Microbial Populations and Suppression of Dollar Spot Disease in Creeping Bentgrass with Inorganic and Organic Amendments

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ABSTRACT

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Alginate, ammonium nitrate, Bovamura, Milorganite, Ringer Lawn Restore, Ringer Greens Super, Ringer Turf Restore, Sandaid, sewage sludge and sulphur-coated urea were evaluated from 1991 to 1993 on a creeping bentgrass (*Agrostis palustris*) green and a Kentucky bluegrass (*Poa pratensis*) lawn for their effects on soil bacterial and fungal populations and dollar spot disease incidence. Over the 3 yr, fertilizers were applied every 4 wk at recommended rates from early June to September, and once again in November. Application of Ringer fertilizers, ammonium nitrate, and sulfur-coated urea gave rise to significantly higher microbial populations on turfgrass leaves and in thatch and soil than did most other fertilizers. In most experiments, Ringer fertilizers also improved water retention in thatch compared with other treatments. Ringer Greens Super, Ringer Turf Restore, or ammonium nitrate on the creeping bentgrass green significantly suppressed dollar spot disease compared with the other amendments or the untreated control, but for most of the season they did not control disease as well as the fungicide chlorothalonil did.

Nitrate leaching from inorganic nitrogen fertilizers has the potential to be a significant source of nitrate contamination of ground water in urban areas where turfgrass is the major living ground cover (17). Frequent application of inorganic fertilizers such as ammonium nitrate can change the soil pH and consequently may alter soil microbial populations and affect plant growth (19,20,22).

Concerns regarding environmental quality are prompting the development and use of various kinds of organic amendments or fertilizers to reduce or replace inorganic fertilizer and synthetic pesticide use. Organic amendments have been reported to suppress soilborne plant pathogens (6,15). Recently developed organic turf amendments, such as Ringer Lawn Restore, Ringer Greens Super, and Ringer Turf Restore are derived from hydrolysed poultry feather meal, blood meal, wheat germ, potassium sulfate, and bone meal. These products contain microorganisms that may be very important in the biological control of diseases caused by species of *Phytophthora, Pythium, Rhizoctonia,* and *Sclerotium* (18). They may also directly or indirectly affect the decomposition of thatch and nutrient transformations in soil.

Turfgrass thatch is a complex of dead and living roots, stems, and organic debris (2). Excessive thatch accumulation can be detrimental to turf quality and is normally controlled through verticutting and top-dressing (2). High microbial activity is important to thatch decomposition and nutrient recycling in turf (3). Cole and Turgeon (5) reported that 1 g of dry soil or thatch from turf of Kentucky bluegrass (*Poa pratensis* L.) can contain up to 2.8×10^8 bacteria and up to 2.8×10^6 fungi. Berndt et al (3) found that application of a range of organic amendments reduced thatch thickness of Kentucky bluegrass, whereas Mancino et al (11) found that addition of organic amendments increased thatch thickness and soil fungal counts.

Soil bacteria and fungi can increase the availability of plant nutrients in soil, form symbiotic associations with turfgrass roots, produce substances that stimulate plant growth, and protect plants against infection from pathogenic fungi (13). Application of composts to turf introduces or may promote increased populations of antagonistic microorganisms that interfere with the activities of pathogenic fungi (13). Many nonpathogenic soil microorganisms can effectively colonize foliage as well as roots in soil and allow protection of these tissues from infection (13).

Dollar spot disease, caused by *Sclerotinia homoeocarpa* F. T. Bennett, is one of the most common diseases on golf courses (23). Disease incidence and severity may be reduced by maintaining adequate fertility, especially nitrogen level (12). Cook et al (7) and Markland et al (12) found that dollar spot disease was not significantly reduced by inorganic nitrogen fertilizers, but was reduced with application of composted materials such as sewage sludge. Nelson and Craft (14) indicated that Ringer Compost Plus, Ringer Greens Restore, and Sustane (a turkey litter compost) significantly suppressed the severity of dollar spot disease. They found that Ringer amendments performed better than Sustane in suppression of dollar spot disease; however, Hoyland and Landschoot (10) did not find significant differences in dollar spot suppression among Ringer amendments, Sustane, and Milorganite. The lack of clear agreement between these results indicates a need for further studies.

The objective of this research was to evaluate and compare the effects of various organic amendments and turf fertilizer on *(1)* bacterial and fungal populations on turfgrass foliage and in thatch and soil of a creeping bentgrass (*Agrostis palustris* Huds.) green and a Kentucky bluegrass lawn; and *(2)* suppression of dollar spot disease on a creeping bentgrass green.

