stands for chloroplast and F<sub>1</sub> and F<sub>0</sub> relate to the homologous ATP synthase of the mitochondria. The mitochondrial and the chloroplast ATP synthase are essentially identical with similar subunits and subunit stoichiometries. The catalytic subunit is the β subunit of CF<sub>1</sub>. The CF<sub>1</sub> complex lies in the stroma. The CF<sub>0</sub> complex channels protons from the thylakoid lumen to the stroma driving rotation of the 12 c subunits which in turn drives ATP synthesis. The ATP formed is released into the stroma where it is needed for the dark reactions of photosynthesis. VI. Cyclic Photophosphorylation There is an alternative pathway for the electrons arising from PSI giving photosynthesis versatility. The electrons carried in reduced ferredoxin can be transferred to the cytochrome bf complex rather than the ferredoxin-NADP<sup>+</sup> reductase complex as shown below. The electrons then flow back through cytochrome bf to reduce plastocyanin, which then reduces the P700<sup>+</sup> to complete the cycle. The net outcome of this cyclic flow of electrons is the pumping of protons across the thylakoid membrane by the cytochrome bf complex, producing a pH gradient which then drives the synthesis of ATP. This process produces ATP without NADPH generation. In addition PSII does not participate in cyclic photophosphorylation, so O<sub>2</sub> is not generated during this process. Cyclic photophosphorylation only occurs when the NADP<sup>+</sup> concentration becomes limiting, such is the case when there is a very high ratio of NADPH/NADP .

## Nutrients and Minerals involved in Chloroplast Development

### **Manganese**

Manganese is present in most Fe-Mg containing igneous rocks and when solubilized by weathering, forms several secondary minerals mostly pyrolusite (MnO<sub>2</sub>) and manganite (MnOOH). Thus, most soil Mn is insoluble and not available to plant roots. The plant-available form of free Mn in soil solution is the divalent cation Mn<sup>2+</sup> .

Manganese solubility is very much dependent on soil pH, decreasing 100 fold with each unit increase in pH. Consequently Mn is much more soluble and available to plants in acid soils ( $\text{pH} < 7$ ). Here, minerals containing Mn in oxidized form ( $\text{Mn}^{3+}$  or  $\text{Mn}^{4+}$ ) will be reduced to  $\text{Mn}^{2+}$  by acquiring electrons ( $e^-$ ) through the microbial oxidation of organic carbon. To meet the nutritional needs of plants for Mn, the soil solution and exchangeable Mn should be 2-3 ppm and 0.2-5.0 ppm, respectively. In acid soils, soluble Mn may exceed these levels by substantial amounts and become toxic to plants. This is likely in situations where soil minerals contain Mn, the organic matter content is high, the pH is low and the soil is periodically waterlogged (low in free oxygen). Under such conditions, acid inhibition of plant growth

4 Light Photons Photosynthesis Fig. 2. Oxygen evolving mechanism of plant photosynthesis in which water replaces electrons driven by light from chlorophyll through the re-dox action of Mn. may be due primarily to toxic levels of available Mn. Under opposite conditions (parent minerals low in Mn, alkaline pH, low organic matter and good drainage) inadequate supplies of available Mn are likely. Some 13 million acres in 30 states of the US exhibit chronically inadequate levels of Mn. For the turf manager, Mn insufficiency is a more likely problem than its toxicity. Soluble soil Mn is also present in the form of Mn-chelates. These organic chelates are excreted from plant roots or produced during the metabolism of organic residues. They will bind with  $\text{Mn}^{2+}$  but remain in solution as uncharged molecules. Much of the soluble Mn in organic soils will be present in a chelated form. Being soluble, Mn chelates are mobile in the soil and can be drawn to roots via mass flow when plants are removing water from the soil through transpiration. This process can assist in Mn uptake as we will see below. Manganese uptake

Plant roots can absorb Mn primarily as the divalent cation ( $\text{Mn}^{2+}$ ). It enters root cells by crossing their plasma membrane via a

