



Dark green genetic off-type (T-3) appeared in patches on a Florida fairway in 1993, 2 years after Tifway bermudagrass was supposedly planted. The surrounding matrix grass (T-1) is yellowish green.

**Golf course professionals have long been confronted with the problem of off-types in greens and fairways**

# DNA

BY PHIL BUSEY, CHARLIE GUY, AL DUDECK  
AND NIGEL HARRISON

There is a world with perfect lawns in H.G. Wells' *Time Machine*. Time Traveler found the future "air was free of gnats, the earth from weeds or fungi; everywhere were fruits and sweet and delightful flowers..." Predicting advances from technology, Wells pointed out that in the early 1900s nature was "...shy and slow in our clumsy hands. Some day all this will be better organized."

Wells' imaginary future was not altogether desirable. But for better or worse, unprecedented biological change is already here. This article answers basic questions about DNA technology, focusing on DNA in bermudagrass fingerprinting. We'll show that DNA fingerprinting can provide quality assurance, by supporting the certification of Florida bermudagrass planting stock. First let's look at the problem of off-type bermudagrasses.



Dark green off-type (T-3) grown in a container differed morphologically from Georgia foundation Tifway' (T-28), which was yellowish green. T-3 also had abundant inflorescences and pollen, while 'Tifway' did not. Matrix grass (T-1) could not be distinguished morphologically from 'Tifway'.

# Bermudagrass Fingerprinting

## The problem: off-type bermudagrasses

Golf course professionals have long been confronted with the problem of off-types in greens and fairways. Off-types are genetic variants, generally of unknown origin. In a 1975 article, "What is happening to our bermudagrass?" Monty Moncrief recognized the occurrence of off-types. The complaint 20 years ago was that hybrid bermudagrass greens (i.e., Tifdwarf and Tifgreen) were showing patches with different characteristics. Ever since, a growing demand for faster greens, higher number of rounds and better year-round color have made these irregularities more conspicuous. Excessively close mowing may have further exposed genetic variants that would not be obvious at a higher cut. Rising expectations are as much behind the problem as the fact that we are growing grasses developed 35 years ago (i.e., Tifdwarf, Tifgreen and Tifway) that were never meant to be stressed in the way they are today.

Is this really a problem? Yes, it is a serious problem. Tifway and Tifdwarf

Rising  
expectations  
are as much  
behind the  
problem as the  
fact that we are  
growing  
grasses  
developed 35  
years ago

# DNA fingerprinting is any method for identification or comparison based directly on an organism's DNA

(305) 581-0444

**SPREAD-RITE, INC.**  
CUSTOM SPREADING  
FERTILIZER • DOLOMITE • ARAGONITE

LAMAR SAPP

6001 S.W. 19th STREET  
PLANTATION, FL 33317  
MOBILE: (407) 479-9417



## TESTING

- Soils
- Water
- Physical
- Tissue
- Environmental

**Tom Burrows**  
Certified Agronomist  
Turfgrass Specialist

Phone: 407-692-1221  
1741 San Souci, Stuart, FL 34994

CGCS  
Golf Course Supt. - 29 years  
FGCSA Past President  
Penn State - 1960  
Musser Int'l Turf Foundation - VP

are still the most serviceable grasses for Florida golf courses. When planted successfully in pure stands, these grasses are responsive to maintenance and play. If the thousands of dollars spent on golf course operations are applied to pure Tifway and Tifdwarf, then consistently high density and wear tolerance, uniform color and predictable ball roll result. In contrast, the presence of genetic mixtures results in excess maintenance costs, disappointed expectations of golfers and serious challenges to the credibility of golf course superintendents. If you are growing the wrong grass, there's no telling how much money you might throw at it and still not have a golf course on par with the one down the street.

### Where do off-types come from?

Amazingly, we don't know where off-types come from. It is reasonable to presume that some off-types originate from contaminated sprig stock used to plant golf courses. Other off-types may be spontaneous mutations. They and seedling of *common bermudagrass* might also be redistributed on golfers' shoes, golf cart tires and mower parts. Nevertheless, and despite much speculation (Table 1), we

still do not know where the off-types come from, how they may be prevented or if they can be prevented. DNA fingerprinting offers us the potential to understand the problem and solve it.

### What is DNA fingerprinting?

DNA fingerprinting is any method for identification or comparison based directly on an organism's DNA (*See related story, "What is DNA?," Page 64*) Later we will discuss different DNA fingerprinting approaches, such as RFLPs and PCR profiling. The purpose of any technique is to tell whether a grass source is what it is claimed.

