condition) exceeded that occurring in BM containing sucrose (photo-mixotrophic condition) for several plants (e.g., carrot and lettuce) if given an adequate CO₂ level (Fig. 4). We conducted our studies with different species and obtained significant growth increases using ultra-high levels of CO₂. This work suggests that other species should now be tested using the inexpensive CO₂ flow-through system presented herein with similar CO₂ levels.

Literature Cited

- Buddendorf-Joosten, J.M.C. and E.J. Woltering. 1994. Components of the gaseous environment and their effects on plant growth and development in vitro. *Plant Growth Regulat.* 15:1-16.
- Buddendorf-Joosten, J.M.C. and E.J. Woltering. 1996. Controlling the gaseous composition in vitro—description of a flow system and effects of the different gaseous components on in vitro growth of potato plantlets. *Sci. Hort.* 65:11-23.
- Cournac, L., B. Dimon, P. Carrier, A. Lahou, and P. Chagvardieff. 1991. Growth and photosynthetic characteristics of *Solanum tuberosum* plantlets cultivated in vitro in different conditions of aeration, sucrose supply, and CO₂ enrichment. *Plant Physiol.* 97:112-117.
- Desjardins, Y., F. Laforge, C. Lussier, and A. Gosselin. 1988. Effect of CO₂ enrichment and high photosynthetic photon flux on the development of autotrophy and growth of tissue cultured strawberry, raspberry and asparagus plants. *Acta Hort.* 230:45-53.
- Enoch, H.Z. and B.A. Kimball. 1986. Carbon Dioxide Enrichment of Greenhouse Crops. vol. 1. CRC Press, Inc., Boca Raton, Fla.
- Figueira, A., A. Whipkey, and J. Janick. 1991. Increased CO₂ and light promote in vitro shoot growth and development of *Theobroma cacao*. *J. Amer. Soc. Hort. Sci.* 116:585-589.
- Fujiwara, K., S. Kira, and T. Kozai. 1992. Time course of CO₂ exchange of potato cultures in vitro with different sucrose concentrations in the culture medium. *J. Agr. Met.* 48:49-56.
- Fujiwara, K.S. and T. Kozai. 1995. Physical microenvironments and its effects, p. 319-369. In: J. Aitken-Christie, T. Kozai, and M. Lila Smith (eds.). *Automation and environmental control in plant tissue cultures*. J. Kluwer Academic Publishers, Boston.
- Fujiwara, K., T. Kozai, and I. Watanabe. 1988. Development of a photoautotrophic tissue culture system for shoots and/or plantlets at rooting and acclimation stages. *Acta Hort.* 230:153-158.
- Jeong, B.R., K. Fujiwara, and T. Kozai. 1993. Carbon Dioxide enrichment in autotrophic micropropagation: Methods and advantages. *HortTechnology* 3:412-414.
- Kozai, T. 1988. Autotrophic (Sugar free) tissue culture for promoting the growth of plantlets in vitro and for reducing biological contamination, p. 39-51. In: T.W. Flegel, G. Tharun, and Y. Yuthavonh (eds.). *Intl. Symp. Appl. Biotechnol. Small Ind. Dev. Developing Countries*, 21-23 Sept., Bangkok, Thailand.
- Kozai, T., H. Oki, and K. Fujiwara. 1987. Effects of CO₂ enrichment and sucrose concentration under high photosynthetic photo fluxes on growth of tissue-cultured cymbidium plantlets during the preparation stage. *Symp. Florizel on Plant Microprop.* in *Hort. Ind.*, Arlon, Belgium. p. 135-141.
- Prusky, D., H. Hamdan, R. Ardi, and N.T. Keen. 1996. Induction of biosynthesis of epicatechin in avocado suspension cells treated with an enriched CO₂ atmosphere. *Physiol. Mol. Plant Pathol.* 48:171-178.
- Treat, W.J., C.R. Engler, and E.J. Soltes. 1989. Pine in a modified Fermentor using a novel impeller. *Biotech. Bioeng.* 34:1194-1202.
- Yorio, NC., R.M. Wheeler, and R.C. Weigel. 1995. Effect of irradiance, sucrose, and CO₂ concentration on the growth of potato (*Solanum tuberosum* L.) in vitro. *NASA Tech. Mem.* 110654:1-19.

