Distinguishing off-types in Tifway and Tifdwarf bermudagrass

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Interim report, July 1995 through May 1996

Objective and Brief Summary

The research will determine the feasibility of distinguishing off-types in Tifway and Tifdwarf bermudagrasses.

We have found that DNA banding patterns are powerful in distinguishing off-types from Tifway. Samples of fairway off-types from different golf courses can be matched by their RAPD patterns. Therefore, the off-types appear not to have originated on the various golf courses, but were carried in as planting stock.

For the greens, in contrast, few DNA bands distinguish Tifdwarf from its apparent off-types. We are retesting recollections of one interesting off-type, T-74, which appears to have several distinctive DNA banding pattern differences from Tifdwarf. If we can show again that off-types did not originate on, but were carried to, a golf course, this would minimize the role of recurring mutation as a source of off-types. Morphology data are complementing DNA banding pattern data.

Background

The main idea of the proposal is that DNA banding patterns (i.e., RAPD mark

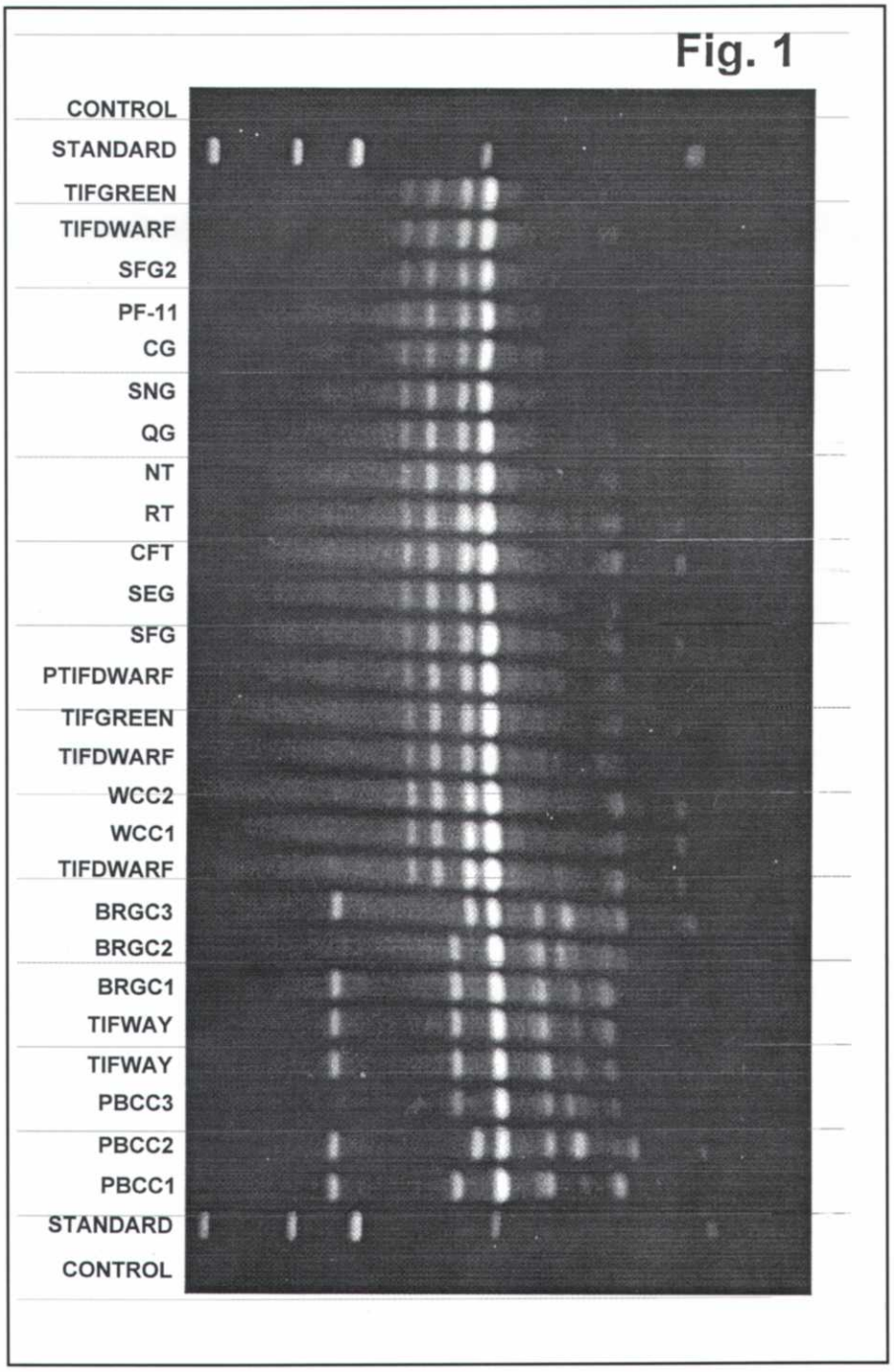

Fig. 1. *Image of PCR amplification products from 26 bermudagrasses, based on primer AK18. ("Standard" refers to a molecular size reference, and is not grass DNA.) At the top, the banding patterns for 18 greens bermudagrasses (Tifgreen, Tifdwarf, SFG2. . .Tifdwarf) were indistinguishable. In striking contrast, the banding patterns for 8 fairway grasses at the bottom of the image (BRGC3, BRGC2. . .PCC1) varied.*

ers) might distinguish bermudagrasses, thereby helping in quality control.

RAPD markers provide a relatively inexpensive genetic identification tool, but the method is prone to possible errors. To generate diagnostic banding patterns, varying-size fragments of sample DNA must be multiplied or "amplified" using "primers," short pieces of DNA that recognize a specific DNA sequences from the unknown grass. Because amplification is a sensitive step in the RAPD process, we have attempted to control error by selecting primers that are consistent, and we have exchanged primers and procedures between our two laboratories, at Gainesville and Fort Lauderdale. Morphological traits, including chromosome number, are being developed as a potentially faster and cheaper method of prescreening for genetic off-types.

