Black layer is a condition where a darkened stratum exists in the root-zone of golf putting greens. It was the number one malady of greens in the 1980s because its nature was unknown and severe turf injury was associated with it. Researchers at Michigan State University (MSU) hypothesized that black layer was an accumulation of metal sulfides (MeS) linked to release of hydrogen sulfide (H$_2$S) by sulfur-reducing bacteria (SRBs) in response to low redox potential. Turf injury likely resulted from H$_2$S toxicity. Elemental sulfur (S$_0$) was thought to contribute to black layer, and nitrate (NO$_3$) could be a control. Researchers at MSU sought to test this hypothesis. The presence of MeS was verified in 	extit{in situ} black layers from putting greens at 26 golf courses by spot testing with a solution of sodium azide (Na$_3$N$_3$) and iodine (I$_2$). Black layer and H$_2$S were linked when H$_2$S and MeS were produced after $^{35}$SO$_4^{2-}$ was injected into intact soil cores from an 	extit{in situ} black layer. Adding molybdate (MoO$_4^{2-}$) with the label demonstrated involvement of SRBs. MeS-based black layers resulted and redox potential as pH was lowered when S$_0$ was applied to waterlogged sand. Nitrate (NO$_3$) poised redox high enough to avert release of H$_2$S. Black layer was MeS. Applying S$_0$ contributed to black layer because it reduced redox and stimulated H$_2$S. The release of H$_2$S by SRBs was controlled by poisoning redox with NO$_3$ and limiting S$_0$. Fertilizing with NO$_3$ and limiting S$_0$ were effective controls for black layer.

**Keywords:** Agrostis palustris Huds., creeping bentgrass, redox potential, sulfate, sulfate reduction, sulfide, sulfur, sulfur-reducing bacteria

**Abbreviations:** H$_2$S, hydrogen sulfide; MeS, metal sulfide; NO$_3^-$, nitrate; SO$_4^{2-}$, sulfate; $^{35}$SO$_4^{2-}$, radioactive sulfate; $^{35}$S$^0$, radioactive elemental sulfur; S$^-$, sulfide; S$_0^-$, elemental sulfur; SRBs, sulfur-reducing bacteria

**INTRODUCTION**

Black layer is a term used in the turfgrass management industry that describes the presence of a dark colored layer or banding in the soil profile of certain putting greens ([Fig. 1](#1)). In some instances the black layer is very distinct, while in other cases it is diffused. In some greens it appears near the surface of the soil, and in other greens it exists several inches down in the soil profile. Thickness of the black layer can vary from several millimeters to several centimeters or more. Sometimes the entire soil profile is blackened. Usually, the smell of rotting eggs accompanies black layer, and exposure to air typically causes black layer and the associated smell to disappear within a few hours.

Regardless of its appearance, a severe decline in the quality of the turf is frequently, but not always, associated with black layer. This decline ranges from thinning and bronzing of the turf to outright loss (Scott 1986; Berndt et al. 1987). Root growth is typically limited and soil infiltration is often compromised (Brown 1987). Death of the turfgrass has been strongly associated with the black layer condition at many golf courses (Scott 1986; Chaltas 1987). For this reason black layer has been referred to as ‘the black plague’ (Scott 1986) and ‘the black death’ (Anonymous 1988). Due to its mysterious nature (Brown 1987), black layer was called the number one malady of creeping bentgrass (Agrostis palustris Huds.) putting greens in the late 1980s (Scott 1986). Rankin (1988) called black layer ‘the topic of the year for 1987 in the US turfgrass industry’.

There was a great deal of speculation as to the nature, cause, and control of the black layer, with disagreement (Berndt 1990). Information regarding black layer as published in the popular literature by various golf organizations, turfgrass researchers, and golf course superintendents caused a conflicting view of the problem (Chaltas 1987). This review serves to introduce readers to the concept of the
black layer condition as it occurs in putting greens, and to clarify its true nature, cause, and control.