### How can DNA fingerprinting help?

Golf course facing reconstruction, replanting and new construction have quandaries such as, "How do we know we are getting clean planting stock?" and "How do we know we're going to remain clean over the next five years?" DNA fingerprinting is offered as a key to understanding the problem, and it may provide a solution. This is because DNA fingerprinting can be used as a quality control step in the production and distri-

YOUR "ONE-STOP" SOURCE  
FOR AMERICA'S LEADING  
GOLF SURFACES & SUPPLIES!

**PARTAC®**  
GOLF COURSE  
TOP-DRESSING



HEAT TREATED  
FLEXIBLE GOLF COURSE MARKERS  
TURF DRAG MATS & BRUSHES  
SAFETY FENCING & BALL NETTING

**TYPAR®**

GEOTEXTILES & TURF BLANKETS

**Terra-Green®**

THE POROUS CERAMIC  
SOIL CONDITIONER

NEW **PARTAC®** NEW  
COLORED CERAMIC  
**GREEN SAND**

**DIVOT REPAIR MIX**  
PARTAC® GREEN SAND BLENDED  
WITH PARTAC® PREMIUM  
TOP-DRESSING FOR EXCELLENT  
GERMINATION AND  
DEEP GREEN COLOR!

THE BEST DIVOT REPAIR MIX AVAILABLE!

AVAILABLE NATIONWIDE  
**800-247-2326**  
IN N.J. 908-637-4191

PARTAC PEAT CORPORATION  
KELSEY PARK, GREAT MEADOWS, N.J. 07838  
ORLANDO (FL) MEMPHIS (TN) RENO (NV)

HIGHLIGHT GOLF HOLES,  
IMPROVE VISIBILITY,  
& MAINTAIN CONSISTENCY

U.S. GOLF HOLE  
**TARGETS™**



**ACCU-TECH™**  
PRECISION CUP CUTTERS

**TerraFlow™**  
Drainage Systems

AND MANY MORE  
GOLF SPECIALTY PRODUCTS

# *The advantage of DNA testing over other methods is its capability of being faster and more accurate*

bution of planting stock. It makes sense that this be done on a statewide basis before the grass ever hits the market as a part of certification. Otherwise, each superintendent and greens committee is left to do it piecemeal.

If DNA testing can show a prospective planting stock to be authentic, then this is extra assurance that it will perform predictably. (In practice, one would use DNA fingerprinting to reject rather than accept planting stock.) DNA fingerprinting is appropriate because it has great power to distinguish genetic off-types. DNA fingerprinting is so powerful that it gives scientists a way to quantify degrees

of relationship. This is helpful in determining whether off-types are mutations or seedling variants. The advantage of DNA testing over other methods is its capability of being faster and more accurate than other methods. As we will explain later, however, a sound program needs to be three-pronged, considering chromosomes, morphology and DNA.

Despite the capabilities of new technology, nobody should promote DNA fingerprinting as a cure-all. Even in this new age, Monty's excellent practical recommendation, "constant surveillance is necessary," is good advice.

## **Is DNA fingerprinting a specific method?**

No. There are two general approaches, RFLPs and PCR profiling. RFLPs (restriction fragment length polymorphisms) analysis is more precise, but usually more expensive. They work directly with specific regions of an organism's DNA. In contrast, PCR approaches usually amplify random regions of the DNA, using the Polymerase Chain Reaction (PCR). This lab procedure copies minute traces of DNA to produce 2, 4, 8, 16, 32, 64, 128 and eventually millions of copies. So much DNA is made that it is readily visible in the presence of fluorescent stain.

## Storage Tanks from A to Z



Orchid Island G.C.

- \* *"Get a good, reputable tank company . . ."*
- \* *" . . . Knowledge and guidance ultimately saved me \$30,000."*  
Scott Bell, Bent Pine G.C.
- \* *"First Rate. Couldn't have asked for more."*  
Jack Orr, Orchid Island G.C.

- Underground Tank Removals
- Aboveground Tank Installations
- Remedial Actions
- Statewide Service

### **Brevard Oil Equipment, Inc.**

1557 Cooling Avenue  
Melbourne, Florida 32935

800-472-7670 • (407) 259-4181

***"The Golf Course Specialists"***



*Stoloniferous off-type (T-75) surrounded by matrix grass (T-74) on a Florida green. Both T-74 and T-75 differed in DNA profile from Georgia foundation 'Tifdwarf' and from one another.*

**Often, to detect the small amount of DNA, the visualization process uses radioactive isotopes**

Both RFLPs and PCR profiling methods produce banding patterns, much like the bar codes used at the supermarket check-out. Characteristic differences in banding patterns tell two grasses apart.

