Nutrient Salts and Toxicity of Black-layer

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Black-layer or black-plug layer is a common disorder of high-sand-content golf greens characterized by development of an interrupted or continuous subsurface blackened layer in the sand (2, 4, 5, 6). The layer is typically associated with noxious odors, and the turf may show symptoms of chlorosis, wilting, thinning, and eventual death. The layer may be initiated by a consortium of bacteria or by cyanobacteria that produce biofilms in the sand that impede the drainage of water (4, 6). The biofilm creates anaerobic conditions and provides organic matter that support the proliferation of sulfate-reducing bacteria and the subsequent development of the black-layer.

The precise cause of death of creeping bentgrass growing on black-layered sand is unknown. The potential production of hydrogen sulfide by sulfate-reducing bacteria may be one of the toxic components of black-layer development (1, 7). It is not uncommon, however, to find healthy grass growing on black-layered sand. This suggests that the toxicity of the layers may be variable. It has also been observed that the roots of *A. palustris* can grow through and clear the black-layer formed by the interaction of cyanobacteria and sulfate-reducing bacteria (5). Studies on the physical structure of black-layer have revealed vertical cavities in the layer through which grass roots grow with at least 3-mm of clear sand between the root and the blackened sand (2).

There are two primary prerequisites for the formation of black-layer by sulfate-reducing bacteria in high-sand-content greens: anaerobic sites in the sand and metabolizable organic matter. The nature of the toxicity of black-layer is unclear and may be variable and/or dependent on factors associated with the site or the management practices applied to the turf. Preliminary research has been initiated to determine if various elemental substances commonly used on golf greens can influence the toxicity of black-layer formed by the interaction of cyanobacteria and sulfate-reducing bacteria.

Nutrients and Toxicity

Black-layered sand was produced from the combination of cyanobacteria of the genera *Nostoc, Oscillatoria,* or *Phormidium* combined with the sulfate-reducing bacterium *Desulfovibrio desulfuricans*. All black-layered sand columns and the non-black-layered control columns received the same standard salt solution (3) supplemented with iron, sulfur, or lime.

Salts. Dry weight loss in the black-layered sand was 51% of the no-organism salts control in response to the combinations of *Nostoc* + *D. desulfuricans*, 59% for *Phormidium* + *D. desulfuricans*, and 79% for *Oscillatoria* + *D. desulfuricans*. These observations suggest that the toxicity of black-layered sand formed from the interaction of different species of cyanobacteria and *D. desulfuricans* can differ. These preliminary observations indicate that the cyanobacterium *Nostoc* in combination with *D. desulfuricans* produces a more toxic black-layer than that resulting from other cyanobacteria. *D. desulfuricans* alone failed to produce a black-layer and had no effect on plant growth relative to the no-organism salts control.

Salts + sulfur. Dry weight of plants from the salts + sulfur no-organism control did not differ from that from the no-organism salts control. All combinations of cyanobacteria and *D. desulfuricans* moderately decreased dry weight relative to the no-organisms sulfur control. All decreases were less than that in response to the same organism combinations in the salts control. The combination of *Nostoc* + *D. desulfuricans* stilled caused the greatest decrease at 78% of the no-organisms sulfur control. *D. desulfuricans* alone in nonblack-layered sand responded to the sulfur by decreasing dry weight to 55% of the no-organism sulfur control. This observation suggests that *D. desulfuricans* can damage turfgrasses long before there is any visible sign of black-layer formation in the sand.

Salts + iron. Dry weight of plants from the salts + iron no-organism control increased dramatically relative to that of the no-organism salts control and salts + sulfur control. Dry weight of the grasses in the no-organism salts + iron control were about 140% greater than that in the no-organisms salts control and salts + sulfur control. The various combinations of cyanobacteria and *D. desulfuricans* decreased dry weight relative to the stimulated plants in the no-organisms salts + iron control. *Nostoc*

+ *D. desulfuricans* caused the greatest decrease in dry weight (54%). *D. desulfuricans* alone in nonblack-layered sand responded to the iron by decreasing dry weight to 54% of the no-organism salts + iron control. Iron also increased the intensity of the black coloration of the black-layer; the intensity of blackening, however, was not necessarily correlated with an increase in toxicity of the layer.

Salts + lime. Dry weight of plants from the salts + lime no-organism control was less than that from any of the other no-organism controls (salts, sulfur, and iron). This response was believed due to a pH that reach 9.0 or higher. Plant dry weight increased in black-layered sand produced by the combinations of *Oscillatoria*, *Phormidium*, or *Nostoc* with *D. desulfuricans* to 149, 142, and 138%, respectively, of the no-organisms salts + lime control. However, these increases in dry weight were still substantially lower than that of the same organism combinations in the salts, sulfur, and iron no organisms controls. Hence, the effect of the lime on the dry weight of plants growing in black-layer was equal to or less than those growing in sulfur or iron treatments.

Root Growth and Black-layer Persistence

All plants transplanted into black-layered sand columns survived. Roots of plants growing in blackened sand that received the salts control, salts + sulfur, salts + iron, and salts + lime produced clear channels in the sand that surrounded roots as they grew downward in the column. By the end of the 10-wk growing period the only blackened sand occurred in the bottom of the sand columns below the tips of the extending roots. The blackened region below the root tips in sand columns receiving salts + iron remained intensely black as compared with the sand columns receiving the salts control, salts + sulfur, or salts + lime.

The clearing of blackened sand by *A. palustris* roots has been observed in previous studies (2, 5). Cullimore et al. (2) showed that the physical structure of black-layer consisted of a series of vertical columnar structures and lateral plates. It was further observed that vertical cavities were present, through which roots grew with at least a 3-mm zone of clear sand between the root and the blackened sand. The observations of roots growing through black-layer or clearing black-layered sand suggest that the toxicity of black-layers may be variable.

References

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