TABLE 2. MANGANESE FUNCTIONS IN HIGHER PLANTS INCLUDING TURFGRASSES

Essential component of two enzymes:

- $\text{O}_2$  evolving component of photosynthesis (PS II)
- Mn-containing superoxide dismutase (MnSOD)

Activating cofactor for 3-5 enzymes required for:

- Respiratory metabolism of organic acids (TCA cycle)
- RNA synthesis in chloroplasts (RNA polymerase)
- Shikimic acid pathway (aromatic amino acid synthesis)
- Lignin synthesis in roots (cell wall peroxidases)
- Lipid synthesis including chlorophyll and carotenoids
- Regulating auxin levels and isoprenoid synthesis (GA)

Required for root elongation and lateral root formation specific transporter protein following an electrical gradient (cell interior is more negative than the cell wall). Other divalent micronutrient cations, such as Copper ( $\text{Cu}^{2+}$ ) and Zinc ( $\text{Zn}^{2+}$ ), do not compete with  $\text{Mn}^{2+}$  for membrane transport sites although they do compete with each other (Bowen 1969). However, the abundance of several macronutrients on cation exchange sites within the cell walls of root cells (the apoplast) can influence Mn absorption by roots. Manganese is much less available from soils of high pH. This is in large part due to the high concentrations of Calcium and Magnesium ions ( $\text{Ca}^{2+}$  &  $\text{Mg}^{2+}$ ) in such soils. When

$\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  dominate cation exchange sites in root cell walls, there are few places for less abundant ions like  $\text{Mn}^{2+}$  to be retained. Since cations absorbed by roots are drawn mostly from those present within the cell wall matrix, any less abundant ions (including most micronutrients) will have a difficult time reaching the plasma membrane when they are vastly outnumbered by basic divalent cations. This is the reason why most micronutrient metals are less available in soils of neutral or alkaline pH. In acid soils, the principle competing cation is Hydrogen ( $\text{H}^+$ ) and it is not held on exchange sites as tightly as most divalent cations. However, if other cations such as aluminum ( $\text{Al}^{3+}$ ), potassium ( $\text{K}^+$ ) or other micronutrients greatly outnumber  $\text{Mn}^{2+}$  even in acid soils, Mn may become deficient. Even so, the most common method for increasing Mn availability to plants is to apply acid fertilizers such as  $(\text{NH}_4)_2\text{SO}_4$  or urea. The availability and absorption of Mn from soils is more influenced by microbial activity than is any other. Most soil Mn is insoluble and not available to plant roots. To be absorbed by plants, Mn must be reduced to the divalent  $\text{Mn}^{2+}$  ion and this requires the action of microbial metabolism (Marschner 1995). Under acid conditions, Mn-containing minerals will slowly be solubilized and several ionic To meet the nutritional needs of plants for Mn, the soil solution and exchangeable Mn should be 2-3 ppm and 0.2-5.0 ppm, respectively. acids and phenolics) to the soil immediately adjacent to the roots (rhizosphere). These exudates along with dead root cells (root cap cells, root hairs, epidermal and cortical cells) fuel microbial activity and, especially when the soil is waterlogged, supply electrons to reduce Mn ions to the plant-available  $\text{Mn}^{2+}$  form. This ion can then be absorbed by root cells as described above. In grasses, there is a cooperative mechanism for acquiring Mn by plants experiencing an Iron (Fe) deficiency. Because of its low solubility and the inability of membrane transporters to accommodate trivalent ions,  $\text{Fe}^{3+}$  poses some special problems in its availability to plants. Grasses have a unique strategy for making  $\text{Fe}^{3+}$  ions available for root uptake. In response to low Fe stress, grass roots are induced to release special amino acids called phytosiderophores that chelate free  $\text{Fe}^{3+}$  allowing it to move to root surfaces and be absorbed (Fig. 1). While phytosiderophores have a very high affinity for  $\text{Fe}^{3+}$  and root cells have enhanced transporter capacity for the Fe-phytosiderophore complex, other micronutrient cations can also be chelated by phytosiderophores and become mobilized within the rhizosphere. Thus when Fe is deficient,  $\text{Mn}^{2+}$  can be chelated by phytosiderophores and diffuse to the root surface where it is absorbed into the root. The Mn-phytosiderophore forms of free Mn and  $\text{Mn}(\text{OH})_n$  will be released to the soil solution. Under such conditions, most plant roots will discharge low molecular weight exudates (organic acids, sugars, amino complex is not absorbed as readily as is that formed with  $\text{Fe}^{3+}$ . However, this Fe deficiency induced mechanism for capturing micronutrient ions can make nutrients other than Fe more available to grasses. Manganese serves a number of functions in plants (Table 2). Its most important roles are as an essential