MATERIALS AND METHODS

Treatments and experimental design. This 3-yr study was started in early June 1991 on an 11-yr-old cv.Penncross creeping bentgrass putting green and on a 6-yr-old cv. Ram I Kentucky bluegrass lawn. The experimental site was located at the Cambridge Research Station, University of Guelph, Guelph, Ontario. The soil was a native Fox sandy loam (Brunisolic Gray Brown Lurisol) (76.9% sand; 17.0% silt; 6.1% clay; 1.7% organic matter; pH 5.2 as measured in calcium chloride; CEC 12.5 c/kg). The green was mowed daily at 5 mm height and the lawn was mowed twice a week at 5 cm height. The clippings were removed and the grass was irrigated as needed. The creeping bentgrass was core-cultivated in early September 1992 with the cores removed.

Various organic amendments and turf fertilizers were evaluated on both creeping bentgrass and Kentucky bluegrass from 1991 to 1993 (Table 1). All fertilizers were distributed by hand as uniformly as possible to 1×2 m plots at recommended label rates (Table 1) every 4 wk from early June to early September and once again in late November. Control plots were those to which no amendment was applied.

Separate experiments were conducted in adjacent turf areas. Eight fertilizers

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Table 1. Fertilizers, distributors and rates of treatment applications, summer 1991 through summer 1993

Fertilizer	Distributor	Annual Rate (kg N/100 m^2)
Alginate (1-0-2) (Norwegian kelp meal)	Imported by Bio-corp International, 225 Bradwick Dr. Unit 2, Concord, Ontario	0.5
Ammonium nitrate (34-0-0)	C.F. Industries Inc., Chicago, Illinois	2.2
Bovamura (converted from dairy manure)	Farmura LTD, Kent, England. Distributed by PBI/Gordon Corporation, Kansas	1.5
Milorganite (6-2-0) (activated sewage sludge)	Milorganite Division, MMSD Milwaukee, Wisconsin	1.5
Ringer Greens Super (10-2-6)	Ringer Corporation, 9959 Valley View Road, Minneapolis, Minnesota 55344	2.2
Ringer Lawn Restore (9-4-4)	Ringer Corporation, 9959 Valley View Road, Minneapolis, Minnesota 55344	2.2
Ringer Turf Restore (10-2-6)	Ringer Corporation, 9959 Valley View Road, Minneapolis, Minnesota 55344	2.5
Sandaid (1-0-2) (granular sea plant meal)	Emerald Isle Ltd, 2153 Newport Rd., Ann Arbor, Michigan 48113	0.5
Sewage sludge	Department of Public Works, Windsor, Ontario	1.5
Sulfur-coated urea (35-0-0)	Brussels Agromart Ltd., Brussels, Ontario	2.6

with an untreated control were used in the first experiment to evaluate microbial populations and thatch thickness for creeping bentgrass, and, in the second experiment, nine fertilizers for Kentucky bluegrass. A third experiment designed to assess dollar spot disease severity on creeping bentgrass consisted of six fertilizers, one fungicide, and an untreated check. All experiments were laid out in a randomized complete block design with four replicates. Baseline measurements of thatch thickness and microbial populations on turfgrass leaves and in thatch and soil were taken in early June 1991 just before the treatments were first applied.

Moisture content and thatch thickness. Samples of soil, thatch, and turfgrass were collected in late August each year with a 2.5-cm-diameter soil sampler and trimmed to a soil depth of 6 cm. Water was withheld 2–3 days before sampling for all plots. Five cores were sampled from each plot and thatch thickness was measured. The cores were then stored at 4 C for analyses of microbial population and moisture content the next day.

Over 5 g of thatch or soil of the sample mixtures from each plot were placed separately into paper bags for oven drying at 105 C overnight. The samples were allowed to cool for 1 hr before reweighing. Moisture was calculated on a percent dry weight basis.

Dilution-plate procedures. Bacterial and fungal population densities were determined from fresh turfgrass, thatch, and soil using the dilution-plate technique (4). One gram of soil (2 cm below soil-thatch interface), thatch, or turfgrass from a mixture of five plugs was placed into an autoclaved 35-ml test tube with 10 ml of sterile deionized water. The test tubes were mixed with a vortex mixer (Vortex Genie Model 550-G) for 10 sec. A series of 10^{-3} to 10^{-7} dilutions were made for each sample. A suspension (1 mL) was placed into a petri dish (9 cm in diameter) with a micro-pipette. There were three subsamples from each plot and three replications for each dilution series.