SOME INITIAL VIEWS ON THE BLACK LAYER

A golf course superintendent in northern Michigan was the first to publicly write about the black layer (Scott 1986). In his writing, Scott stated that a turf problem of major consequence was facing a growing number of golf courses throughout the country, and that very little was known about it. He wrote that the black layer problem begins as a drastic drop in percolation, and then graduates to the formation of a black, offensive-smelling, anaerobic layer somewhere in the soil profile of putting greens, and that death of the turf plants ultimately results. Scott stated that the causes were uncertain, the effects devastating, and the cure unknown, and he concluded that all of the turfgrass experts would participate in the final analysis of the black layer problem because it appeared to be much too complex for one individual or one research experiment to remedy.

Scott’s article (1986), along with commentary provided by the Golf Course Superintendents Association of America (GCSAA) (Chaltas 1987) generated a great deal of interest in the black layer problem, and thus prompted the proliferation of a number of articles in the popular literature by various research groups. The United States Golf Association (USGA) concluded that coupling the filling of soil pore spaces with water to warm soil temperatures and the presence of organic matter causes anaerobic conditions to set in, resulting in occurrence of the black layer and eventual death of the turf plants.

Analysis of sub-surface black layers by Lubin (1987) led to the observation that metal ion concentrations were found to be higher in black layer than elsewhere in the soil profile, and that these metals in high enough concentrations were likely to be toxic to turf plants. Lubin reported that root growth stopped at the black layer, and severe turf damage was seen in its presence. He also reported that soluble phosphorus (P) appeared to be low in a number of black layer soil samples and in corresponding plant tissues, and the black layer problem appeared to be most prevalent on golf courses where application of P was minimized in order to control seed-head production in annual bluegrass (Poa annua L.). Further, the application of soluble P appeared to alleviate black layer problems at a number of golf courses in the southeast, probably by forming metal-P precipitates of low solubility and by stimulating root growth. Thus, Lubin concluded that the black layer was related to low levels of P, and that addition of soluble P would function in controlling the effects of black layer. For that reason, Lubin suggested that applications of soluble P compounds, which strengthened root systems and complexed with soil metals, were needed for managing greens affected by the black layer.

Beard (1987) suggested that the increased incidence of black layer in the mid-1980s was related to intense top-dressing with sand onto a relatively impermeable base, use of 100% sand in new putting green root zone construction, and increased fungicide use. He wrote that ordinarily, placing sand over an impermeable clayey soil only partially solves a compaction problem. He said the soil still lacks downward percolation, but this need not be a problem in semi-arid climates if proper irrigation is applied. However, Beard also suggested that serious excess-soil-water problems may occur in humid to high rainfall climates regardless of irrigation practices. He also wrote that a 100% sand root-zone provides minimal buffering, which can lead to drastic shifts in soil chemical and biological properties that leave the soil susceptible to serious problems. Beard additionally contended that excessive use of fungicides on a 100% sand root-zone can cause significant shifts in microbial populations, and when combined with lack of internal drainage can lead to serious soil problems, such as black layer.

According to Burpee and Anderson (1987, 1988) the origin of black layer could be traced to the movement and accumulation of silt and clay particles into distinct strata within the soil profile of golf greens. They wrote that the existence of aberrations in particle size distribution (PSD) within the soil profiles could lead to development of poorly drained, anaerobic soils, and that layers of differing PSD play a role in black layer development. Burpee postulated that some unique physical property must develop in the soil profile that allows for the occurrence of black layer. They concluded that PSD was not homogeneous between black layers and zones below black layers particularly with respect to the very coarse sand and coarse sand fractions. Layers of differing PSD exist in golf greens in Ontario and these layers play a role in the development of black layer. Such may be related to topdressing with material that does not match the rootzone in terms of PSD.

THE ALGAE HYPOTHESIS

Couch (1987) wrote that the black layer was an anaerobically-induced decline of turfgrass related to the saturation of the soil. He contended that the onset of anaerobiosis was brought on by 1) prolonged periods of rainfall, or 2) im-

Fig. 1 Black layer in the soil profile of a creeping bentgrass putting green in northern Michigan (top). Damage to a putting green from the black layer (bottom). Black layer affected both the visual quality and the functional quality of the green (from Berndt et al. (1987, 1989), with kind permission, Golf Course Management).
paired infiltration related to problems with initial construction or an aerification program that included improper selection of sand type for topdressing. He suggested that anaerobiosis could be rapidly accelerated by the accumulation of surface algae, which impedes infiltration. Thus, in Couch’s view algae could be an important factor in the development of the black layer condition. Further, Couch stated that elements in their reduced state may be taken up more rapidly by plants that can form toxic metabolites by becoming toxic, and that under anaerobic conditions the root systems of plants do not function properly, and their ability to absorb water and nutrients may be reduced significantly. Also, anaerobic microbes can produce toxic metabolites that cause the untimely death of roots or an unthrifty growth of the overall plant. Couch concluded that the condition that causes plant death is anaerobiosis, and the black layer is a by-product of this activity.

