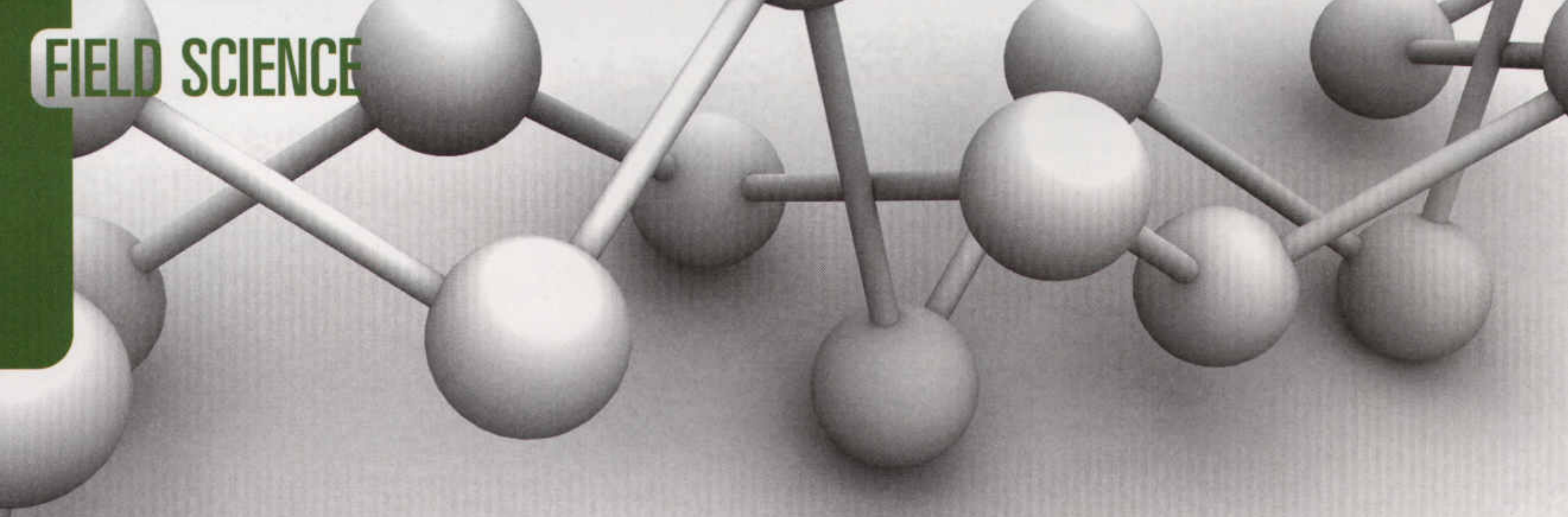


Playing defense vs. free radical damage

By Mike Winkenhofer and Jeff Haag

The goal of every sportsturf manager is to not only provide a safe playable surface, but to also provide aesthetically pleasing green turfgrass. To achieve the latter involves a reciprocal balance between soil, fertility, moisture, temperature, humidity, grass species used, mowing techniques, cultural practices, preventing reactive oxygen species (free radicals) damage from occurring, and cooperation from Mother Nature. This article discusses what free radicals are, factors that cause free radical damage, and mechanisms you can use to prevent development and alleviate their existence if they do develop.

What are free radicals?

Most turfgrass managers know that research has shown that free radicals, if not quickly converted to water and ground-state oxygen by antioxidants, can bleach chlorophyll, and can damage lipids, proteins, and DNA inside cells of the turfgrass plant.

So exactly what are free radicals? Typically, stable molecules contain pairs of electrons. When a chemical reaction breaks the bonds that hold the paired electrons together, free radicals are produced. Free radicals 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. This chain reaction or accumulation of reactive oxygen species in plants is generally ascribed to several possible sources, which can be attributed to environmental causes such as drought, heat, ultraviolet light, airborne photooxidants, or chemicals such as herbicides. Accumulation of reactive oxygen species is central to plant response to several pathogens. The free radicals (reactive oxygen species) are singlet, hydroxyl, superoxide, and hydrogen peroxide.

There is a catch-22 to light. We know that light is necessary for photosynthesis to occur but it can also play a part in free radical formation. When photosynthetic organisms are exposed to ultraviolet radiation, significant, irreversible damage to important metabolic processes within the cell may occur (such as lesions in DNA and inhibition of photosynthesis). Through these reactions and others, radical forms of oxygen are often created. Many studies suggest that this damage is due to oxidative stress resulting from either UV-A or UV-B or both.

