BY JEFF HAAG

Grow playable, healthy turf

A look at factors that damage chloroplasts and the defenses that protect them

The goal of every turfgrass manager is to provide a playable surface and aesthetically pleasing green turfgrass. Achieving the latter involves a reciprocal balance between soil, fertility, moisture, temperature, humidity, grass species, mowing techniques, cultural practices and cooperation from Mother Nature. All these aspects have to be working in sync for turfgrass to perform properly and be appealing colorwise.

Protecting and strengthening chloroplasts would seem like the logical action to take because this is where chlorophyll, a pigment that gives turfgrass its green appearance, is developed.

The most important characteristic of plants is their ability to photosynthesis – to make their own food by connecting light energy into chemical energy. This process is carried out in specialized organelles called chloroplasts. A photosynthetic cell contains anywhere from one to several thousand chloroplasts. The electrons from chlorophyll molecules in photosystem II replace the electrons that leave chlorophyll molecules in photosystem I.

Located inside the chloroplast are thylakoid membranes where light reactions take place. This is where chlorophyll is found, therefore, there's a synergistic relationship between keeping the chloroplasts and the thylakoid membranes as healthy as possible.

There are events that can be harmful to chloroplasts and thylakoid membranes, as well as necessary components that can prevent damage to them.

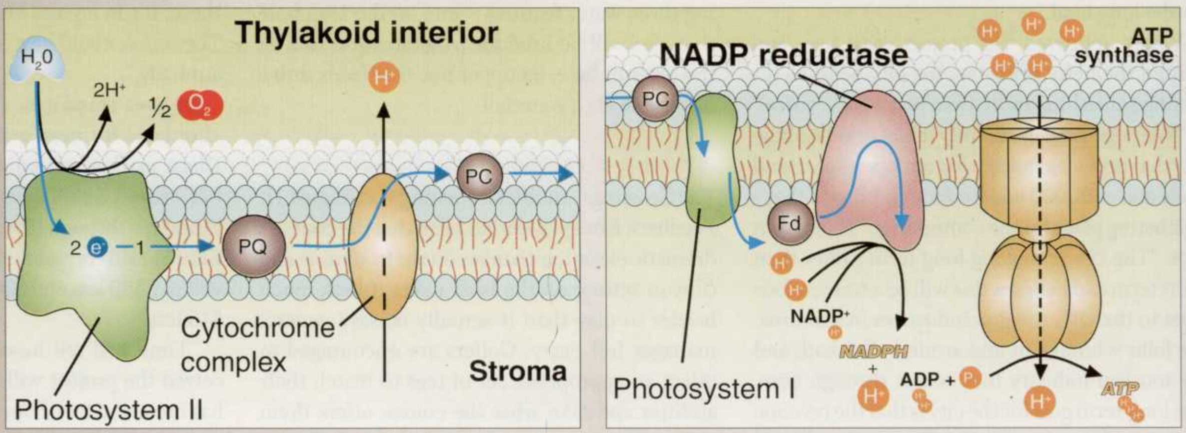
FREE RADICALS

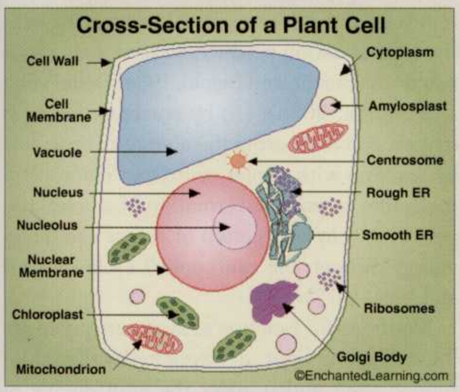
One event that can damage chloroplasts is the development of free radicals. The medical profession has shown that free radicals can cause diseases in the human body. Likewise, turfgrass managers know that research throughout the past several years has shown free radicals can damage lipids, proteins and DNA inside cells of turfgrass plants, including chloroplasts.

