BLUE-GREEN ALGAE AND BLACK LAYER

Researchers throughout the country have proposed theories on the causes of the black layer destroying sand greens. Last month, LANDSCAPE MANAGEMENT looked at some of these theories. This month, we examine preliminary research conducted at Iowa State University.

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The preliminary studies presented here on the role of blue-green algae and anaerobic bacteria in black layer formation were begun in the fall of 1986. Our initial studies examined the ability of *Oscillatoria* to colonize sand and to produce mucilage that would ultimately restrict water infiltration into the sand.

These studies were conducted with silica (quartz) and calcareous (10.6 percent calcium) sands placed in modified culture tubes.

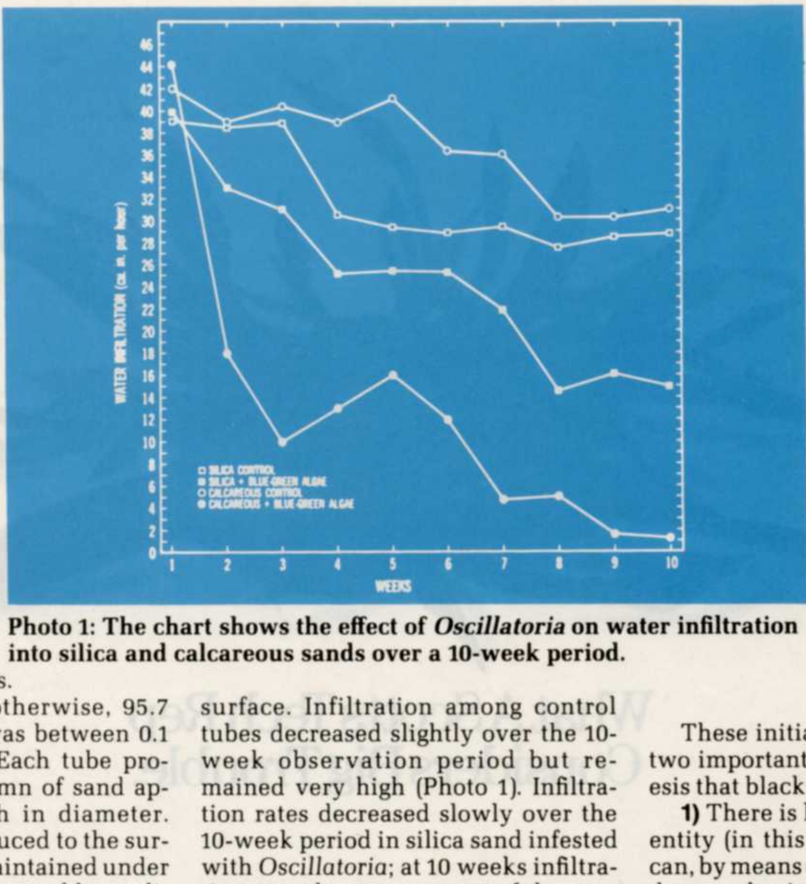

Photo 1: The chart shows the effect of *Oscillatoria* on water infiltration into silica and calcareous sands over a 10-week period.

Unless specified otherwise, 95.7 percent of the sand was between 0.1 and 1.0 mm in size. Each tube provided a six-inch column of sand approximately one-inch in diameter. The algae were introduced to the surface of the sand and maintained under flourescent lights with weekly applications of a weak nutrient solution. The tubes were flushed with distilled water between nutrient solution applications. *Oscillatoria* was grown 10 weeks on the sand columns, and infiltration measurements were taken every seven days (Photo 1).

Initial water infiltration rates of the columns ranged from 39.1 to 45.2 cu. in. per hour for both the silica and calcareous sands at the time *Oscillatoria* was introduced to the sand's surface. Infiltration among control tubes decreased slightly over the 10-week observation period but remained very high (Photo 1). Infiltration rates decreased slowly over the 10-week period in silica sand infested with *Oscillatoria*; at 10 weeks infiltration was about 54 percent of the control (Photo 1). Infiltration of the calcareous sand infested with *Oscillatoria* was severely reduced; at 10 weeks infiltration was 4.8 percent (1.5 cu. in/hr) of the control (Photo 1).

Infiltration was more severely affected on calcareous than on silica sand. Most species of blue-green algae grow most successfully in alkaline conditions and may use calcium carbonate. The initial slowing (first five weeks) of water infiltration by the algae seems due to their physical blockage and sealing of the sand's surface.

Ten weeks later...

By 10 weeks, however, the algae can be removed from the sand's surface and the infiltration rate remains the same. This suggests that the extra-cellular by-products of the algae (mucilage) gradually fill the pore space in the sand until water movement is seriously impaired. The mucilage products are visible in drainage water collected from the tubes.

These initial observations support two important aspects of the hypothesis that black layer is of biotic origin.

1) There is little doubt that a biotic entity (in this case blue-green algae) can, by means of its life processes, produce a physical problem in the sand mix profile that impairs water movement.

2) Organic substances produced by the algae that are responsible for the impaired water movement are hydrophilic. They seem to be responsible for establishing a perched water table in the top two to three inches of the sand mix that may establish the anaerobic zone needed for black-layer development.

Studies in progress are intended to

repeat these observations with more precision and to examine the effects of iron, sulfur, lime ($CaCO_3$), gypsum and the common sugars found in grass tissues on proliferation of algae and the rate at which they interfere with water infiltration.

The odor's origin

Some of the odors associated with black-layered greens may come from the algae. A variety of odors are produced by the different algal species being examined. These odors also may be changed by the substratum on which the algae are growing.