Effect of Natural Organic Nitrogen Fertilizers on Hybrid Bermudagrass Growth

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ADDITIONAL INDEX WORDS. *Cynodon*, turfgrass, golf courses

SUMMARY. Eco, Milorganite, Ringer, and Sustane natural organic fertilizers, alone or combined with the synthetic organic fertilizer isobutylidene diurea (IBDU), were compared with IBDU alone for their effect on a 'Tifdwarf' hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burt-Davy] golf course putting green. Over the 2-year study period, no consistent differences were observed among the fertilizer treatments on the turfgrass growth parameters of quality, clipping weights, or root weights.

Hybrid bermudagrass (*Cynodon dactylon* x *C. transvaalensis*) grows all year on golf course putting greens in southern Florida. Due to the extensive rainfall received in this subtropical climate, slow-release rather than quick-release nitrogen fertilizer sources are commonly used to reduce nitrogen leaching and maintain turfgrass quality (Sartain, 1982; Snyder et al., 1981). Products developed from urea, such as ureaformaldehydes, sulfur-coated urea, and isobutylidene diurea (IBDU), are examples of slow-release synthetic organic nitrogen fertilizers.

Other alternatives to quick-release nitrogen sources are natural organic nitrogen fertilizers. These fertilizers

are slow-release materials also, because they depend on soil microorganisms to break down the proteins, amino acids, and other complex organic compounds into nutrient forms available to the plant. Some of the slow-release synthetic organic fertilizers also depend on microbial breakdown for release of available plant nitrogen. Ureaformaldehyde reaction products are an example (Turner and Hummel, 1992).

In the United States, for a fertilizer to be described as a natural organic fertilizer, it must conform to the Association of American Plant Fertilizer Control Officials definition of "materials derived from either plant or animal products containing one or more elements (other than carbon, hydrogen and oxygen) that are essential for plant growth" (Meister, 1995). Examples of products used to make natural organic fertilizers include turkey litter, sewage sludge, alfalfa, blood meal, and bone meal.

Since a golf course superintendent in southern Florida must maintain optimum bermudagrass quality every day of the year, practical use of a natural organic fertilizer all year depends on the availability of the nutrients. Southern Florida's subtropical (USDA Zone 10) climate should be conducive to the use of such a fertilizer since winter temperatures sustain microbial activity. This study was initiated to evaluate the effect of natural organic fertilizers, either used alone or with a synthetic organic fertilizer, on growth parameters of 'Tifdwarf' hy-

brid bermudagrass on a golf course putting green.

Materials and methods

The field experiment was conducted from May 1992 through May 1994 at the Univ. of Florida's Fort Lauderdale Research and Education Center on an established 'Tifdwarf' bermudagrass research golf green built with a root-zone mix composed of 80% sand and 20% Canadian sphagnum peat (by volume). The area was vertically mowed and topdressed at least once per month, with depth of vertical mowing dependent on thatch layer thickness. Topdressing material was composed of the same material as the root-zone mix. The turfgrass height was maintained at 4.7 mm by mowing six times weekly. The area was irrigated as needed to maintain the best possible quality. A fertilizer blend containing IBDU and Milorganite as the nitrogen sources had been used routinely since 1990 at the rate of 784 lb/acre (879 kg·ha⁻¹) of nitrogen per year, applied at 2-week intervals.

Nine treatments of natural and synthetic organic fertilizers were used (Table 1). Each plot was 2 × 3 m with four replicate plots per treatment in a randomized complete-block design. The control fertilizer was made by mixing IBDU, a synthetic organic nitrogen source, with potassium magnesium sulfate to obtain an 8N-0P-8K fertilizer blend, a standard blend used on putting greens in southern Florida. One set of four treatments was the natural organic fertilizers used indi-

vidually as the sole nitrogen source. A second set of four treatments mixed the natural organic fertilizers with the IBDU blend, 1:1 for nitrogen content. Because the Milorganite fertilizer did not contain potassium, potassium magnesium sulfate was applied when the Milorganite was applied to these treatment plots to obtain a 1N : 1K ratio.