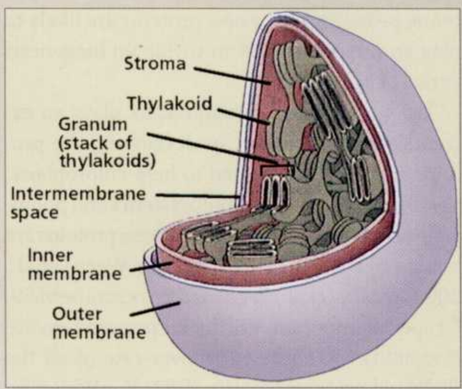
Typically, free radicals are stable molecules that contain pairs of electrons. When a chemical reaction breaks the bonds that hold the paired electrons together, free radicals are produced. They contain an odd number of electrons, which make them unstable, short-lived and highly reactive. As they combine with other atoms that contain unpaired electrons, new radicals are created, and a chain reaction begins (Droge, 2002; Haag, 2005).

This chain reaction, or accumulation of reactive oxygen species, in plants is generally ascribed to several possible sources (Klessig and Malamy, 1994; Corpas et al.,2001; Desikan et al., 2001; Blokuna et al., 2003): cell-wall-bound perxidases, membrane-located NADPH oxidases, amine oxidases, xanthine oxidase, chloroplastic electron transport chains, mitochondrial electron transport chains, and peroxisomal fatty acid B-oxidation, which includes the H_2O_2-generating argyl-coenzyme A oxidase steps (Couee et al., 2006). These sources can be attributed to environmental causes such as

The electrons from chlorophyll molecules in photosystem II replace the electrons that leave chlorophyll molecules in photosystem I. Images: Sinauer Associates and WH Freeman







There is a synergistic relationship between keeping chloroplasts and thylakoid membranes as healthy as possible. Images: Sinauer Associates and WH Freeman.

drought, heat, and ultraviolet light, or chemicals such as herbicides (Haag, 2005).

Accumulation of reactive oxygen species is central to plant response to several pathogens. One of the sources of reactive oxygen species is the chloroplast because of the photoactive nature of the chlorophylls (Kariola et al.,2005). The free radicals, or reactive oxygen species, are singlet, hydroxyl, superoxide and hydrogen peroxide.

LIGHT

There's a catch-22 with light. Light is necessary for photosynthesis to occur; however, it also can play a part in the degradation of chlorophyll.

When photosynthetic organisms are exposed to ultraviolet radiation, significant, irreversible damage to important metabolic processes within the cell might occur (such as lesions in DNA and inhibition of photosynthesis). Through these reactions and others, radical forms of oxygen are often created. Many reports suggest this damage is because of oxidative stress resulting from UV-A, (Dring et al., 1996, Jeffrey and Mitchell, 1997, Turcsanyi and Vass, 2000) UV-B (Teramura and Ziska, 1996, Gotz et al., 1999, Mazza et al., 1999, hideg et al., 2000, Estevez et al., 2001) or both (Krause et al., 1999, Muela et al., 2000, Vega and Pizzaro, 2000, Laloi et al., 2006).

Photosynthetic light absorption and energy usage must be kept in balance to prevent formation of reactive oxygen species in the chloroplasts. Drought causes stomatal closure, which limits the diffusion of carbon dioxide to chloroplasts and thereby causes a decrease of carbon dioxide assimilation in favor of photorespiration that produces large amounts of hydrogen peroxide (Noctor et al., 2002). Under these conditions, the probability of singlet oxygen production at phtosystem II and superoxide production of photosystem I is increased (Niyogi, 1999; Foyer et al., 2005). These can cause direct damage or induce a cell suicide program (Tambussi et al., 2000).

It has been known for a long time wavelengths in the ultraviolet-B region of the spectrum are effective in inactivating photosynthesis, and the molecular target is photosystem II (Jones and Kok, 1966., Chen and Gallie, 2005). An excess of light brings about the inactivation of oxygenic photosynthesis, a phenomenon known as photoinhibition (Powles, 1984), and the molecular target of photoinhibition is photosystem II, a thylakoid multisubunit pigment-protein complex (Bergo et al., 2003). The major effect of ultraviolet-B light on the thylakoid proteins is the breakdown of the reaction centre D1 protein (Trebst and Depka, 1990; Friso et al., 1994; Barbato et al., 1995).