#### **How does DNA fingerprinting work?**

Both RFLPs and PCR approaches are founded on recognition sequences contained in the organism's DNA. (See related story, "What is DNA?" Page 64) A recognition sequence is a region of DNA with a particular sequence of nucleotides, much like a signature or a pass code. Whereas RFLPs use both enzymes and complementary DNA sequences to do the recognizing, PCR methods use only the complementary property of DNA itself to do the recognizing.

For RFLPs, each enzyme used recognizes only a particular DNA sequence, and then cuts the DNA fragment at that point. For PCR, recognition occurs during the amplification, or copying, of DNA, in which specific primers are used to start the process of copying DNA. Primers

bind specifically with "complementary" sequences. Each method produces characteristic fragments of consistent length, using recognition sequences at each end of the fragment. The recognition sequences are sometimes portrayed as "bookends."

#### **Is one method of DNA fingerprinting best?**

No. The usefulness of a particular DNA fingerprinting method is based on its cost, reproducibility and power to discriminate. RFLPs involve a hybridization process that is laborious and requires a high level of technical skill. Often, to detect the small amount of DNA, the visualization process uses radioactive isotopes that require safety containment. In contrast, PCR is rapid, requires a lower level of technical skill and does not require the use of radioisotopes.

Recipe improvements in PCR profiling are constantly being made, and their use depends on the situation. The first two documented methods were RAPD (random amplified polymorphic DNA)



Repeating occurrence of bermudagrass genetic off-types on a Florida green reduced the putting quality.



## **COMPUTER-EXACT CUSTOM MIXES.** **EVERY ORDER. EVERY TIME.**

Every order received by **Golf Agronomics** is prepared in our fully computerized machine. Double screening capabilities ensure that your first mix portion is the same as your last.

**Golf Agronomics** meets all your top dressing needs with its ability to customize your mix with a variety of soil amendments

including: Dolomite • Hi-Cal Wetting Agents • Charcoal Humic Acid • Minor Elements Rock Phosphate • Gypsum and Customer products.

**Golf Agronomics** donates a % of every ton of top dressing sold to the FGCSA. Since its opening, **Golf Agronomics** has donated in excess of \$17,000. Our sincere thanks to all of our customers.



(800) 626-1359  
(813) 955-4690 Fax  
P.O. Box 3455  
Boynton Beach, FL 33424

# *An important safeguard is blind testing, where the scientists do not know the true identities of the material being evaluated*

**Scientists are still making improvements to this new approach of DNA fingerprinting.**

and AP-PCR (arbitrarily primed PCR), both from 1990; another method, DAF (DNA amplification fingerprinting), was introduced in 1991. The main characteristics distinguishing the methods are the length of the primer, the primer concentration, the choice of gel and method of staining for displaying banding patterns. Some methods borrow aspects from other methods.

Scientists are still making improvements to this new approach of DNA fingerprinting. Presently there is no universal standard for PCR profiling. Typically, a particular laboratory uses only one method. Consequently, there are few objective comparisons among methods. The goal of choosing a method is to produce powerful, repeatable results from the same plant materials. So, in adopting a PCR profiling method, it must be shown to work in different laboratories. Internationally, most scientists use an adaptation of RAPDs. During 1994 and 1995, according to a major literature citation service, there were over 350 publications citing the use of RAPDs.

## **Are there pitfalls?**

Yes, several. A comparison is only as good as the sample, and only as good as the standard with which it is compared. If only one sprig was sampled, it might check out to be the expected grass, but the entire remainder of the field could be a contaminant. To be 100% sure, each of the million of sprigs in the field would have to be DNA fingerprinted. If one wanted even 95% certainty that the sprig field source was 95% pure, it would require 59 DNA fingerprints, cumulatively costing about \$20,000 at current prices.

Costs could be reduced by various means. Sample pooling might help sometimes, but excessive pooling would reduce the sensitivity of the test, creating confusion. A thorough look at the genetic makeup of a field of grass is costly beyond imagination, so DNA fingerprinting must be used judiciously. Morphological markers and chromosome counts are less costly, and are therefore the front lines for off-type detection. The hybrid bermudagrasses are all supposed to have 27 chromosomes, while common bermudagrasses (in-

cluding most seedling variants) have 36 chromosomes.

## **What safeguards are needed?**

An important safeguard is blind testing where the scientists do not know the true identities of the material being evaluated. No procedure is 100% reliable, either because of inconsistent chemical reactions, human error or unexplained sources of variation. Another safeguard, mentioned previously, is to effectively repeat a procedure in different laboratories, and get the same results.