component of the oxygen (O<sub>2</sub>) evolving complex in photosynthesis and as an activator cofactor of several major enzymes involved in numerous metabolic sequences. Manganese also plays a role in root elongation and lateral root initiation possibly by regulating the auxin levels along the root axis. Some of these functions that are most significant for turfgrass performance will be discussed in this section.

Oxygen evolution in photosynthesis Because of the central role played by photosynthesis in the life of all green plants, the requirement for Mn in the oxygen evolving complex of Photosystem II in the photosynthetic electron transport chain assumes primary importance. Virtually all oxygen in the atmosphere is derived from photosynthesis and its Mn requiring oxygen evolving complex. Thus, all aerobic life including human life is absolutely dependent on this Mn facilitated biochemical process. The electrons used in photosynthesis to reduce carbon dioxide (CO<sub>2</sub>) to carbohydrates [(CH<sub>2</sub>O)<sub>n</sub>] ultimately are taken from the oxygen in water (H<sub>2</sub>O). When two molecules of water are so CO<sub>2</sub> + 2H<sub>2</sub>O > (CH<sub>2</sub>O)<sub>n</sub> + O<sub>2</sub> + H<sub>2</sub>O oxidized (lose 4e<sup>-</sup>), one molecule of oxygen gas (O<sub>2</sub>) is produced as a byproduct and the 4e<sup>-</sup> reduce an electron carrier that eventually will reduce the C of one CO<sub>2</sub> to one carbohydrate C. The oxygen of water does not easily surrender its electrons and it will only do so in the presence of a very strong oxidant (substance having a powerful affinity for electrons). This strong oxidant forms when a special chlorophyll molecule receives a quantum of light energy that drives off an electron from the excited chlorophyll to a nearby electron acceptor. This begins the photosynthetic electron transport pathway. The chlorophyll that has lost an electron must obtain another or it will become further oxidized and destroyed. Electrons are provided from a nearby protein that contains a cluster of four Mn atoms each capable of donating an electron to the oxidized chlorophyll. When each Mn atom has donated an electron (become oxidized from Mn<sup>2+</sup> to Mn<sup>3+</sup> or Mn<sup>4+</sup>), these four oxidized Mn atoms together represent an oxidant so strong that they can strip electrons from the oxygen of water. To reduce the four Mn atoms, four electrons are needed and that requires the oxidation of two water molecules. 2H<sub>2</sub>O + 4Mn<sup>3+</sup> > 4Mn<sup>2+</sup> + O<sub>2</sub> + 4H<sup>+</sup> The Mn cluster is now ready again to deliver electrons to chlorophyll when it becomes oxidized through photoactivation. Thus, the Mn-containing oxygen evolving complex allows the flow of electrons to continue as light drives electrons from chlorophyll ultimately to reduce CO<sub>2</sub> or Manganese-containing superoxide dismutase (Mn-SOD), Mn is the core atom (prosthetic group) in an enzyme that catalyzes the destruction of a potentially damaging oxygen free radical, superoxide (O<sub>2</sub><sup>-</sup>). Wherever O<sub>2</sub><sup>-</sup> is involved in biochemical reactions and a source of electrons is present (reducing agents), the probability of O<sub>2</sub><sup>-</sup> being formed is great. To eliminate this toxic radical, a family of SOD enzymes has evolved each employing a different metal as its prosthetic group. The Fe-SOD and CuZn-SOD are most active in chloroplasts with the latter also found in mitochondria. The Mn-SOD is not so widely distributed among plant families and is most active in mitochondria and in peroxisomes