One must question whether ultraviolet-B radiation will become an even more serious factor in the future. The depletion of the stratospheric ozone is causing renewed concern about the increased level of ultraviolet-B radiation reaching the earth's surface (Smith et al., 1995). It's also known exposure to environmental ozone can cause significant damage to turfgrass by imposing conditions of oxidative stress (Chen and Gallie, 2005; Grimes et al., 1983; Schraudner et al., 1998). This might be the case because we're seeing a gradual increase in yearly temperatures throughout the world and an increase in skin cancers in humans. How it affects crops and turfgrass plants in the future remains to be seen.

SENESCENCE

Senescence results in massive levels of cell death, but the purpose of senescence isn't cell death; rather death only occurs when senescence has been completed. Senescence occurs in two stages. The first stage is reversible, and the cells remain viable throughout. The second stage results in cell death (Buchanan-Wollston et al., 2003; McGlaughlin and Smith, 1995; Mothes et al., 1960; Riefler et al., 2006; Venkatrayappa et al., 1984).

The key enzyme in the pathway to chlorophyll degradation during senescence appears to be pheophorbide *a* oxygenase. The activity of pheophorbide *a* oxygenase increases dramatically during senescence, implicating this enzyme as a control point in the process (Buchanan-Wollston et al., 2003; Hortensteiner et al., 1998). Light absorption by pheophorbide *a* oxygenase also is believed to cause the production of singlet oxygen (Pruzinska et al., 2005), which is a free radical.

Because senescence is reversible, it suggests that fully developed chloroplasts retain enough genetic information to support regreening and chloroplast reassembly.

CALCIUM AND POTASSIUM

From a nutritional standpoint, there are various nutrients and compounds that can be applied in the process of strengthening and defending chloroplast damage.

Because the chloroplasts and thylakoid membrane are located inside the plant cell, the first line of defense would seem to be to strengthen the plant cell by keeping calcium and potassium at optimal levels. Calcium plays a key role in strengthening the cell walls of the turfgrass plant, while potassium helps strengthen cell walls inside the turfgrass plant, which makes it harder for physiological problems to occur inside the cell wall (Haag and Serrato, 2006).

With regard to calcium applications, add a light amount of zinc along with the calcium because zinc helps calcium to translocate to the cell walls (Haag and Serrato, 2006).

AMINO ACIDS

Amino acids are the building blocks of proteins. Under optimal conditions, proteins are able to perform the normal physiological function to synthesize amino acids, but intensively manicured turfgrass, such as golf courses and athletic fields, are rarely operating under optimal conditions because of stress caused by low mowing heights and traffic (Haag and Serrato, 2006).

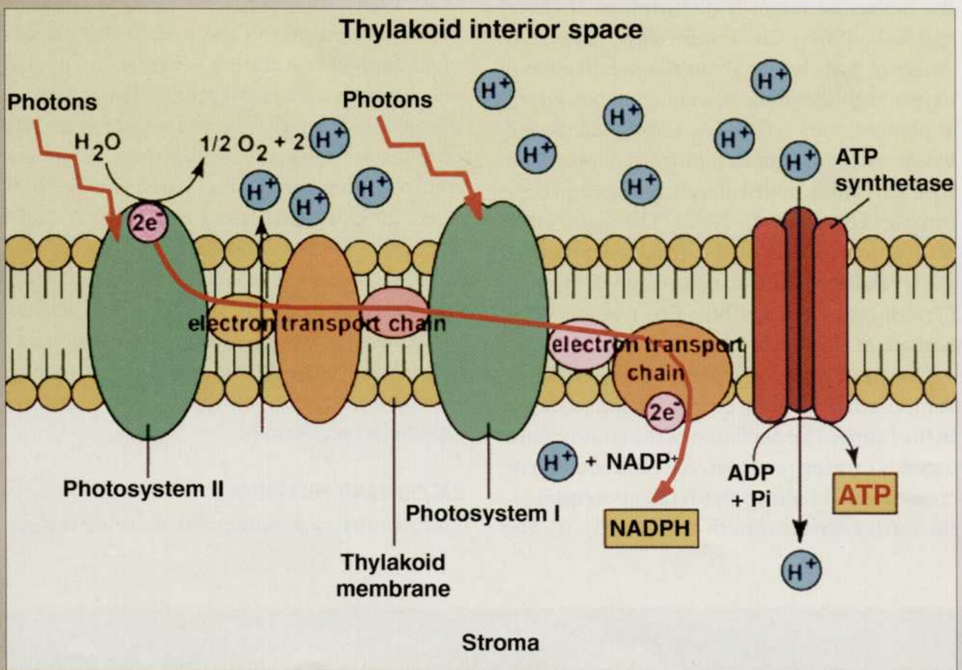
To date, 154 proteins in the turfgrass plant have been identified – 76 (49 percent) are integral membrane proteins. Twenty-seven new proteins without known functions, but with predicted chloroplast transit peptides, have been identified – 17 (63 percent) are integral membrane proteins. These new proteins are likely to play an important part in thylakoid biogenesis (Friso et al., 2004).