Work Completed

We have completed the screening of 130 primers ("Stage 1" in the proposal), have tested their repeatability ("Stage 2"), have exchanged 11 primers between locations (beginning of "Stage 3"), and have completed the application of primers to a population of 26 grasses ("Stage 4," which was planned for the second year). We have further retested selected primers on six additional fairway samples. We have initiated a study of morphology ("Supplement"), including a replicated grow-out, and preliminary work on roottip chromosomes. (For an explanation, see the original proposal.)

Work Remaining

We will complete the retesting of exchanged primers on bermudagrass which can be distinguished ("Stage 3") and complete the morphological study of all 26 bermudagrasses, including chromosome numbers, and complete a final report by July 1997.

Results Thus Far

We found several primer-derived markers for distinguishing fairway off-types, but few markers for distinguishing greens off-types. This is apparent in the image for primer AK18 (*Fig. 1*). The banding patterns for 18 greens bermudagrasses (Tifgreen, Tifdwarf, SFG2. . .Tifdwarf) were indistinguishable. The banding patterns for 8 fairway grasses (BRGC3, BRGC2. . .PCC1) varied. As another example, primer 719 (*Fig. 2*) showed that the dominant matrix grass on fairways of two golf courses was indistinguishable from the Tifway foundation, but two off-types from fairways of each golf course were not Tifway. Furthermore, the off-types matched across golf courses, indicating that they had been propagated and planted from a common source, possibly as a contaminant, and not through recent mutation or seedling variation. the genetically matched bermudagrasses were also similar morphologically, and the off-types produced abundant pollen, so they must be tetraploids ($2\underline{n} = 36$). We found that a uniform, desirable, fairway grass (T-20) from a third golf course matched the genetic signature of the dark-green