Plots were fertilized every 2 weeks. A total of 784 lb/acre of nitrogen was applied per year. The control and Milorganite treatment plots received an equivalent amount of potassium. The amount of potassium applied to the other treatment plots varied based on the fertilizer source. Phosphorus, as triple super phosphate, was applied twice each year to the control treatment plots at 109 lb/acre. As with potassium, the amount of phosphorus and other nutrients applied to the remaining treatment plots varied based on the fertilizer source (Table 1).

To reduce variability between batches of natural organic fertilizers, a 2-year supply of each material was obtained and stored in an air-conditioned room in the laboratory. In December 1993, a water pipe broke and flooded the room, damaging the Eco fertilizer. The manufacturer was unable to supply us with new material until March 1994. Therefore, no Eco fertilizer was applied for 3 months from 22 Dec. 1993 to 16 Mar. 1994.

Turfgrass quality ratings were determined based on observation of grass color and density using a scale of 1 to 10, with 10 representing turf with

Table 1. Synthetic and natural organic fertilizer treatments applied to a 'Tifdwarf' bermudagrass putting green in southern Florida.

Fertilizer treatment	Nutrient ratio			Supplier	Source of nutrients
	N	P	K		
Eco	7.0	0.4	4.2	Sinnamon Brothers, Camrose, Alberta, Canada	Dehydrated alfalfa, sunflower seed hull ash, bone meal, blood meal, canola meal, and soybean meal
Sustane ²	5.0	0.9	3.3	Sustane Corp., Bloomington, Minn.	Aerobically composted turkey feather litter
Ringer ²	8.0	0.9	6.7	Ringer Corp., Minneapolis	Hydrolyzed poultry feather meal, wheat germ and soybean meal
Milorganite	6.0	0.9	0	Milorganite Division, MMSD, Milwaukee	Activated wastewater sludge
IBDU blend ³ (control)	8.0	0	8.0	Vigoro Industries, Inc., Winter Haven, Fla.	isobutylidene diurea, potassium magnesium sulfate

²Sustane and Ringer were the only fertilizer materials evaluated that are amended with microorganisms.

³The control treatment (synthetic organic nitrogen) was a blend of IBDU (8% N) and potassium magnesium sulfate (8% K and 4% Mg). The mixture of a natural organic nitrogen source with the IBDU blend was done at a 1:1 ratio for nitrogen.

Table 2. Effect of natural and synthetic organic nitrogen fertilizer treatments on 'Tifdwarf' bermudagrass quality ratings.

Fertilizer treatment	Quality rating ^a								
	1992		1993		1994				
	3 Nov.	30 Dec.	21 Apr.	6 May	10 Feb.	23 Feb.	10 Mar.	23 Mar.	7 Apr.
Eco ^b	5.3 b	5.6 b	7.5 a	7.5 a	4.0 b	4.0 b	3.0 b	4.0 b	5.0 c
Sustane	6.1 a	5.6 b	7.4 b	7.4 b	5.0 a	4.8 b	5.5 a	5.5 a	6.4 b
Ringer	5.8 ab	5.6 b	7.5 a	7.5 a	5.0 a	4.8 b	5.5 a	5.5 a	6.1 b
Milorganite	5.0 b	5.8 ab	6.5 c	6.5 c	5.0 a	4.9 b	5.5 a	5.5 a	6.4 b
Eco ^b /IBDU blend	5.5 ab	5.8 ab	7.5 a	7.5 a	4.0 b	4.0 b	3.0 b	4.0 b	5.3 c
Sustane/IBDU blend	6.1 a	6.0 a	7.5 a	7.5 a	5.0 a	4.9 b	5.5 a	5.5 a	6.1 b
Ringer/IBDU blend	5.4 ab	6.0 a	7.5 a	7.5 a	5.0 a	5.9 a	5.5 a	5.5 a	7.0 a
Milorganite/IBDU blend	5.8 ab	6.0 a	7.5 a	7.5 a	5.0 a	4.9 b	5.5 a	5.5 a	6.1 b
IBDU blend (control)	6.1 a	6.0 a	7.5 a	7.5 a	5.0 a	4.8 b	5.5 a	5.5 a	6.1 b
Pr > F	0.0380	0.0106	0.0001	0.0001	0.0001	0.0053	0.0001	0.0001	0.0001