The major effect of ultraviolet-B light on the thylakoid proteins is the breakdown of the reaction centre D1 protein. One must question whether ultraviolet-B radiation will become an even more serious factor in the years to come. The depletion of the stratospheric ozone is causing renewed concern about the increased level of ultraviolet-B radiation reaching the Earth's surface, and it is known that exposure to environmental ozone can cause significant damage to turfgrass by imposing oxidative stress.

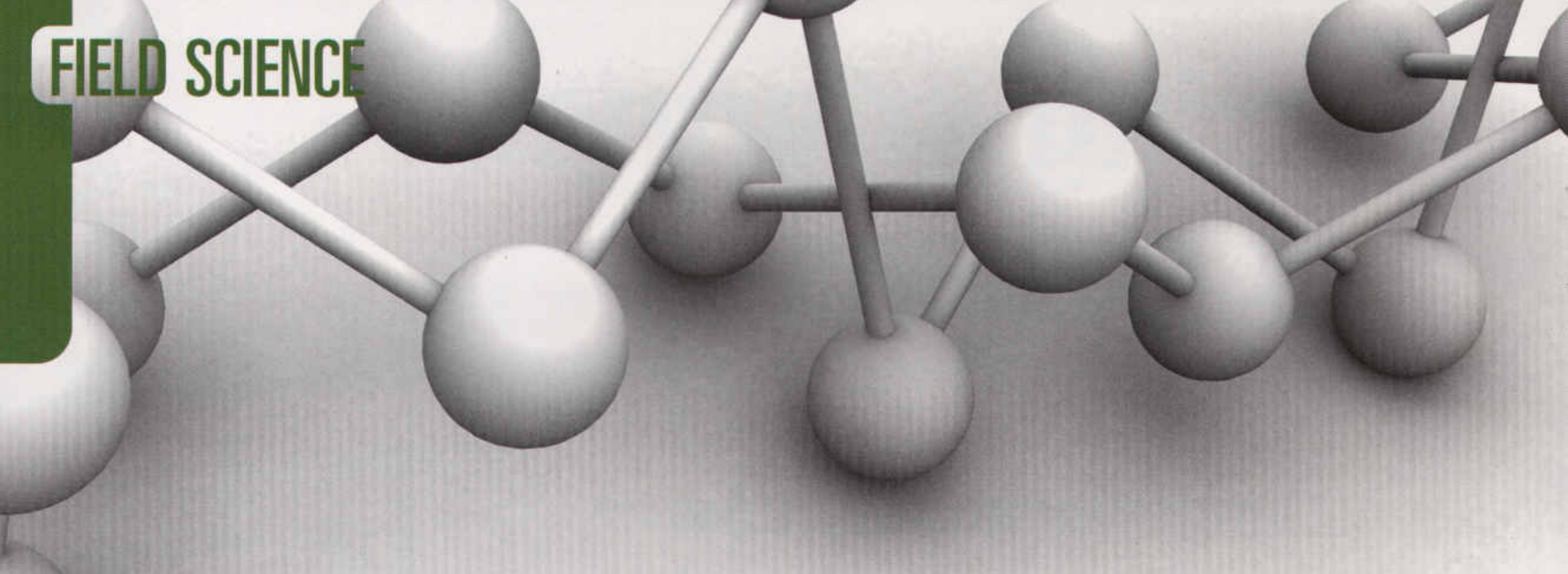
Photosynthetic light absorption and energy use must be kept in balance to prevent formation or reactive oxygen species in one important component of the turfgrass cell where chlorophyll is developed, the chloroplast. In the case of drought for example, it causes stomatal closure, which limits the diffusion of carbon dioxide to chloroplasts and thereby causes a decrease in CO? assimilation in favor of photorespiration that produces large amounts of hydrogen peroxide.

Under these conditions the probability of singlet oxygen production at Photosystem II and superoxide production of Photosystem I is increased. These may cause direct damage, or induce a cell suicide program. We suspect this to be the case as well since we are currently seeing a gradual increase in yearly temperatures across the world, and an increase in skin cancers in humans. How it affects turfgrass plants in the years to come remains to be seen.

Defense mechanisms: antioxidants

The antioxidants a-tocopherol (Vitamin E), ascorbic acid (Vitamin C), carotenoids (B-Carotene), vitamin B6, and mannitol contained in some biostimulants all play a vital role in scavenging free radicals. Applications of biostimulants, which supplement plant hormones, have been shown to enhance the presence of various antioxidants in the leaves, reducing stress and promoting faster recovery from injury. Applying this type of biostimulant product to sports fields several weeks before the beginning of play and following up with regular applications during the season can enhance the presence of antioxidants for stress reduction and recovery.