Hodges (1987a) stated that the black layer disorder was related to water problems and to anaerobic conditions, and that it might be best described as an anaerobic decline as suggested by Couch. Initial research performed by Hodges suggested that the black layer was devoid of aerobic microbes, but that species of *Pythium* and of the algae *Nostoc* were common on black layered greens. He stated that blue-green algae were of particular interest because once blue-green algae were established on a green the prerequisites for black layer formation may be set into motion. He contended that extra-cellular mucilage produced by the algae gradually plugs soil pore spaces reducing infiltration and drainage. In effect, Hodges suggested that a biologically induced perched water table was created by the accrual of algae at the greens surface, and that the mucilage also serves as a substrate for anaerobic bacteria and some fungal species. Hodges concluded that the black layer condition seemed to be the result of an abnormal biological system involving blue-green algae, anaerobic bacteria, and fungi, and was set into motion by the physical characteristics of high sand content greens and the cultural regimes required to maintain them.

**THE SULFATE REDUCTION HYPOTHESIS**

Researchers at Michigan State University (MSU) proposed a direct link between black layer formation and dissimilatory sulfate (SO$_4^{2-}$) or sulfur (S$^0$) reduction (Berndt et al. 1987). These researchers hypothesized that the black layer was physically composed of accumulations of metal sulfides (MeS) linked to the release of hydrogen sulfide (H$_2$S) via the activities of sulfur-reducing bacteria (SRBs) in response to low redox potential. Conditions black layer was most likely induced by water-logging, excessive microbial respiration, or reduced oxygen (O$_2$) diffusion, or combinations of these. These researchers also suggested that applied S$^0$, which was commonly used on putting greens in an attempt to lower the pH of rootzone soil, may contribute directly to the development of the black layer by inducing low redox potential and also stimulating release of H$_2$S and accrual of MeS. Many studies black layer was created under sand conditions and also contain high levels of S compounds, including S$^0$. Further, the researchers proposed that the decline in the quality of the turf was likely a result of H$_2$S toxicity.

Other researchers supported the SO$_4^{2-}$ reduction hypothesis. Rankin (1988) contended that the condition which resulted in black layer was lack of air in the root-zone coupled with the presence of S from fertilizer or organic manure. Many fertilizers and pesticides contain sulfur and thus could contribute to water-logging. In these cases, reduced oxygen tension was created by water-logging occurring via factors such as compaction, layer development, poorly managed irrigation, absence of drainage, and run-off from surrounding areas. When Rankin analyzed black layer deposits on tensiometer cups that had been placed in an Australian black layer he reported that the black deposits were composed of iron sulfide (FeS) and manganese sulfide (MnS). His analysis showed that there was a 10x increase in manganese (Mn$^+$) where the increase in iron (Fe$^+$) was less dramatic. Thus, Rankin concluded that the black layer was predominantly MnS, and that the black layer deposits were simply the end of a chemical road involving S and waterlogging.

Smith (1987, 1988) also suggested that the black layer was ultimately produced from dissimilatory SO$_4^{2-}$ reduction involving SRBs and water-logged conditions. He wrote that where layers of the soil become water-logged anoxic conditions develop which favor the activities of anaerobic bacteria. Smith concluded that the black layer development were heavy rainfall, perching of water tables, ponding, and physical layer development in the soil profile, and that excess water was important in initiating black layer, but the key element in the black layer reaction was probably S$^0$. Lastly, he wrote that amendments with S$^0$, ammonium sulfate (NH$_4$)$_2$SO$_4$, or iron sulfate (FeSO$_4$) are contraindicated when black layer is present or suspected.