^aValues are means of four replicate plots. Means separation within a column by Waller-Duncan k ratio *t* test, *P* = 0.05. No significant differences were observed for any other dates during the 2 year study.

^bEco fertilizer and Eco/IBDU fertilizer blend were not applied from 22 Dec. 1993 to 16 Mar. 1994 due to unavailability of the Eco material.

a dark green color and uniform dense stand. The plots were rated about every 2 weeks, 1 week after each nitrogen application.

Turfgrass clippings, from plots that had not been cut for 48 h, were collected once each month from a 1-m² area in the center of each plot. Clippings were dried at 60°C for 72 h and then weighed. When possible, quality ratings and clippings were obtained on the same day or subsequent days.

Root weights were obtained every 3 months, beginning in November 1992. At each sampling date, two 6-in-diameter (15-cm) × 4-in-deep (10-cm) samples were obtained from each end of each plot, for a total of four subsamples per plot. Root samples were not collected randomly within the plot because samples collected from the center of a plot would have resulted in voids that would have interfered with clipping weight evaluations. A 0.5-in (1.25 cm) cap was cut from the top of the sample and then discarded to re-

move leaf tissue and most of the thatch layer. Samples were then processed with a commercial root washer (Gillison Hydropneumatic Elutriation System, Benzonia, Mich.) using 760-mm primary and secondary sieves. The accumulated material was dried at 80°C for 36 to 48 h and then weighed. Weights from the four subsamples of each plot were added together to obtain the total root weight per plot. Resulting "holes" from sampling were filled with topdressing material.

Analysis of variance was performed using the GLM procedure (SAS Institute Inc., Cary, N.C.). Means were compared using the Waller-Duncan k ratio *t* test.

Results and discussion

No differences in turfgrass quality were observed among any of the treatment plots on 30 of the 44 rating dates. Differences were observed on the other 14 rating dates, but significant differences (*P* ≤ 0.05) among treatments were observed on only 9

(20%) rating dates (Table 2).

No consistent association between a fertilizer treatment and quality ratings was obtained for the entire 2-year study. During the first year, on the dates when significant differences were observed, the use of one or more natural organic amendments as the sole nitrogen source resulted in lower quality ratings compared to the IBDU control. Milorganite, applied as the sole nitrogen source, was associated with these lower quality ratings on three of the four dates. On two dates (23 Feb. and 7 Apr. 1994), fertilizer treatments resulted in significantly higher quality ratings than the IBDU control treatment. On both dates, that treatment was the Ringer/IBDU blend.

As would be expected, significantly lower quality ratings were observed for Eco alone and Eco/IBDU blend fertilizer treatment plots from February 1994 through mid-April 1994 due to nonapplication of these materials. About 6 weeks after the last

Table 3. Effect of natural and synthetic organic nitrogen fertilizer treatments on 'Tifdwarf' bermudagrass clipping weights during the second year of the study.