Carotenoids (B-Carotene). In terms of its antioxidant properties, carotenoids can protect Photosystem I and Photosystem II in one of four ways: by reacting with lipid peroxidation products to terminate chain reactions; by scavenging singlet oxygen and dissipating the energy as heat; by reacting with triplet or excited chlorophyll molecules to prevent formation



of singlet oxygen; or by dissipation of excess excitation energy through the xanthophyll cycle.

Xanthophylls function as accessory pigments for harvesting light at wavelengths that chlorophyll cannot, and transfer the light energy to chlorophyll, but they also absorb excess light energy and dissipate it in order to avoid damage in what is termed the xanthophyll cycle.

a-tocopherol (Vitamin E). This 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. The antioxidant properties of Vitamin E are the result of its ability to quench both singlet oxygen and peroxides, although Vitamin E is a less efficient scavenger of singlet oxygen than B-Carotene, it may function in the thylakoid membrane to break carbon radical reactions by trapping peroxyl radicals.

Vitamin E also has the ability to donate two electrons, which results in opening of the chromanol ring to form the corresponding tocoquinone derivative. These combined molecular characteristics allow Vitamin E to protect polyunsaturated fatty acids from lipid peroxidation by scavenging lipid peroxyl radicals that propagate lipid peroxidation chain reactions in membranes.

Ascorbic Acid(Vitamin C). It is generally believed that maintaining a high ratio of ascorbic acid is essential for the scavenging of free radicals, and is needed in high concentrations in the chloroplasts to be effective in defending the turfgrass against oxidative stress. Although ascorbic acid can directly scavenge the free radicals superoxide and singlet oxygen, probably the main benefit that ascorbic acid plays in the prevention of free radicals is that it is an excellent scavenger of the hydroxyl radical. The hydroxyl radical is dangerous to turfgrass because it can inhibit carbon dioxide assimilation by inhibiting several Calvin cycle enzymes.

Vitamin B6. Apart from its function as a cofactor, Vitamin B6 is also thought to act as a protective agent against reactive oxygen species (free radicals), such as singlet oxygen. Vitamin B6 is also the master vitamin in processing amino acids, and plays a very 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 of light-induced loss of Photosystem II activity, as well as the degradation of the D1protein, which we will discuss later. It has further been demonstrated that when mannitol is present in the chloroplasts, it can protect turfgrass against oxidative damage by the hydroxyl radicals. The Senn, Senn, and Senn Company manufactures a biostimulant called N.O.G. (Natures Organic Growth) that contains and excellent source of mannitol, along with the other antioxidants mentioned.

Amino Acids. As we know, amino acids are the building block of proteins. Under optimal conditions proteins are able to perform the normal physiological function to synthesize amino acids, but intensively manicured turfgrass such as sportsturf are rarely operating under normal conditions due to low mowing heights and traffic stress placed upon them.

The topical 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, and cytochrome b559. Of special importance is the D1 protein because it exhibits the highest turnover rate of all the thylakoid proteins, and is also highly vulnerable to the free radical *singlet oxygen*.

Humic acids are another compound that has shown to contain antioxidant properties that promote the scavenging of free radicals. The added benefits are that they also increase the availability of micronutrients, phosphate, and potassium to the plant, and enhance the chlorophyll content of turfgrass. Humic acid also has been shown to stimulate root initiation due to the auxin-like activity they contain, which is most likely due to their ability to inhibit indoleacetic acid (IAA) oxidase breakdown.

There is new evidence that carbon now plays a role in the overall development of the turfgrass plant leaf, that a reduction in carbon also reduces photosynthetic activity which reduces carbohydrate availability to the turfgrass plant, and there is also new evidence to suggest without proper amounts of carbon in the chloroplast that proper development of the turfgrass plant cannot occur.

There is also further evidence to suggest that, if there is an abundant source of carbon in the thylakoid membranes inside the chloroplasts, it can be mobilized for use as an energy source during senescence. As we discussed earlier, light absorption by the enzyme pheophorbide an oxygenase, the key enzyme in the pathway to chlorophyll degradation to senescence, can cause the development of the free radical singlet oxygen. ■

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3-D Molecule illustration courtesy of istockphoto.com