that are involved in photorespiration. The MnSOD enzyme is the major SOD in several groups of rhizosphere bacteria. Plants transformed to over-produce Mn-SOD experienced less chlorophyll degradation and mitochondrial leakage when grown in high light. These enzymes permit most grasses to grow in full sunlight without experiencing photo-oxidative damage. Mn and phenol biosynthesis leading to lignin. Lignin is a complex polymer of unsaturated six-sided ring structures that are produced in cell walls and give plant tissues great strength. The highly lignified vascular cells and fibers of trees enable them to reach great heights and withstand considerable mechanical stress. Lignin also contributes to the strength of grass stems and leaves and its presence in root cell walls helps resist pathogen. Manganese serves as activator for several enzymes of the shikimic acid pathway that leads to the synthesis of aromatic (phenolic) amino acids (phenylalanine and tyrosine). These are the starting products for the synthesis of phenolic acids and alcohols that are produced in response to attack by pathogenic fungi and constitute a major disease defense mechanism. These substances are also the building blocks of lignin molecules that are assembled in cell walls through the action of Mn-containing peroxidases. Manganese plays a central role in several reactions required for the synthesis of phenolic compounds and its function cannot be substituted by any other element. Manganese insufficiency results in dull chlorotic leaves that not grow rapidly. As is the case for most micronutrients, deficiency symptoms of Mn are subtle and not easily recognized. For turfgrasses, physical deficiency symptoms are almost useless for diagnosing problems. Such symptoms are poorly defined and their presence invariably indicates that substantial damage has already occurred. Consequently I will not attempt to describe the appearance of Mn deficient turf but rather concentrate on the impact insufficient Mn can have on turfgrass growth and performance. The most common turf response to inadequate Mn is a markedly reduced growth rate of both shoots and roots. This occurs because the oxygen evolving complex of photosynthesis is highly sensitive to low Mn levels and when it malfunctions, photosynthetic rates decline sharply. This results in a reduced supply of carbohydrates that in turn depress amino acid and protein synthesis and the growth of cells. As a result, nitrate, phosphate and other nutrients may accumulate because they cannot be utilized in plant growth. Root growth is also inhibited because photosynthetic energy from shoots (sugars) is not available to support root growth. Thus, even though roots are the first to receive whatever Mn might be available from the soil, resources from the shoots are not sufficient to support much growth. Increased disease incidence is another symptom of Mn deficiency. Because Mn plays many critical roles in the biosynthesis of phenolics and lignin, grasses deficient in Mn are unable to respond to pathogen attack by producing phytoalexins that would inhibit spore germination and block fungal invasion. As a result, disease outbreaks are more frequent and difficult to control. Many soil fungi that normally would not be pathogenic or only weakly so will cause disease in Mn deficient plants. This

makes disease identification more difficult and reduces the effectiveness of fungicides. In short, the grass is unable to do its part in resisting infection and has no chance of growing out of an infection. You might suspect that Mn supplies are low when turf receiving acid generating fertilizers exhibits less disease. Fertilizer materials such as  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{NH}_4\text{NO}_3$  and urea tend to acidify the rhizosphere making Mn more soluble and available to grass roots. If liming promotes an increase in disease incidence, again you might suspect that Mn supplies are marginal. Because Mn is not readily mobilized within a plant (it does not translocate well in the phloem), new leaf growth is most likely to become chlorotic when Mn is deficient. This symptom can easily be confused with that resulting from an Fe deficiency. Iron deficiency causes emerging new leaves to be bright yellow and continue growing at a reasonable rate. Manganese insufficiency results in dull chlorotic leaves that do not grow rapidly. Soil conditions that would promote Mn deficiency (elevated pH, high organic matter and carbonate enrichment) would also tend to reduce the availability of Fe. However, deficiencies of Mn are less common in turf than are those of Fe. Turf grown on soil may experience Mn toxicity. Any program that would acidify soils ( $\text{NH}_4$ -fertilizers, sulfur sources) could make Mn increasingly available and reach toxic levels. As mentioned above, Mn toxicity is part of the acid soil toxicity syndrome but this can usually be avoided by maintaining the soil at pH 5.5 or higher. Turf grown on artificial media (sand based greens) is prone to suffer from inadequate Mn unless it is applied in fertilizer or top dressing. The materials from which artificial greens are constructed will contain very little Mn and sufficient amounts will not likely be provided as contaminants in fertilizers or most other soil amendments. Thus, Mn should be considered as part of a micronutrient management program.