**NATURE OF METAL SULFIDE**

Metal sulfides readily form when hydrogen sulfide (H$_2$S) is released to the soil (Atlas and Bartha 1980). For example, FeS (trolite) forms at $p$H $> 5.63 + 0.12$ log SO$_4^{2-}$ - 2.21 $p$H, provided that sufficient H$_2$S and Fe$^{2+}$ are available (Lindsay 1979):

$$\text{H}_2\text{S (g)} \rightleftharpoons 2 \text{H}^+ + \text{S}^2- \quad \log K^o = 20.92$$

$$\infty-\text{FeS} \rightleftharpoons \text{Fe}^{2+} + \text{S}^2- \quad \log K^o = -16.21$$

There are two pathways by which H$_2$S becomes available in soils, including mineralization of organic S and dissimilatory reduction of inorganic S (Paul and Clark 1996). Dissimilatory reduction of inorganic S occurs via the respiratory activities of SRBs where compounds like SO$_4^{2-}$ or S$^0$ are utilized as terminal electron (e-) acceptors (TEAs) in the respiratory pathway (Smith 1981; Berndt 1990; Paul and Clark 1996). A thorough review of the dissimilatory sulfate reduction process is presented online by Rabus et al. (2000). Prerequisites for the formation of H$_2$S include low redox potential (Connell and Patrick 1968; Connell and Patrick 1969; Vainshtein and Gogotova 1987; Berndt and Vargas 1992, 1996; Paul and Clark 1996), the presence of suitable e- donors (Postgate 1951; Goldhaber and Kaplan 1975; Badzioń et al. 1978; Smith 1981; Dicker and Smith 1985; Ustalo and Maier 1986; Berndt and Vargas 1992, 1996; Paul and Clark 1996), and the presence of suitable e- acceptors (Cappenberg 1974; Jorgensen and Fenchel 1974; Goldhaber and Kaplan 1975; Biebl and Pfenning 1977; Smith 1981; Berndt and Vargas 1992, 1996; Paul and Clark 1996).

**INITIAL EXPERIMENTS**

To test their hypothesis several experiments were conducted (Berndt 1990). Initially, glasshouse experiments were performed at MSU involving sand, elemental sulfur (S$^0$), and crude cultures of SRBs (Berndt et al. 1987). In these initial studies black layer formed in as little as 4 days when sand was treated with S$^0$ and lactate (C$_3$H$_5$O$_3$Na) then inoculated with crude cultures of SRBs and water-logged. Black layers readily formed when S$^0$ was applied at a rate of 48 kg ha$^{-1}$. Higher levels of S$^0$ intensified the black layer, but where no S$^0$ was added no black layer formed, even though sand was water-logged for 33 days or longer. Experimental black layers were also created by amending sand with various S-containing products including sulfur coated urea fertilizer (SCU), ferrous ammonium sulfate (Fe(NH$_4$)$_2$(SO$_4$)$_2$-H$_2$O), ferrous sulfate (FeSO$_4$-7H$_2$O), and calcium sulfate (CaSO$_4$) or gypsum. Formation of experimental black layer was prevented by concurrent addition of calcium nitrate (Ca(NO$_3$)$_2$), potassium nitrate (KNO$_3$), sodium nitrate (NaNO$_3$), sodium molybdate (NaMoO$_4$), and sodium azide (NaN$_3$).

That MeS was present in these initial experimental black layers was verified by spot testing with a solution of
sodium azide (NaN₃) and iodine (I₂) per the procedure described by Feigl et al. (1972). For this procedure 5-6 g black layer soil was mixed with 10 mL or more solution on a 50 mm or larger watch-glass. The solution was non-reactive except when MeS was present. The MeS acted catalytically to drive production of NaI according to the reaction:

\[ 2 \text{NaN}_3 + I_2 \rightarrow 2 \text{NaI} + 3 \text{N}_2 \]

This procedure relied on observing the evolution of N₂ from the solution as fine bubbling, which was a positive response for presence of MeS.