Standards are another sensitive issue, because it must be shown that the source standard (either breeder's or foundation stock) is itself consistent. Besides, the same standard should be used every year. There is currently no single national repository for maintaining the source material of released cultivars. At the University of Florida we are carefully maintaining source materials, which we have propagated from a single sprig.

## **How might DNA fingerprinting provide a research solution?**

The first step is to document, through scientific publication, that a problem exists. This has never been done!

Next, DNA fingerprinting, in combination with morphology and chromosomes, should be used to discover the variation among bermudagrass off-types. By systematic procedures, we can test alternate hypotheses for their origin (Table 1). If we judge that off-types occur commonly as spontaneous mutations, then golf course superintendents and greens committees will be so informed.

If recurring mutations are the source of the problem, then they may not be preventable. This is probably not so, however, because there exist 30-year-old greens showing no evidence of off-types.

## **How might DNA fingerprinting provide a solution for the golf course?**

DNA fingerprinting should be considered first as a tool for certification of source material, secondly for the validation of a grower's field and as

# DNA fingerprinting can be a helpful tool in certification

a last resort for the golf course. While there is legal rationale for DNA fingerprinting a golf course, this will not correct the source of the problem. Without certification and other quality control efforts, the issue of off-types will continue to be debated and unresolved. The solution is for turfgrass certification inspectors to use chromosome counts, morphology and DNA testing as a routine quality control step, before grass is put on the market.

## What can we do meanwhile, before research is completed?

Four things: references, accountability, personal inspection and performance. (1) Request a list of 1-3 year-old plantings from prospective grassing contractors. (2) Request written documentation on where the source grass originated. (3) Personally inspect prospective source fields, hopefully having the opportunity to look at areas where the grass has been mown closely over sev-

eral months. (4) Include appropriate performance specifications in the bid with timelines for inspection of quality and consistency and an appropriate remedy (hence, a performance bond.)

## Conclusions

DNA fingerprinting can be a helpful tool in certification, if the technology is powerful and repeatable for bermudagrass off-types. DNA fingerprinting would help ensure the consistency of source plant material and sprig stock, and it can be used with chromosome counts and simple morphology.

It is important to show that the technique really works on bermudagrass off-types, and to document the presence and nature of off-types. This has never been done. We believe it will work. We also believe that it will give us a treasure of practical information on how to maintain the purity of improved bermudagrass.

**While there is legal rationale for DNA fingerprinting a golf course, this will not correct the source of the problem**

## Duststorms Got Members Trading Carts For Camels?



After you've done everything else right, why take short-cuts on course maintenance with ag-grade fertilizers? Sunniland's custom blends are formulated specifically for your course. Uniformly granular, too, eliminating hazardous dust on the course, your equipment, pools and members! The course will look superb and so will you. We knew you wouldn't mind if we cleared the air on course maintenance, so phone our Sunniland Golf Products Representative today.



Post Office Box 8001 • Sanford, Florida 32772-8001 • Phone (407) 322-2421 or (800) 432-1130



**Table 1.**

Examples of hypothetical origin and genetic nature for off-type bermudagrasses. There is not a single instance in which any of these hypotheses has been either proved or disproved. Some suggested explanations are more probable than others.

ORIGIN	GENETIC NATURE		
	MUTATIONS	SEEDLINGS	CONTAMINATION
History of Planting Source;	Spontaneous mutation occurred once in breeder's plots, 35 years ago, and no effort was subsequently made to repropagate from a single sprig;	Bermudagrass seeds blew in from a pasture to the certified sprig field and seedlings were unknowingly propagated;	Sprig grower allowed runners of different cultivars to creep across ditches separating adjacent fields; fields were mown high, and this variation went undetected
Golf Course Construction	Dozer operations caused mechanical stress on underground bermudagrass rhizomes, causing them to mutate into new genetic variations	A little bird flew over the golf course one day, dropping a seed. A few weeks later, the seed germinated, forming a plant which eventually took over the golf course	Fumigant was unable to penetrate deeply enough to kill dormant rhizomes of 'Common,' because a high organic content tied up MeBr near the surface
Planting and Grow-in	An herbicide was used which is known to be a mitotic arrester, thus potentially mutagenic; a combination of chromosome breakages and endopolyploid cells resulted in new genetic variations	'Common' bermudagrass seeds were tracked in from adjacent rough; because soil was primarily bare, these had an opportunity to germinate and become established	Planting crew used the same truck to haul 'Tifdwarf' and 'Tifway' sprigs, without cleaning between loads. 'Tifway' was inadvertently carried over in the lot of 'Tifdwarf.'
Golf Course Operations	Greens were exposed to UV light, which caused turf cells to mutate spontaneously	In a one-in-a-million occurrence, 'Tifway' produced an unreduced gamete, which was fertilized by pollen from 'Common' bermudagrass growing along an adjacent highway	Golfers, golf car tires or mower parts carried seeds or sprigs from rough; cup cutter inadvertently redistributed contaminants