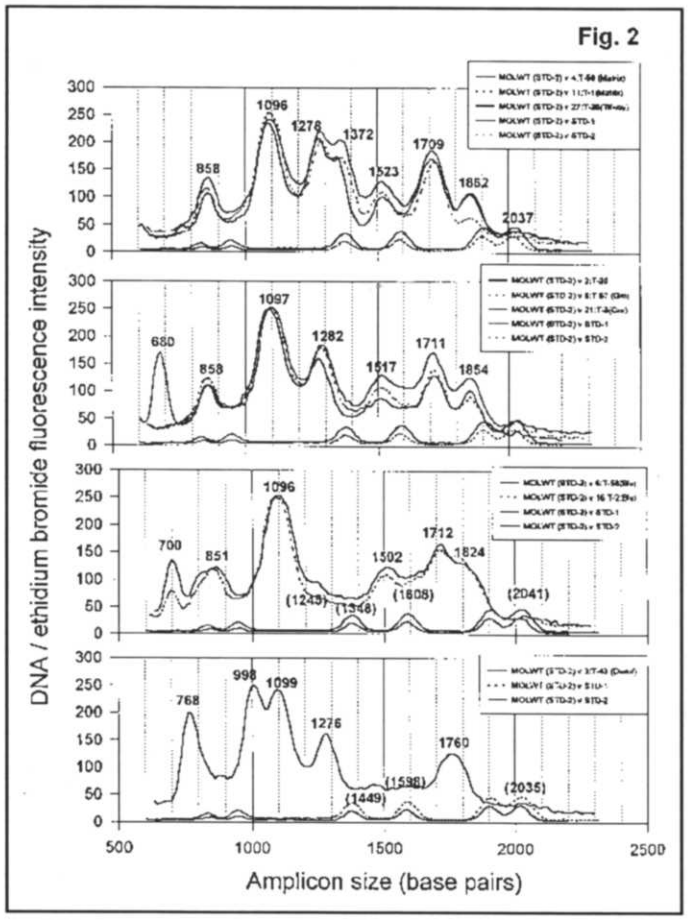

Fig. 2. Comparison of DNA profiles of nine bermudagrasses from PCR amplification with UBC primer 719. Ethidium bromide fluorescence intensity in an agarose gel was resolved at 75 dpi (dots per inch) in a digitized scan of an enlarged photograph, maximizing digital contrast. This is the second amplification in a series, hence the designation 719-2. The profiles of similar grasses (e.g., Tifway = T-35 and matrix grasses T-1 and T-56) are superimposed. Another 17 grasses were indistinguishable from Tifdwarf, thus they are not represented. Towards the bottom of each panel are the profiles of the two molecular weight standards. Amplicon sizes of the sample bands were estimated as a log-quadratic function of migration distance. The function was derived by lest-squares regression of the known base pair sizes of the standards (831, 947, 1375, 1584, 1904 and 2027) on their respective migration distances. All peaks were fitted iteratively using a Gaussian amplitude curve with a smoothing coefficient of 11 (PeakFit, Jandel Scientific, San Rafael, CA).

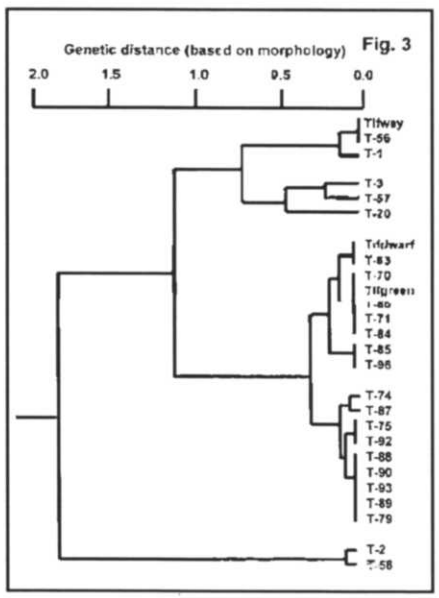

***Fig. 3.** Phenogram of genetic relatedness of 26 bermudagrass samples based on three morphologic traits (stolon thickness, internode length, and number of inflorescences per pot). Cluster analysis was performed on the matrix of Euclidean genetic distances by the unweighted pair-group method (METHOD = AVE, the CLUSTER procedure of SAS 6.03, The SAS Institute, Cary, NC). Bermudagrass samples with a genetic distance close to zero (e.g., Tifway T-1 T-56) were not statistically different. Grasses T-2 and T-58 branched close to the main trunk, which reflects heavy weighting for the prolific seedhead production of those two samples.*

off-type from the other two golf courses. Morphological data (e.g., leaf texture and pollen production) indicated that many other fairway bermudagrass accessions are not Tifway. Clustering based on morphological differences (internode length, stolon thickness, and number of inflorescences) brought unknown fairway bermudagrasses together in plausible groups, consistent with the original field observations (*Fig. 3*). Subsequent DNA profiling of six more fairway variants showed a repetition of similar patterns, which resulted in a clustering of genetic relationships (*Fig. 4*). The results from DNA complemented the morphology.