Preparation of dilution plating media for bacteria (yeast extract agar) and fungi

Table 2. Fertilizer effect on microbial populations (propagules per gram of fresh grass, dried thatch, and soil) and moisture contents of thatch and soil of creeping bentgrass turf

	Bacteria ($\times 10^6$)			Fungi ($\times 10^4$)			Moisture (%)[y]	
Treatment	Grass	Thatch	Soil	Grass	Thatch	Soil	Thatch	Soil
Ammonium nitrate	375 b[z]	222 ab	67 bcd	217 a	109 abc	62 a	105 c	22 a
Bovamura	125 d	168 bc	64 bcd	83 b	54 bc	17 b	104 c	19 a
Control	48 ef	83 d	39 d	53 b	42 bc	17 b	102 c	20 a
Milorganite	107 ed	79 d	36 d	78 b	52 bc	23 b	100 c	19 a
Ringer Greens Super	955 a	280 a	114 a	232 a	149 a	47 a	133 a	21 a
Ringer Turf Restore	409 b	255 a	89 abc	214 a	115 ab	45 a	120 b	23 a
Sandaid	25 f	105 cd	43 d	39 b	37 c	17 b	106 c	20 a
Sewage sludge	32 f	75 d	49 cd	66 b	45 bc	22 b	100 c	20 a
Sulfur-coated urea	225 c	229 ab	102 ab	116 b	79 abc	18 b	102 c	19 a

[y] Moisture content calculated on a dry weight basis.
[z] Means with same letters within column are not significantly different at $P = 0.05$ according to Duncan's multiple range test. Each number is the mean of combined data of 3 yr with four replicates in each year.

(rose bengal streptomycin agar) followed that of Black et al (4) with slight modifications. After autoclaving and cooling to 45 C, approximately 15 ml of media were poured into the plates. Control plates were amended with 1 ml of sterile deionized water. The plates were incubated for 4 days in darkness at room temperature, and plates with 30–300 colonies were selected for counting. The number of microbes per gram of oven-dried soil or thatch or per gram fresh weight of grass was then calculated.

Evaluation of dollar spot disease. Fertilizers were first applied on 4 June 1991 to plots for dollar spot evaluation and followed by 1 cm of irrigation to water fertilizers into thatch. The fungicide Daconil 2787 F (96 ml/100 m^2, 75% a.i. chlorothalonil) was used for comparison with the fertilizers and was applied from early June to late August every 14 days with a bicycle-wheel-mounted air pressure sprayer. To ensure a base level of disease pressure, the creeping bentgrass was inoculated in late June of 1991–1993. The inoculum for dollar spot disease was prepared by incubating *Sclerotinia homoeocarpa* on autoclaved cereal grains for 2–3 wk. The inoculum was dried overnight and chopped with a mixer into small particles (<2 mm). Inocula from five strains of the fungus were mixed together, and 2 g of the inocula were mixed with 10 g of sand for uniform distribution to each plot. Total number of dollar spot patches larger than 1 cm in diameter were counted every 2 wk starting in early July.

Statistical analyses. All data were first analyzed using the Univariate procedure of SAS (21). The number of colonies of bacteria and fungi were transformed to a log scale [log (x + 1)] before statistical analysis. Analyses of variances for microbial populations and moisture contents were performed on pooled measurements over the three years and followed by Duncan's multiple range test if significant differences were found at $P = 0.05$. The LSD test at $P = 0.05$ was used to compare differences in the mean number of dollar spot patches among different treatments.

RESULTS AND DISCUSSION

Microbial populations for creeping bentgrass. Significant treatment differences were found for microbial populations on leaves, in thatch, and in soil of creeping bentgrass (Table 2). Turf treated with Ringer Greens Super had the highest ranking for fungal and bacterial populations on grass leaves, in thatch, and in soil. Treatments with ammonium nitrate and sulfur-coated urea in addition to Ringer amendments also ranked higher for microbial populations on turfgrass, in thatch, and in soil than most of the other amendments. Mancino et al (11) also found a increase in fungal populations by Ringer Greens Restore.