The application of amino acids plays an extremely important part in developing the proteins specifically designed to help chloroplasts, thylakoid membranes, photosystem I and photosystem II to function properly. These proteins are known as D1, D2 CP43, CP47 (de Weerd et al., 2002, Zheleva et al., 1998) and cytochrome b559. Of special importance is the D1 protein because it exhibits the highest turnover rate of all the thylakoid proteins (Matto, 1984; VonWettstein et al., 1995; Prasis et al., 1992; Aro et al., 1993; Schuster et al., 1988) and is highly vulnerable to singlet oxygen (Barber, 1984), a free radical.

ANTIOXIDANTS

The antioxidants a-tocopherol (vitamin E), ascorbic acid (vitamin C), carotenoids (B-carotene), vitamin B6 and mannitol in some biostimulants play a vital role in scavenging free radicals (Barna et al., 2003) and helping protect chloroplasts, thylakoid membranes inside the chloroplasts, photosystem I and photosystem II.

The best biostimulant that I've encountered to date is the N.O.G. product.



The image to the left depicts how the electron transport chain moves through the thylakoid interior. Image: Gary E. Kaiser, Ph.D.

CAROTENOIDS (B-CAROTENE)

In terms of its antioxidant properties, carotenoids can protect photosystem I and photosystem II in one of four ways: (i) by reacting with lipid peroxidation products to terminate chain reactions (Burton and Ingold, 1984; DellaPenna and Pogson, 2006); (ii) by scavenging singlet oxygen and dissipating the energy as heat; (iii) by reacting with triplet or excited chlorophyll molecules to prevent formation of singlet oxygen, or (iv) by dissipation of excess excitation energy through the xanthophyll cycle (Mathis and Kleo, 1973).

Xanthophylls function as accessory pigments for harvesting light at wavelengths that chlorophyll can't and transfer the light energy to chlorophyll. But, they also absorb excess light energy and dissipate it to avoid damage in the xanthophyll cycle.

A-TOCOPHEROL (VITAMIN E)

A-tocopherol (vitamin E) is considered a major antioxidant in chloroplasts in at least two different but related roles. It protects photosystem II from photoinhibition and thylakoid membranes from photooxidative damage (Havaux et al., 2002; Havaux et al., 2005; Delong and Steffen, 2002, Flohe and Traber, 1999). The antioxidant properties of vitamin E are the result of its ability to quench singlet oxygen and peroxides (Fryer, 1992; Sattler et al., 2006).

Although vitamin E is a less efficient scavenger of singlet oxygen than B-carotene, it might function in the thylakoid membrane to break carbon radical chain reactions by trapping peroxyl radicals (Fryer, 1992; Burton and Ingold, 1984; Mathis and Kleo, 1973).

ASCORBIC ACID (VITAMIN C)

It's generally believed maintaining a high ratio of ascorbic acid is essential for the scavenging of free radicals (Mitler, 2002) and are needed in high concentrations in the chloroplasts to be effective in defending the turfgrass against oxidative stress (Noctor and Foyer, 1998).

Although ascorbic acid can directly scavenge the free radicals superoxide and singlet oxygen, the main benefit ascorbic acid plays in the prevention of free radicals is that it's an excellent scavenger of the hydroxyl radical (Blokhina et al., 2002; Yoshida et al., 2006). The hydroxyl radical is dangerous to turfgrass because it can inhibit carbon dioxide assimilation by inhibiting several Calvin cycle enzymes (Asada, 1996).