We found DNA patterns from six primers that may distinguish some greens grasses (including foundation standards, trade types, and off-types). The banding patterns for some primers were unstable among extractions from the same grass, producing spurious results. Only one primer, CG119, showed banding pattern variation among more than three greens grasses. One dwarf-type grass, T-74, was distinguishable, using any of four primers, from Tifdwarf and all other greens grasses. However, this result awaits confirmation at Fort Lauderdale. Therefore, 36 new samples were recollected from the same golf course, in the expectation that we might be able to show the repeated occurrence of T-74 across different greens. While the RAPD patterns for fairway bermudagrasses are strong and consistent, we need to cautiously retest those on greens bermudagrasses, because of repeatability problems inherent in the RAPD method. Among green bermudagrasses, there were differences in stolon thickness ($P < 0.05$) and internode length ($P < 0.0001$). Surprisingly, the foundation Tifdwarf and foundation Tifgreen clustered together, while several trade types and off-types clustered together (*Fig. 3*). These results are very interesting and encouraging.

Possible Significance

While this work is ongoing, several possible conclusions are anticipated:

1. The sparseness of RAPD markers which distinguish among greens bermudagrasses, e.g. Tifdwarf and its off-types, is consistent with their possible origin as point mutations. PCR-based DNA profiling, such a RAPDs markers, may not presently be a practical means of

identification for greens bermudagrasses.

2. Morphological variations were detectable among greens bermudagrasses, at the 0.01% probability level, thus real genetic differences exist. In other words, based on preliminary data, not all products labelled as Tifdwarf are really Tifdwarf.

3. The abundance of RAPD markers which distinguish among fairway bermudagrasses, their contrasting morphology, and the presence of abundant pollen in the off-types, is consistent with their origin as seedling variations.

4 The recurrence of matching genetic off-types on fairways from different golf courses is consistent with their having been planted, not arising after planting. If we can show the same for greens, then it will support the idea of a need for stepped-up quality control in the expansion of plant material.

5. At this time, an absolute assurance of genetic purity does not exist; rather, the Greens Committee should be aware that off-type variations typically are noticed several years after bermudagrass areas are planted, even after the most diligent research by those involved in the purchase of planing material. Once noticed in established playing areas, off-types ten to become more prevalent over time.

6. It may be possible to use this technique to distinguish genetic variants that are superior to Tifway for use on Florida golf courses.

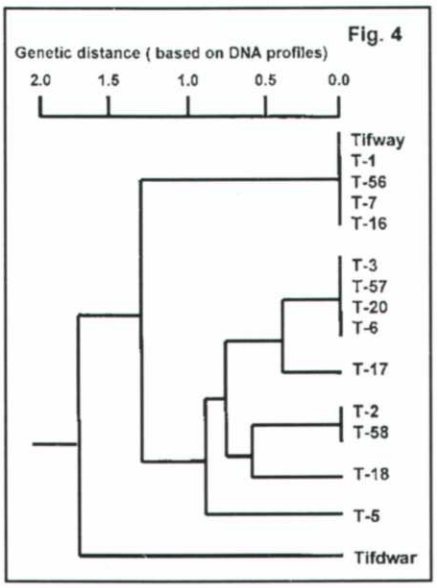

Fig. 4. Phenogram of genetic relatedness of 15 bermudagrass samples based on 25 RAPD markers and five common bands derived from seven primers. A pairwise index of genetic distances was obtained (Nei and Li, 1979) from a presence-absence matrix. Cluster analysis was performed on genetic distances by the unweighted pair-group method. Bermudagrass samples with a genetic distance of zero (e.g., Tifway = T-35, T-1, T-56, T-7 and T-16) were identical for the presence or absence of all 25 RAPD markers. T-17 was relatively close to the cluster of T-3, T-57, T-20 and T-6, differing in the presence or absence of only 3 bands. Bermudagrass samples which branched far to the left of the main trunk (e.g., Tifdwarf = T-43, genetic distance = 1.733) had many (between 12 and 18) band differences from all other samples.

Response of 'Tifdwarf' Bermudagrass to Seaweed-Derived Biostimulants

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Edited by Joel Jackson from a paper accepted by the refereed journal HorTechnology.