Volk and Horn (24) reported that certain organic amendments were markedly inferior to inorganic fertilizers such as sulfur-coated urea, and that turf response to some organic amendments such as digested sludge was negligible. Fertilizers such as sulfur-coated urea have nitrogen release rates strongly affected by microbial activity (1). In our studies, Alginate and Sandaid generally gave no significant effects on microbial populations. These materials are derived from kelp or other sea plant meals and contain a low nitrogen content with controlled release characteristics; thus, it was not unexpected that these amendments showed no major effects on microbial populations in our study. For most tests, microbial populations were not altered significantly by Bovamura or Milorganite compared with the control.

Many factors may be involved in the increased populations of microorganisms from certain treatments. Increased plant growth through increased nutrients, especially nitrogen, may be a major factor. Increased microbial populations may also be related to microbial and biological properties of organic fertilizers that may themselves contain high populations of microorganisms or that may stimulate native populations in the turf. Nelson (13) has discussed in much greater detail the effects of composts on microbial populations and disease suppression in turfgrass.

Table 3. Fertilizer effect on microbial populations (propagules per gram of fresh grass, dried thatch, and soil) and moisture content of thatch and soil of Kentucky bluegrass turf

	Bacteria ($\times 10^6$)			Fungi ($\times 10^4$)			Moisture (%)[y]	
Treatment	**Grass**	**Thatch**	**Soil**	**Grass**	**Thatch**	**Soil**	**Thatch**	**Soil**
Alginate	158 c[z]	248 cd	73 a	55 abc	94 a	20 a	61 b	18 a
Ammonium nitrate	217 bc	329 bcd	56 a	59 abc	129 a	33 a	59 b	18 a
Bovamura	117 c	195 d	55 a	46 c	108 a	24 a	61 b	20 a
Control	211 bc	248 cd	61 a	52 bc	84 a	26 a	58 b	18 a
Milorganite	154 c	247 cd	64 a	50 bc	109 a	30 a	60 b	19 a
Ringer Lawn Restore	438 a	645 a	87 a	69 a	124 a	37 a	73 a	20 a
Ringer Turf Restore	316 ab	418 b	64 a	65 ab	106 a	32 a	78 a	20 a
Sandaid	113 c	280 bcd	89 a	49 bc	100 a	27 a	63 b	19 a
Sewage sludge	123 c	247 cd	64 a	61 abc	104 a	24 a	58 b	19 a
Sulfur-coated urea	166 c	376 bc	69 a	50 bc	93 a	23 a	60 b	20 a

[y] Moisture calculated on dry weight basis.
[z] Means with same letters within column are not significantly different at $P = 0.05$ according to Duncan's multiple range test. Each number is the mean of combined data of 3 yr with four replicates in each year.

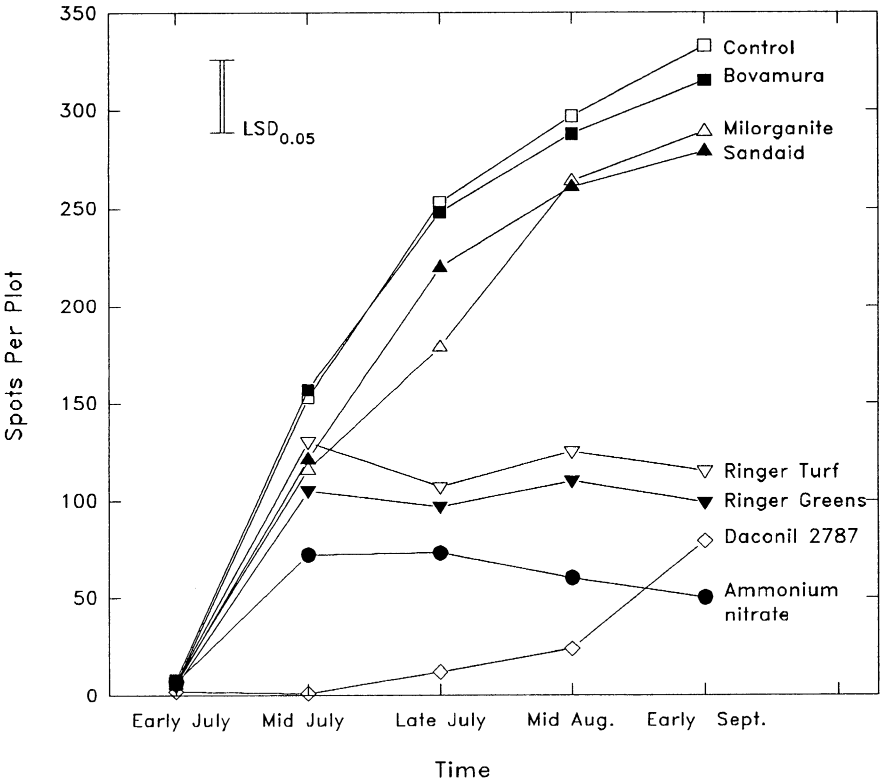

Fig. 1. Effect of fertilizers and a fungicide on development of dollar spot disease on creeping bentgrass. Each data point is the average of ratings over 3 yr. Fertilizer treatments applied monthly and the fungicide Daconil 2787 applied biweekly from early June to early September, 1991–1993.