The species of *Oscillatoria* and *Nostoc* being examined produce an earthy or swampy odor on the sand columns when supplied nutrient solution only.

It is interesting, however, that if a carbon source is provided, such as fructose or glucose (sugars commonly found in grasses), *Oscillatoria* will generate large quantities of gas with a distinct sulfur odor. The gas is generated in quantities great enough to lift the algal culture off the surface of the sand column. The nature of the gases generated by the algae and their potential importance in black layer and the turf's death is unknown.

It is suspected, however, that some hydrogen sulfide may be generated. Also, the mucilage of some algae may produce polysaccharides that contain sulphate groups. These polysaccharides also may emit a sulfur odor. Some preliminary analyses of C_1 to C_3 hydrocarbons indicate that small quantities of methane, ethane, ethylene and some still unidentified gases are also associated with the growth of the blue-green algae on the sand columns. The decrease in water infiltration, the production of mucilage, and the evolution of potentially toxic gases by blue-green algae still fail to explain black layer formation.

Bringing in bacteria

After the 10 weeks of infiltration studies and observations on mucus production and gas evolution, there was no indication that black layer was developing in the sand columns. At this point, anaerobic bacteria were introduced into the sand columns. The reduced water infiltration caused by the accumulation of mucilage and other organic by-products of the algae would seem to provide a substratum for the bacteria and a potentially anaerobic (or at least very poorly aerated) environment.

The mixed cultures of bacteria were injected into the sand columns with a syringe; 5 ml. of bacterial suspension was placed two inches below the sand's surface and the algal colony. The sand columns were maintained as previously described.

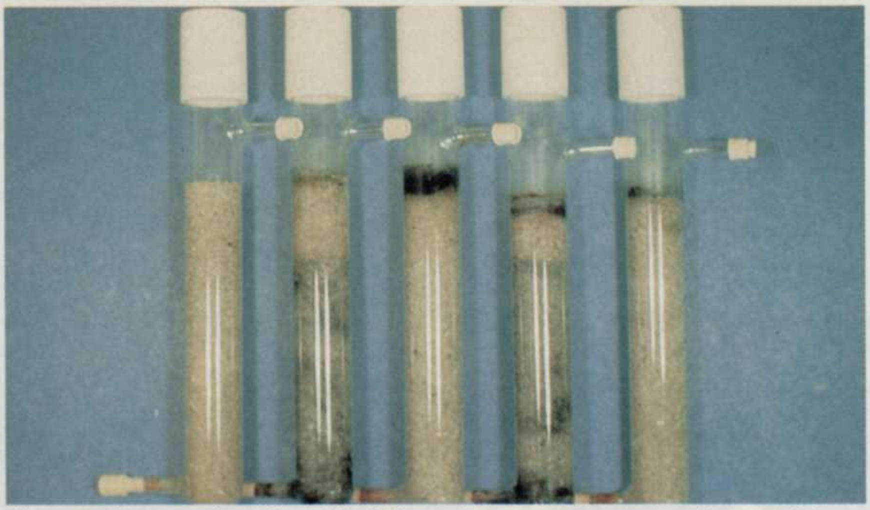

Photo 2:The test tubes show black layer formation in coarse silica sand (0.5 to 2.0 mm) in response to the combination of blue-green algae and bacteria. *From left to right:* control, *Oscillatoria* isolate OS-1, *Oscillatoria* isolate OS-2, *Nostoc* isolate NS-1 and unknown.

The columns were unwrapped at two-week intervals to determine if black layer was developing. Between six and eight weeks after introducing the bacteria, some uneven darkening of the sand was observed. Between 10 and 12 weeks, well developed black zones were well established in several sand columns (Photo 2). Blackening was most visible in the silica sand (due to greater color contrast). Development in calcareous sand seemed somewhat slower and was somewhat less distinct (probably due to less color contrast) (Photo 2). The substance(s) responsible for the blackening are unknown at this time.

Sensitivities

However, the black layer seems sensitive to air. When well-aerated water is flushed through the tubes in large quantities, the blackening seems to fade, but will regenerate when flushing is stopped. Formation of the black layer in the sand columns seems to require both algae and bacteria and takes between five and six months to form under laboratory conditions.

To date, blackening has not been formed by algae or bacteria alone. It is possible, however, that provided with an alternate substratum (other than algal mucilage) and poorly aerated conditions, bacteria might still function to form the black layer.

Results of these preliminary studies support the hypothesis that black layer can result from an abnormal microbiological ecosystem consisting of algae and bacteria on high-sand content greens. It is possible that other factors may also contribute to black layer formation, but the biotic system can account for the extensive viability found in black-layered greens; that is, infiltration and drainage, variations in odors, variation in developmental patterns and variations in toxicity to the turf.

Modern turf demands

Why the problem exists and has proliferated on high-sand content greens is not entirely clear. The organisms being examined in our research are not inherently damaging to turf; however, it seems that cultural systems which have evolved over the last 20 years on golf greens favor their abnormal development. Demands placed on the high-sand content green to provide the kind of surface wanted for today's golf game may be greater than the turf and its growing media can provide. This intense management may be responsible for the abnormal proliferation of algae, bacteria and fungi.

The time may be upon us when we may be forced to reevaluate the demands in realistic terms of what a living turf can, or cannot provide.

In the meantime, for the superintendent plagued with the black layer problem, minimizing irrigation (provided Mother Nature cooperates), increasing aerification, and raising mowing heights to the extent that the golfers will tolerate may help slow the damage done by black layer. **LM**

ACKNOWLEDGEMENTS: Preliminary research presented in this article was supported in part by the O.J. Noer Research Foundation, the Iowa Golf Course Superintendents Association and the Iowa Turfgrass Institute.