With respect to algae, initial research at MSU suggested that it may contribute to black layer secondarily (Berndt et al. 1987). In the initial greenhouse experiments, algae (Nostoc spp.) were found only in experimental units to which S₀ was added. In every case the algae appeared after the formation of black layer. Where no S₀ was added no black layer formed and no algae appeared even when experimental units were water-logged for 33 days or longer, suggesting that algae was not the basic cause of black layer and that application of S₀ may contribute to formation of both black layer and algae.

The MSU researchers did acknowledge that the cell walls of algae contain high levels of sulfate-esters, which probably serve as excellent e⁻ donors for the SO₄²⁻ or S₀ reduction process. Research from Iowa State University (Hodges 1987b, 1989) examined the ability of the alga Oscillatoria to colonize columns of sand. Hodges wrote that after 10 weeks the rate of water infiltration on silica sand inoculated with algae were 54% of the control, and rates of infiltration on calcareous sands were 4.8% of the control. Further, removal of surface algae did not regenerate water infiltration in either sand. Hodges concluded that algal polysaccharides must have contributed to plugging the sand profile. However, he reported that the black layer did not develop even though water infiltration was reduced by colonization with algae. In his experiments, the black layer developed only after subsequent inoculation with mixed cultures of anaerobic bacteria. Twelve to 16 weeks after inoculation well developed black zones became visible. Hodges concluded that both algae and SRBs, such as Desulfovibrio spp., and 5-6 months incubation time were necessary for black layer to form, and that S₀ and/or S-containing products would stimulate black layer formation.

As a result of the initial experimentation, researchers at MSU concluded that MeS-based black layers readily formed in response to application of S₀ and other S-containing compounds. Nitrates prevented formation of experimental black layers. Further, algae could be involved in formation of black layer but it was not considered the basic cause.

**EXAMINING IN SITU BLACK LAYERS**

In support of the initial greenhouse research a survey of naturally occurring in situ black layers from the putting greens at 26 golf courses located in lower Michigan and northern Ohio was conducted. All samples from all of the in situ black layers tested positive for the presence of MeS by the spot testing protocol. These black layers emitted foilt odor and disappeared after exposure to air for several hours.

In addition to the survey, radioactive ³⁵SO₄²⁻ was used as a tracer to determine if H₂S was released in intact soil cores taken from an in situ black layer present in the root zone profile of a ‘Penncross’ creeping bentgrass (Agrostis palustris Huds.) putting green. Radioactive ³⁵SO₄²⁻ has been used to measure rates of SO₄²⁻ reduction in marine sediment model systems by Jorgensen and Fenchel (1974), in the sediments of a eutrophic lake in Michigan by Smith (1981), and in Black Sea silts by Sorokin (1962). This is the only known report citing the use of ³⁵SO₄²⁻ as a tracer in intact putting green soil cores (Berndt and Vargas 2006).

When 10⁻⁴ M ³⁵SO₄²⁻ with a specific activity of 1.554 x 10⁶ Bq mg⁻¹SO₄²⁻ was injected into an intact soil core it reduced to ³⁵S₂⁻ at a mean rate of 7.1 nM S cm⁻³ soil day⁻¹ (Berndt and Vargas 2006). As a result H²S was released to the soil and a MeS fraction subsequently formed and accrued. About 32% of the label reduced into ³⁵S₂⁻, and between 80-95% of the ³⁵S₂⁻ was in a MeS fraction, leaving 5-20% as free H₂S. This was proof that SO₄²⁻ was reduced in an in situ MeS-based black layer and that H₂S was released to the soil.

Introducing azide (N₃⁻), which is a non-selective biocide, and molybdate (MoO₄²⁻), which is a specific inhibitor of SRBs, along with the ³⁵S label diminished the rates of reduction. When N₃⁻ at 10% w/v was injected with the label the rate of reduction was found to be only 0.03 nM S cm⁻³ soil day⁻¹, which proved that the reduction was biological in nature. When molybdate (MoO₄²⁻) at 10% w/v was injected with the label the rate of reduction was calculated to be only 0.01 nM S cm⁻³ soil day⁻¹, which proved that SRBs were responsible for the observed cycling.