VITAMIN B6

Apart from its function as a cofactor, vitamin B6 is also thought to act as a protective agent against reactive oxygen species, such as singlet oxygen (Bilski et al.,2000; Chen and Xiong, 2005; Ehrenshaft et al.,1999; Drewke and Leistner, 2001). Vitamin B6 is also the master vitamin in processing amino acids and plays an important role in developing proteins specifically designed to help chloroplasts, thylakoid membranes, photosystem I, and photosystem II to function properly.

MANNITOL

The antioxidant mannitol has the ability to protect and quench two damaging free radicals: singlet oxygen and hydroxyl. Singlet oxygen is damaging because it can react with proteins, pigments and lipids and is thought to be the most important species for light-induced loss of photosystem II activity, as well as the degradation of the D1 protein (Krieger-Liszkay, 2004). It has been demonstrated that when mannitol is present in the chloroplasts, it can protect plants against oxidative damage by the hydroxyl radicals (Senn, 1987; Shen, 1997).

MANGANESE AND MAGNESIUM

Both of these nutrients are attached to the chlorophyll molecule that's located inside the chloroplasts. These two nutrients play a part in making turfgrass greener by helping develop chlorophyll. They also transport other vital nutrients and are responsible for many enzymatic functions and help prevent chlorophyll degradation in the cells (Haag and Serrâto, 2006).

CARBON

There's new evidence carbon plays a role in the development of the turfgrass plant leaf, and that a reduction in carbon reduces photosynthetic activity, which reduces carbohydrate availability to the turfgrass plant. There's also new evidence to suggest proper development of the turfgrass plant can't occur without proper amounts of carbon in the chloroplast (Raines and Paul, 2006). There's more evidence to suggest that, if there's an abundant source of carbon in the thylakoid membranes inside the chloroplasts, it can be mobilized for use as an energy source during senescence (Graham and Eastmond, 2002).

HUMIC ACIDS

Humic acids are another compound that contain antioxidant properties that promote the scavenging of free radicals. The added benefits of humic acid are that they increase the availability of micronutrients, phosphate and potassium to the plant and enhance the chlorophyll content of turfgrass.

Humic acids also stimulates root initiation because of the auxin-like activity they contain, which is most likely because of their ability to inhibit indoleacetic acid oxidase breakdown (Haag, 2005; Haag and Serrato, 2006). **GCI**

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Editor's note: Literature cited in this article can be found on GCI's Web site, www.golfcourseindustry.com, posted with this article.

IMPACT ON THE BUSINESS

The plant health dilemma

BY PAT JONES

The evolution of turfgrass science is, in many ways, similar to the evolution of human medical science. In less than a century, we've gone from the most rudimentary forms of nutrition and treatment (manure and heavy metal fungicides like cadmium and mercury) to feeding plants with key elements and treating pathogens that cause disease with highly specific and environmentally benign inoculants. It's not too different than medical science moving from leeches and bleeding to lasers and non-invasive surgery in the same time frame.

BACK TO BASICS

One of the bigger trends during the past decade has been the "back to basics" approach of combining traditional granular fertilization with liquid/foliar nutrition programs that are tailored to meet the needs of the turf and soil. The article above focuses on a core aspect of this trend – finding the right combination of nutrient elements to feed the plant what it needs to thrive.

By identifying the key aspects of photosynthesis and making sure your program meets those needs, you can make an excellent start toward creating a customized "diet" that fits your turf's photosynthetic requirements. More importantly, by understanding the basic nature of plant self-feeding, you can think about your nutrition program at its most fundamental level.

IT'S ELEMENTAL, WATSON

The author effectively points out how a variety of elements and proteins help build up turf health from the inside out. Calcium, potassium, amino acids and various vitamins are all photosynthetic enhancers. The question is, how do you know which to use and in what combination?

The answer lies in a combination of soil and tissue testing and trial and error. By establishing a baseline and working with your local university, consultants and colleagues, you can determine the best mix of supplements. It's no different than getting a physical exam or blood test from you doctor to determine the best combination of diet, exercise, nutrition and preventative medicines for your own health.

RETURN ON INVESTMENT

Many of the articles that we run in GCI's research section offer immediate opportunities for cost-savings or instant improvement in turf quality or health. This is a different approach. The payback here is that, by understanding the building blocks of healthy plants, a superintendent can look at his nutrition program with a new set of eyes and develop a long-term approach.

In the long run, healthy turf is the best defense against pathogens and pests. **GCI**