Microbial populations for Kentucky bluegrass. Similar to the treatments on creeping bentgrass but at a smaller magnitude, Ringer amendments, especially Ringer Lawn Restore, gave significantly higher bacterial counts on leaves and in thatch of Kentucky bluegrass than most other treatments (Table 3). Bacterial populations in soil and fungal populations in thatch and soil did not significantly differ among the treatments.

Moisture contents and thatch thickness. The moisture content of thatch from plots treated with Ringer amendments was significantly higher than that of thatch from plots treated with other fertilizers on both creeping bentgrass and Kentucky bluegrass (Tables 2 and 3). These results suggest that Ringer amendments, especially Ringer Greens Super, may impart greater moisture retention capabilities for thatch, but the reason for this is unknown and needs further study.

On creeping bentgrass, mean thatch thickness ranged from 15.1 mm to 19.4 mm, and for Kentucky bluegrass from 11.8 mm to 16.3 mm. For nearly all treatments, thatch thickness did not change significantly over the 3 yr on either creeping bentgrass or Kentucky bluegrass, possibly because increased plant growth and thatch production were balanced by increased thatch decomposition through increased microbial populations (3). On creeping bentgrass, only Ringer Greens Super was found to alter thatch thickness significantly, a decrease of 2.6 mm after 3 yr. This contrasts with the report of Mancino et al (11), who found an increase in creeping bentgrass thatch thickness after treatment with Ringer Greens Restore .

Dollar spot disease on creeping bentgrass. Dollar spot incidence was not significantly different for fertilizer treatments in early and mid-July (Fig. 1). From late July to early September, ammonium nitrate and Ringer amendments significantly reduced the incidence of dollar spot disease on creeping bentgrass compared with other amendments and the untreated control. The ammonium nitrate treatment showed the best control of dollar spot disease compared with other fertilizers and amendments.

The results of this study support those of Nelson and Craft (14) who found that Ringer Compost Plus and Ringer Greens Restore significantly suppressed dollar spot disease development and gave over 60% control. Gallant (9) also reported that top-dressings amended with Greens Restore gave a reduction of dollar spot disease.

The mechanism by which Ringer amendments suppress dollar spot disease is unknown. Increased turf growth from increased nitrogen level could affect expression of dollar spot symptoms by allowing the grass to outgrow or recover from infections. The higher microbial population on turfgrass and in thatch from the Ringer treatments may compete or antagonize pathogenic organisms such as *S. homoeocarpa* and protect plants from infection (18). Bovamura, Milorganite and Sandaid provided less nitrogen than Ringer treatments or inorganic sources at the rates applied (Table 1) and they were ineffective in suppression of dollar spot disease (Fig. 1). Nelson and Craft (15) found that the ammonium form of nitrogen and not the nitrate form was related to dollar spot disease suppression.

The competitive and antagonistic effects on pathogenic organisms from the increased microbial populations in thatch and soil and on turfgrass may be related to the suppression of dollar spot disease in this study. O'Neill (16) and Nelson and Craft (15) found that composts autoclaved right before application also have disease suppressive properties, suggesting that stimulation of microbial populations by sterile amendments may be related to disease suppression. Couch and Bloom (8) found that, below field capacity, increased soil moisture contents correlated with decreased dollar spot incidence, and thus a higher moisture content in thatch treated with Ringer amendments could also have contributed to the reduction of dollar spot incidence.

Our results indicated that nitrogen levels in fertilizers and amendments play a major role in both microbial populations and disease suppression; however, because we did not apply all amendments and fertilizers at a standardized rate, the effect of nitrogen was confounded. On the other hand, the organic amendments are more than mere nitrogen carriers: the organic structures and microbial populations give organic amendments a complex nature, and when combined with a turfgrass system, the cause-effect relationships may be difficult to elucidate. The effects of organic amendments on turfgrass characteristics such as microbial populations and disease expression require much more study.

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