When ³⁵SO₄²⁻ was introduced into disturbed black layer soil from a ‘Penncross’ creeping bentgrass green within a reactor vessel it was reduced at a rate of 116 nM S cm⁻³ soil day⁻¹. Rates of reduction were significantly higher in disturbed soil than in intact soil. Percentage of the added label released as H₂S was after 24 h was 6.4%. The value for accrued S₀ was 25.2%. This translated into a mean residence time of 3.0 days and a residence half-life of the label of 1.6 days.

From this research it was concluded that most, but not necessarily all, black layers were composed of MeS. Further, dissipatory reduction of SO₄²⁻ was an active pathway producing both the release of H₂S and accrual MeS. Also, the observed reduction of SO₄²⁻ was biological in nature, involving unspecified genera of SRBs. Both SO₄²⁻ and S₀ were actively reduced in black layer soil.

**HYDROGEN SULFIDE IS TOXIC TO CREEPING BENTGRASS**

Hydrogen sulfide is known cell poison disrupting cellular respiration (Atlas and Bartha 1980). That H₂S was involved in the turf decline frequently observed with black layer was investigated by subjecting established cores of ‘Penncross’ creeping bentgrass to exposure to dissolved H₂S at a level of 1,000 mg H₂S L⁻¹. Turf aerial tissue was visibly stunted after only 12 h exposure. After seven days the bentgrass was dead, with turf turning from deep green to straw brown. Root tissue was also visibly stunted and necrotic compared to the control. Thatch and exposed roots took on a blackened appearance characteristic of black layer. Exposing bentgrass to the no-H₂S control had no observable effect.

It was concluded from this research experiment that H₂S is highly toxic to ‘Penncross’ creeping bentgrass. Thus, the release of free H₂S in the rootzone of putting greens, which has been demonstrated, has the potential to cause the decline in turf quality frequently observed with the black layer.

**SULFUR LOWERS REDOX POTENTIAL**

Researchers at MSU considered that low redox potential in soil is typically induced by water-logging, excessive microbial respiration, and reduced rates of oxygen (O₂) diffusion. They also considered that S₀ or S-containing compounds applied to putting greens could directly generate low redox because the oxidation of S₀ scavenges O₂: $S_0 + 3/2 O_2 + H_2O \leftrightarrow H_2SO_4$.

That the addition of S₀ to putting greens results in anaerobiosis and eventually the black layer was disputed (Couch 1987). Couch (1987) wrote that none of the assumptions as offered by Berndt et al. in their 1987 article was correct. He stated that there is no scientific evidence that S₀ or S from fertilizers currently used in turfgrass management either causes or contributes to the development of anaerobiosis, and that S₀ is not a factor in the development of anaerobi-
POISING REDOX POTENTIAL WITH NITRATE

Release of H$_2$S in soil is a process that requires low redox potential (Connell and Patrick 1968, 1969; Vainshtein and Gogotova 1987; Berndt and Vargas 1992, 1996; Paul and Clark 1996). Previous research at MSU demonstrated that low redox potentials can occur in the rootzone soil of putting greens as a result of application of S$^0$ (Berndt and Vargas 1992). It was reported that nitrate (NO$_3^-$) stabilizes redox potentials at +0.2 to +0.4 volts in lake waters, muds, and in submerged soils and prevents release of H$_2$S (Ponnampuruma 1972). Chemical oxidants have also been used to improve the quality of rice growing in H$_2$S-problem soils in Taiwan (Ponnampuruma et al. 1965). Research was conducted to determine if NO$_3^-$ could be used to retard the reduction of turf soil and in turn prevent the release of H$_2$S when applied at a level suitable for fertilizing fine turfgrasses.

The effect of S$^0$ or S$^0$ + NO$_3^-$ on redox potential as pe + pH and the accrual of free H$_2$S or MeS was measured on each of 15 consecutive days (Berndt and Vargas 1996). Applying S$^0$ to soil at a rate of 48 kg ha$^{-1}$ caused a significant reduction in pe + pH (Table 1). It also resulted in the accrual of MeS at an estimated rate of 2.67 mg kg$^{-1}$ soil day$^{-1}$ and the accrual of free H$_2$S at a rate of 0.80 mg kg$^{-1}$ soil day$^{-1}$. In contrast, the pe + pH of the soil with NO$_3^-$ at a rate of 48 kg ha$^{-1}$ N from calcium nitrate (Ca(NO$_3$)$_2$) + 4H$_2$O with the S$^0$ was poised at a point high enough to delay the release of H$_2$S by 5-6 days. Only after the pe + pH began to fall at about day 9 of the study did MeS begin to accrue at a diminished rate of 0.54 mg kg$^{-1}$ soil day$^{-1}$. Accrual of free H$_2$S where NO$_3^-$ was applied was not observed.