Fertilizer treatment	Clipping wt (grams)*													
	1993							1994						
	21 Apr.	28 May	30 June	28 July	26 Aug.	30Sept.	5 Nov.	5 Dec.	12 Jan.	9 Feb.	9 Mar.	6 Apr.	4 May	20 May
Eco ¹	3.14 bc	3.14 ab	7.22 ab	6.90	6.17	3.65	1.57	1.82 c	2.51 c	1.26 e	0.33 d	0.88 c	5.81 d	2.78 d
Sustane	3.10 bc	2.25 cd	6.33 b	6.61	5.77	3.37	2.09	2.13 bc	2.64 c	2.24 d	1.69 c	2.89 b	7.79 c	4.33 b
Ringer	2.82 cd	3.02 abc	7.33 ab	6.41	6.13	3.17	1.32	2.33 abc	2.67 c	2.17 d	1.97 bc	3.54 b	7.76 c	3.87 bcd
Milorganite	2.08 d	1.76 d	5.80 b	6.14	5.08	2.83	1.32	2.38 abc	2.85 c	2.81 bc	1.63 c	3.37 b	8.63 bc	4.41 b
Eco ¹ /IBDU blend	3.46 bc	3.28 ab	9.18 a	8.06	6.23	3.60	1.51	2.28 abc	3.00 bc	1.07 e	0.33 d	1.16 c	5.56 d	2.86 cd
Sustane/IBDU blend	3.46 bc	2.70 bc	7.60 ab	8.01	7.04	3.30	1.72	1.89 c	2.80 c	2.80 bc	1.88 bc	2.88 b	7.76 c	4.17 b
Ringer/IBDU blend	3.97 ab	3.72 a	8.19 ab	7.40	7.77	3.38	1.89	2.77 a	4.10 a	3.97 a	3.11 a	4.98 a	12.47 a	6.16 a
Milorganite/IBDU	3.56 bc	3.22 ab	8.29 ab	7.68	6.28	3.55	1.51	2.72 ab	3.49 b	3.01 b	2.29 b	3.42 b	9.77 b	5.03 ab
IBDU blend (control)	4.58 a	3.77 a	9.43 a	7.64	6.43	3.17	2.04	2.25 abc	2.92 bc	2.26 cd	1.48 c	2.48 b	7.41 c	4.08 bc
Pr>F	0.0005	0.0004	0.0344	0.6972	0.0662	0.9208	0.4656	0.0109	0.0002	0.0001	0.0001	0.0001	0.0001	0.0003

^aValues are means of four replicate plots. When applicable, means separation within a column by Waller-Duncan k ratio *t* test, *P* = 0.05.

^bEco fertilizer and Eco/IBDU fertilizer blend were not applied from 22 Dec. 1993 to 16 Mar. 1994 due to unavailability of the Eco material.

Eco fertilizer application in December 1993, significantly lower bermudagrass quality was observed for those treatment plots. When Eco fertilizer applications were resumed in March 1994, 6 weeks elapsed before the bermudagrass quality in those plots was equivalent to plots treated with other fertilizer materials. If the Eco fertilizer had been applied during the entire study, significant differences in turfgrass quality probably would have been limited to four dates.

Significant differences ($P \leq 0.05$) for clipping weights were obtained among treatments on 16 of 23 (70%) sampling dates. During the first 13 months of the study, the IBDU control treatment resulted in the greatest clipping weights, whereas the lowest clipping weights were associated with the Milorganite treatment. This corresponds with results from a 2-year study on creeping bentgrass in which that Milorganite produced the lowest plant growth rate compared with other natural and synthetic organic nitrogen fertilizers (Peacock and Dipaola, 1994).

Data for the second year of our study (Table 3) illustrate the fluctuations in leaf growth during the year. Clipping weights were significantly reduced for the Eco and Eco/IBDU treatment plots after the applications were suspended due to lack of Eco fertilizer material. These reduced weights correspond with reduced quality (Table 2). Significantly greater clipping weights were associated with Ringer/IBDU treatment plots during the last 5 months of the study. However, significantly greater turfgrass quality for this treatment could only be documented twice during this same time period (Tables 2 and 3).