## What is DNA?

DNA is the blueprint for all life forms, from animals to plants. As a script tells the actor or actress what part to play, DNA tells the organism what it will be: animal, plant, microbe. DNA differences between two organisms can be small or

large. This explains how siblings can be similar, yet not the same. DNA differences also explain how a virus can produce another virus, and an elephant can produce another elephant. ➤

### So DNA is genes?

No, not entirely. Most DNA in plants and animals has no essential purpose in the organism. Most DNA is either repetitive script, or a series of scrambled messages that are no long part of the required genetic script. Sometimes this extra DNA is called *junk DNA*.

### How is DNA organized in the cell?

DNA is a linear text, packaged into chromosomes. Because the DNA molecule is a double helix, there are two strands. One strand is the *code*, which tells the cell what to do, and makes you you. The other strand is a *complementary image*. Both strands are copied to produce two new double-stranded molecules. The copying of DNA is an exact process, due to the exact manner by which two strands bind to one another. This exactness is called *complementarity*, and it results because subunits bind only with certain other subunits.

### How does the cell read and write the DNA text?

As with any language, there are subunits. The basic subunit for talking the language of DNA is the nucleotide. Although DNA has only four kinds of nucleotides, they can be arranged in a multitude of combinations. A few thousand nucleotides are enough to blueprint an important kind of chemical in the cell, a protein. Different kinds of proteins serve as enzyme catalysts and provide other vital functions in the cell. Since human DNA is composed of a sequence of three billion nucleotides, our DNA text easily blueprints the tens of thousands of enzymes needed to create each of us.

### How does knowing the genetic code help identify grasses?

It doesn't. For identifying grasses, the actual function of DNA is pretty much irrelevant. We treat DNA as if it were just like a fingerprint, something for which it doesn't matter if there is a purpose. Some DNA fingerprinting procedures use DNA that is known not to be part of a gene. Why? Because there are lots of those sequences, their degree of repetition can be characteristic of a particular individual, and these sequences show more natural variation than is normally found in essential genes.

## ALGAE ON TURFGRASS A PROBLEM?

TRY



### TRIPLE ACTION 20

E.P.A. REGISTERED ALGAECIDE  
AND FUNGICIDE FOR TURFGRASS

- EFFECTIVE • ECONOMICAL
- CURATIVE • BIODEGRADABLE

For a Free Sample Write or Call:

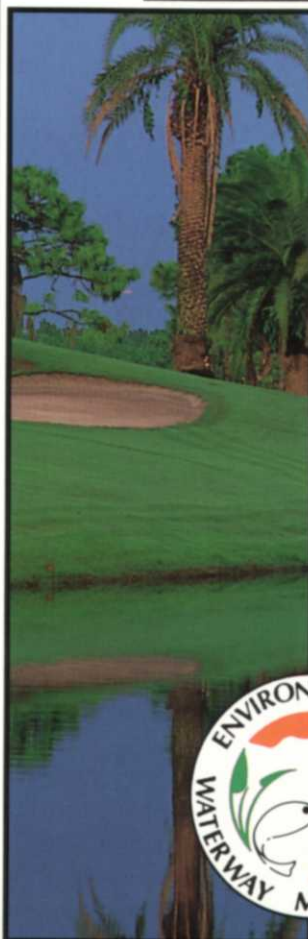


PARKWAY RESEARCH CORPORATION

1-800-256-FOOT

13802 Chrisman Rd.  
Houston, Texas 77039

713-442-9821  
FAX 713-590-3353



### Environmental Waterway Management...

## "Preserving Your Liquid Assets"

Golf Courses have Lakes, Ponds, Canals and Water Everywhere! Many of these water bodies are used for irrigation, fishing swimming, drainage or simply as hazards to the navigating Golfer on your course. If you allow Algae, Grasses and other noxious weeds to grow unchecked, they will rapidly take over your precious waterways. Don't allow your waterway problems to get you in over your head. Just call the experts at Environmental Waterway Mangement.