Sources of manganese

When a Mn insufficiency is suspected, it should be confirmed by a tissue test to determine if it is approaching the critical concentration of 25 ppm. If an addition of Mn is indicated, there are several sources available (Table 3). The material most widely used for the correction of Mn deficiency is  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ . It can be applied through the soil or as a foliar spray.  $\text{MnO}$  is largely insoluble but it can be used effectively as a Mn source if ground finely and incorporated throughout the root zone. There are a number of natural organic Mn complexes and synthetic Mn chelates that are effective sources of Mn when applied as a foliar spray. Manganese application rates generally range from 1 to 25 lbs/acre with the lower rates used as foliar treatments. When soils have a high capacity for binding and immobilizing soluble Mn, foliar applications are strongly recommended. Because Mn will not translocate from leaves to perennial plant organs, several foliar applications may be needed to provide season-long benefits. Frequent mowing and clipping removal will reduce the effectiveness of foliar applications to greens or other intensively managed turf. For such areas, Mn and other micronutrients should be incorporated into a comprehensive turf management program. This could involve an application of Mn with selected pesticide treatments, top dressings, syringings or other

appropriate opportunities throughout the season. On sand-based athletic fields, Mn immobilization within the root zone should not be a problem and less expensive granular Mn sources can be applied during aeration or when the turf is being established. Broadcast topical applications can be made to established turf and incorporated via irrigation. As turf is managed ever more intensely, the chances of micronutrients becoming deficient increase sharply. For elements such as Mn, deficiencies are not easily detected and the turf manager can spend much time and effort trying to identify the cause of problems that are indirectly related to a nutrient deficit. To avoid such problems, a preventive approach might be best. Micronutrient treatments are not costly and if applied properly rarely can cause injury.

## **Magnesium**

Each

chlorophyll molecule is constructed around a magnesium atom. Also, several steps in the biosynthesis of chlorophyll are catalyzed by magnesium requiring ions. In addition, the genetic material, which chlorophyll synthesizing enzymes depend on for expression, is held together by magnesium. In short, magnesium is an essential macronutrient upon which not only chlorophyll synthesis but all plant life depends. The essential roles played by magnesium in plant growth and function are well recognized. Nevertheless, little research has been reported on its importance for turfgrass culture or on how best to make it available to turf. Even the professional turf journals rarely consider the requirements for and management of magnesium in turf. Magnesium in turfgrasses The magnesium content of turfgrass leaves, averaged over seven species, has been reported to be 2.1 g/kg (ppm). This is lower than the concentrations of calcium and phosphorus in turfgrasses by roughly comparable to their content of sulfur. Sufficiency levels of magnesium generally average somewhat greater than 2 g/kg, which suggests that under normal nutritional management, turfgrasses may be chronically deficient in magnesium. This could be a serious matter as we shall discover later. Unlike calcium, magnesium is present in plant tissues in a metabolic pool of between 2-10 mM while that of free  $\text{Ca}^{+2}$  is closer to 0.1-0.2  $\mu\text{M}$  or 10,000 times less than  $\text{Mg}^{+2}$ . This is because  $\text{Mg}^{+2}$  is intimately involved in many metabolic processes and its concentration within the cytoplasm must be maintained within a fairly tight range. The presence of  $\text{Ca}^{+2}$  in the cytosol would effectively compete with  $\text{Mg}^{+2}$  for enzyme binding sites and this Magnesium and calcium are chemically similar. Both bind to cation exchange sites on soil colloids and are retained within the soil against leaching when rain or irrigation water percolates through the soil profile would be disastrous to metabolic function. This cytosolic magnesium concentration is stabilized by a large pool of reserve  $\text{Mg}^{+2}$  maintained within the vacuole where it can reach concentrations of over 100  $\mu\text{M}$ . Considering that the vacuole can constitute 85% of the volume of a mature cell, this represents an enormous reserve supply of magnesium for the cell. This probably explains why magnesium deficiencies do not occur immediately following the withdrawal of available  $\text{Mg}^{+2}$  from the