No significant differences in root weights were obtained among treatments on any of the seven sampling dates. The average root weights across all treatments for each date were as follows: 16 Aug. 1992 = 19.4 g; 30 Nov. 1992 = 14.5 g; 1 Mar. 1993 = 24.6 g; 1 June 1993 = 33.4 g; 6 Sept. 1993 = 26.6 g; 29 Nov. 1993 = 36.7 g; 28 Feb. 1994 = 25.1 g; 23 May 1994 = 36.5 g. Even the lack of a nitrogen fertilizer source (Eco and Eco/IBDU blend) did not result in significant differences among treatments. Since separating small, fine roots from a root-zone mix and topdressing material containing sphagnum peat is difficult, differences may be impos-

sible to obtain. Even incineration of the material collected would not have been useful as the peat was the primary contaminant, and it is organic matter also.

IBDU, a slow-release synthetic organic fertilizer, does not depend on soil microbes for nitrogen release. Fluctuations in microbial activity was apparently not a limiting factor for the use of natural organic fertilizers year-round in southern Florida, since turfgrass quality in plots treated with these fertilizers was equivalent to those treated with IBDU alone. Although Ringer and Sustane fertilizers are amended with microorganisms, this did not significantly alter their effect on turfgrass growth. This has been observed previously with 'Tifway' hybrid bermudagrass (Peacock and Daniel, 1992).

No diseases were observed during this study, and the nonsignificant differences in root weights among treatments might indicate that root health was not affected by the fertilizers. Thatch accumulation was not a problem among treatments, primarily due to good cultural management. Therefore, these reported secondary benefits from the natural organic fertilizers (Agnew 1992; Berndt et al., 1990; Nelson and Craft, 1992; Nus 1993; Sartain and Volk, 1984) could not be evaluated. This study did demonstrate that natural organic fertilizers could be used as the sole nitrogen source (e.g., Milorganite) or the only fertilizer source (e.g., Ringer and Sustane) in a maintenance program for established hybrid bermudagrass putting greens in southern Florida without adverse effects.

Literature cited

- Agnew, M.L. 1992. Natural organic nitrogen sources. *Golf Course Mgt.* 60:70-75.
- Berndt, W.L., P.E. Rieke, and J.M. Vargas, Jr. 1990. Kentucky bluegrass thatch characteristics following application of bio-organic materials. *HortScience* 25:412-414.
- Meister, R.T. (ed.). 1995. *Farm chemicals handbook 1995*. Meister Publishing Co., Willoughby, Ohio.
- Nelson, E.B. and C.M. Craft. 1992. Suppression of dollar spot on creeping bentgrass and annual bluegrass turf with compost-amended topdressings. *Plant Dis.* 76:954-958.
- Nus, J. 1993. Sewage sludge and the turfgrass industry. *Golf Course Mgt.* 61:26-36.
- Peacock, C.H. and P.F. Daniel. 1992. A comparison of turfgrass response to biologically amended fertilizers. *HortScience* 27:883-884.
- Peacock, C.H. and J.M. Dipaola. 1994. Response of creeping bentgrass (*Agrostis palustris*) to natural organic fertilizers, p. 471-476. In: A.J. Cochran and M.R. Farrally (eds.). *Science and golf II: Proc. World Sci. Congr. of Golf*. E&FN Spon, London.
- Sartain, J.B. 1982. Nitrogen management of warm-season turfgrasses, p. 99-108. In: *Symp. on turfgrass fertility: Advances in turfgrass fertility*. Hammer Graphics Inc., Piqua, Ohio.
- Sartain, J.B. and B.G. Volk. 1984. Influence of selected white-rot fungi and topdressings on the composition of thatch components of four turfgrasses. *Agron. J.* 76:359-362.
- Snyder, G.H., E.O. Burt, and J.M. Davidson. 1981. Nitrogen leaching in bermudagrass turf: Effect of nitrogen sources and rates. In: R.W. Sheard (ed.). *Proc. 4th Intl. Turfgrass Res. Conf.*, 19-23 July 1981, Univ. of Guelph, Ontario, Canada.
- Turner, T. R., and N. W. Hummel, Jr. 1992. Nutritional requirements and fertilization, p. 385-439. In: D. V. Waddington, R. N. Carrow, and R. C. Shearman (co-eds.). *Turfgrass*. Agron. Monograph No. 32. Amer. Soc. Agron., Madison, WI.