Table 1 The influence of elemental sulfur (S$^0$) or S$^0$ plus nitrate (NO$_3^-$) on soil redox potential as pe + pH, the release of free hydrogen sulfide (H$_2$S), and the accumulation of metal sulfides (MeS).

<table>
<thead>
<tr>
<th>Day</th>
<th>pe + pH</th>
<th>MeS</th>
<th>H$_2$S</th>
<th>MeS</th>
<th>H$_2$S</th>
<th>S$^0$</th>
<th>S$^0$ + NO$_3^-$</th>
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</table>

In supporting research using radioactive $^{35}$SO$_4^{2-}$ and a reactor vessel (Berndt 1990) it was demonstrated that when NO$_3^-$ was applied at 48 kg ha$^{-1}$ N it was highly effective in reducing the accumulation of both MeS$^{35}$S and free H$_2$S in disturbed black layer soil taken from a ‘Penncross’ creeping bentgrass putting green (Fig. 3). Nitrate effectively diminished the value of the rate of $^{35}$SO$_4^{2-}$ reduction from 52.7 nM S cm$^{-2}$ soil day$^{-1}$ to 4.6 nM S cm$^{-2}$ soil day$^{-1}$.

From this research it was concluded that application of NO$_3^-$ at rates suitable for fertilizing turfgrass was highly effective in retarding the reduction of soil redox potential and decreasing the rate of $^{35}$SO$_4^{2-}$ reduction. Nitrate was effective at reducing the rate of accrual of H$_2$S and MeS because it is energetically more favorable as a TCA than is SO$_4^{2-}$ or S$^0$ (Berndt and Vargas 1996). Fertilizing putting greens with NO$_3^-$ fertilizers should help to keep the redox status of the root zone relatively high, and in turn prevent the release of H$_2$S and subsequent accrual of MeS or H$_2$S.

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Nitrate was thus considered a reasonable preventive control for black layer and the related turfgrass decline.

CONCLUSIONS

The research described above supported the hypothesis that the black layer was an accumulation of MeS produced by dissimilatory reduction of SO$_4$$^{2-}$ or S$^0$ in response to low redox potential. Sulfur-reducing bacteria of unidentified genera were the biological entities ultimately responsible for release of H$_2$S in putting green soils, hence black layer formation, with H$_2$S toxicity. Application of S$^0$ lowered redox potential and stimulated release of H$_2$S and accrual of MeS. Nitrate poised redox potential at a point high enough to avert release of H$_2$S. The key to successfully controlling the release of H$_2$S in putting green soils, hence black layer formation, involved regulating the respiratory activities of SRBs by fertilizing with alternative electron acceptors such as NO$_3^-$. Fertilizing with NO$_3^-$ and withholding S$^0$ were effective best management practices (BMPs) for the black layer.

Reasons for the occurrence of low redox potential in well-drained terrestrial soils like putting greens were less straightforward. This research demonstrated that redox potential can be lowered significantly in putting green soil by simply applying S$^0$. Microbial oxidation of the S$^0$ consumes O$_2$ and increases [H$^+$]$_e$, resulting in a lowering of redox as p$e$ + pH. However, application of S$^0$ is not likely to be the sole factor inducing low redox in putting greens. Other factors such as increased microbial respiration related to turnover of OC, perched water tables related to the development of textural interfaces in the soil profile, waterlogging from intense rainfall or intense irrigation, and reduced O$_2$ diffusion due to the presence of surface algae or saline crusts may also play a role in inducing the anaerobic conditions needed for the release of H$_2$S and the subsequent formation of the black layer.

The black layer should be viewed as an indication that low redox potential exists in the rootzone and that H$_2$S is being released to the soil. If the turf declines when black layer is present it is likely that free H$_2$S is also present in a concentration sufficient to sulfocate or poison turfgrass plants.

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