roots. This also indicates that  $Mg^{+2}$  plays an important role in balancing the ionic charge between vacuole and cytosol and in maintaining favorable water relations between the root and soil. Magnesium shares with calcium the cation exchange sites within the cell walls provided by pectate chains (Hull 1997b). Again because of its stronger ionic bonding,  $Ca^{+2}$  dominates the exchange sites within cell walls but  $Mg^{+2}$  is present to a significant extent (5-10 percent of the total cell magnesium). This also helps maintain a ready supply of magnesium for use within cells when needed. Availability in the Soil Environment Magnesium and calcium are chemically similar elements. In the soil solution, both elements are present as divalent cations ( $Mg^{+2}$  and  $Ca^{+2}$ ). That means they bind to cation exchange sites on soil colloids and are thereby retained within the soil against leaching when rain or irrigation water percolates through the soil profile. The binding strength of cations to exchange sites depends upon the charge strength of the cation and its degree of hydration. Calcium and magnesium have essentially the same charge (+2) but magnesium is more highly hydrated than calcium: diameter of the hydrated ion is 0.64 nm for magnesium and 0.56 nm for calcium. The non-hydrated calcium is slightly larger than the non-hydrated magnesium (0.21 vs. 0.16 nm diameter respectively). This larger amount of water surrounding the magnesium ion tends to insulate its charge making it less effective in binding to cation exchange sites. Consequently,  $Mg^{+2}$  tends to be displaced from exchange sites by  $Ca^{+2}$  when the two ions are present in equivalent amounts. If pH adjustment is achieved by using ground limestone (calcium carbonate =  $CaCO_3$ ),  $Mg^{+2}$  will be displaced from exchange sites and lost during clipping removal if the  $Mg^{+2}$  is absorbed by the turf or leached from the root zone if it is not absorbed by roots. In any event, the amount of  $Mg^{+2}$  available to turf may become compromised if large quantities of calcium are applied with little regard for the magnesium supply. A favorable magnesium status in turf is even more at risk because of the unfavorable competition between  $Mg^{+2}$  absorption by grass roots and other commonly applied cations. The rate of  $Mg^{+2}$  uptake can be strongly depressed by soil cations such as potassium ( $K^+$ ), ammonium ( $NH_4^+$ ),  $Ca^{+2}$  and low pH ( $H^+$ ). Consequently, magnesium deficiency induced by competing cations is a fairly common occurrence. In turf management, there is a long tradition of applying adequate potassium to improve the texture of grass blades and increase disease resistance. Because most turfgrasses grow best at a soil pH range of 6.5-7.0, liming materials rich in calcium are often applied in the more humid regions. In areas where rainfall is limited, the soil pH is often near neutral because the soils are naturally enriched with calcium and potassium. In either case, the soil's ionic environment may not favor the uptake of adequate magnesium by turf even when its presence in the soil is reasonably high. Chemical structure of Mg-Adenosine Triphosphate (Mg-ATP) binding via a magnesium ion to an enzyme protein in the process of transferring the terminal phosphate of ATP to a metabolite. When growth of Mg-limited turf grass plants is stimulated by high nitrogen or

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