Summary. "Tifdwarf" hybrid bermudagrass grown on a putting green in southern Florida was treated for two years with two seaweed-derived biostimulants, Kelpak and PanaSea' Plus. No significant treatment difference were observed in turfgrass quality (44 observation dates) or root weights (eight collection dates) . On only 1 of 22 collection dates for clipping weights was a significant difference obtained among treatments. Although the biostimulants did not enhance plant growth or quality, neither were they harmful to the turfgrass.

The primary concerns of golf course superintendents in southern Florida are the short lengths of hybrid bermudagrass roots and periodic declines in turfgrass quality that can be observed on putting greens year around. Most putting greens in southern Florida are maintained at a height of 4.7 mm or less. This places a stress on the plant since little leaf tissue is present to support photosynthesis. The photosynthetic rate is reduced even further by the reduction in light intensity during overcast, rainy weather typical during summer and fall. As demonstrated for common bermudagrass, low light intensity reduces biomass allocation to rhizomes, an effect that is stronger for short plants, such as those on golf greens. High soil temperatures also increase the shoot:root ratio of bermudagrass. Due to the subtropical climate, the bermudagrass does not become dormant during the winter, but its growth is reduced if extended cool temperature periods occur.

Biostimulants are products that are non-nutritive promoters of growth. Growth can be promoted by stimulating nutrient uptake, chelating nutrients, providing plant growth hormones or enhancing plant hormonal activity. Biostimulants that contain plant growth hormones can be produced synthetically or obtained from natural plant extracts. the latter are primarily obtained for the brown algae family Phaeophyceae, commonly called seaweed or kelp. Applications of seaweed preparations have increased plant growth , including root growth. This plant response is often associated with the presence of plant hormones, but the seaweed extract may also act as a nutrient chelator.

Biostimulants that contain plant growth hormones have benefited cool-season turfgrass under drought stress or salinity stress. They also enhanced growth of creeping red fescue and Kentucky bluegrass seedlings and Kentucky bluegrass sod. Although these biostimulants darken bermudagrass leaf color in temperate climates in the fall, no research has examined the effects of the plant-derived biostimulants on bermudagrass putting greens in a subtropical climate. Our study conducted in southern Florida, evaluated two commercially available seaweed-derived biostimulants, Kelpak and PanaSea' Plus, for their effect of 'Tifdwarf" bermudagrass quality, clipping weight and root weight.

Materials and Methods

A field experiment was conducted from May 1992 through April 1994 at the Fort Lauderdale Research and Education Center on an established 'Tifdwarf' bermudagrass research golf green built with a root-zone ix containing 80% sand and 20% Canadian spaghnum peat moss. The area was vertically mowed and topdressed approximately once per month, with the depth of vertical mowing depending on thatch layer thickness. Topdressing material was the same 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.

The area was fertilized every two weeks using a fertilizer blend containing IBDU™, potassium magnesium sulfate, iron sulfate and manganese sulfate. For both nitrogen (N) and potassium (K), a total of 879 $kg \cdot ha^{-1}$ of each nutrient was applied per year. Phosphorus was applied twice each year at 122 kg $P \cdot ha^{-1}$ per application. This is similar to the average fertility program used in southern Florida.

Treatments included an untreated control, Kelpak applied at 6 wk intervals and PanaSea' Plus applied at 2 wk intervals and at 4 wk intervals. Kelpak contains 0.3N:0.7P:0.6K and is derived from the brown alga Eclonia maima from which several indole compounds have been identified. PanaSea' Plus contains 0.2N:1.3P:1.7K and is derived from numerous seaweeds including Laminaria spp., Chondrus crispus, Porphyra spp., and Ascophyllum nodosum. Both products are liquids.

Biostimulants were applied according to the manufacturers' directions. The first Kelpak application was made as a drench with 1.4 $ml \cdot mm^{-2}$ Kelpak applied in 500 $ml \cdot mm^{-2}$ deionized water; all subsequent Kelpak applications were made as broadcast sprays at 0.3 $ml \cdot mm^{-2}$. PanaSea' Plus treatments were applied as broadcast sprays at 1.3 $ml \cdot mm^{-2}$. Broadcast sprays of both biostimulant products were made in 100 $ml \cdot mm^{-2}$ deionized water. Each plot was 2 m x 3 m with four replicated per treatment in a randomized complete-block design.

Turfgrass quality ratings were deter-

mined based on observation of grass color and density using a scale of 1 to 10 with 10 representing turf with a dark green color and uniform dense stand. The plots were rated approximately every two weeks, one week after each nitrogen application.

Turfgrass clippings, from plots that had not been cut for 48 hr, were collected once each month from a 1 m^2 area in the center of each plot. Clippings were dried at 60C for 72 hr and then weighed. When possible, we tried to rate and collect clippings on the same day or subsequent days.

Root weights were obtained every three months. At each sampling date, two 15 - cm diameter by 10 - cm deep samples were obtained from each end of each plot for a total of four sub samples per pot. Root samples were not collected randomly with the plot because samples collected from the center would have resulted in voids that would have interfered with clipping weight evaluations. A 1.25 cm cap was cut from the top of the sample and then discarded to remove leaf tissue and the majority of the thatch layer. Samples were then processed with a commercial root washer using 760 µm primary and secondary sieves. The accumulated material was dried at 80C for 36-48 hr and then weighed. Weights from the four sub samples of each plot were added together to obtain the total weight per plot. Resulting "holes" from sampling were filled with topdressing material.

Data were analyzed using the ANOVA procedure; the Waller-Duncan k-ratio t test was used to separate means.

Results and Discussion

Quality ratings were obtained on 44 dates. Except for eight dates, there were no quality differences among any plots of any treatment; the entire experimental bermudagrass area was uniform in color and density. Differences among treatments for those eight dates were not significant (data not shown). Clipping weights were collected on 22 dates, but significant treatment differences were obtained only on 27 January 1993 (Table 1). However, there were no quality rating differences among any plots of any treatment on that same date. Root weights were collected on eight dates, and no significant differences were obtained among any treatments on any date (data not shown).

Although other research has demonstrated that turfgrasses respond best to hormonal biostimulants when the turf is under environmental stress, no benefits were observed in our experiment during stressful periods such as extensive rainfall or cool temperatures. For example, 112.5 cm rain was received between 1 June 1993 and 31 October 1993. The turfgrass quality gradually declined during this time period, but no quality rating difference were observed among any treatments.

Researchers who have worked with both cool-season and warm-season turfgrasses have indicated that warm-season turfgrasses do not respond to hormonal biostimulants as well as cool-sea-

Clipping weight (grams)[z]

Application		1992						1993			
Treatment[y]	Interval	29 July	27 Aug.	23 Sep.	28 Oct.	27 Nov.	30 Dec.	27 Jan.	24 Feb.	7 Apr.	21 Apr
PanaSea Plus	2 weeks	7.47	5.20	5.07	1.84	4.10	4.25	2.71 a	3.18	6.42	4.65
PanaSea Plus	4 weeks	8.09	5.45	5.00	1.71	4.22	3.47	2.74 a	3.17	6.05	4.08
Kelpak	6 weeks	7.59	6.18	5.79	1.63	4.00	3.78	2.28 b	2.84	6.27	4.30
Control		7.28	5.47	5.23	1.59	4.19	3.96	2.65 a	3.28	6.62	4.58
Pr>F		0.89	0.43	0.51	0.47	0.96	0.23	0.05	0.84	0.88	0.84

[y]PanaSea Plus application rate was 1.3 ml·m^{-2}; Kelpak application was 0.3 ml·m^{-2}

[z]Values are means of four replicated plots. F values were too small to conduct mean separation teston al dates except 27 January 1993. Mean separation for that date by Waller-duncan k ratio t test (P=0.05)

Table 1. *Effect of Seaweed-derived biostimulant application on 'tifdwarf' bermudagrass clipping weights during the first year of the study (May 1992 through April 1993)*

son turfgrasses, and that the responses are highly variable. In our experiment, a consistent lack of response was observed over the two year study period. Although the seaweed-derived biostimulants did not enhance plant growth or quality, neither were they ever harmful to the turfgrass. Before a golf course superintendent applies these products to all of the putting greens on the course, it would be advantageous to only treat half of two or three greens to determine if a response will be observed. This would save both time and money if there is no response.

Acknowledgements

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