## Genes, Guns, and Turf

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Fill up the tank with Roundup, make a pass over the putting greens, and no worries about *Poa annua* or other weeds. Sound too good to be true? In this case, it might not be.

The first genetically altered turfgrass was transformed about five years ago. Plant transformation can be a tricky and expensive business. Here's how it works in turf and most other monocots: Seed or other plant tissue is placed on a plastic dish (Petri plate) filled with a Jello-like growing medium containing a specific mixture of hormones (auxins and cytokinins). These cells grow and form an amorphous mass of undifferentiated cells called callus (the process called tissue is culture). Microscopic gold or tungsten particles are coated with DNA containing the genes of interest, and fired into the callus from a 22-caliber shell using a device popularly referred to as the "gene gun". One of the genes is likely to be a "selective marker", the other a gene for a desired trait such as herbicide tolerance or drought resistance. The genetic material will be successfully integrated into only a few of the cells (i.e., transformation). The treated callus is placed onto another Petri dish containing a compound that will selectively kill those cells or callus that have not been successfully transformed. The survivors are those into which the genetic material has been successfully placed. These callus are regenerated into plants by growing them on plates of more hormonecontaining media. When large



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enough, the plants are placed in soil and transferred to the greenhouse and/or the field. Here they can be used for breeding programs or for seed production and testing.

Another method for producing genetically modified plants is protoplast transformation. The plant cell walls are digested with enzymes (e.g., cellulases). The protoplasts are incubated with DNA in a glycol:buffer solution. The glycol helps the protoplasts absorb the DNA. Short, intense pulses of electricity (electroporation) are sometimes used to form tiny holes in the protoplast membrane and facilitate uptake of the DNA. The transformed protoplasts are regenerated into callus with the rest of the process being similar to that explained above.

Other transformation processes exist and have been used with great success for other crops. The most common procedure is to insert the genes of interest into Agrobacterium tumefaciens, a bacterial pathogen that causes crown gall on dicots (trees, tomatoes, etc.). The A. tumefaciens is first disarmed by removal of the DNA segment that causes the gall. This method is not used in turf because the bacterium does not infect monocots. A related organism, A. rhizogenes, has been tried with limited success.

The most difficult aspect of genetically modifying turfgrasses from a scientific standpoint has been regenerating the plants using tissue culture methods. The media contain many different ingredients (sugars, salts, hormones) and minor changes cause great effects. In a symposium held at Michigan State University just a few years ago on biotechnology in turfgrasses, many of the presentations focused solely on methods to regenerate turfgrass plants using tissue culture.

One of the most successful scientists was Dr. Lisa Lee, then at Rutgers University. By 1996 she and her colleagues had published research papers on genetic modification of several bentgrass varieties (Lee et al., 1996; Lee and Day, 1998). The main focus was inserting a piece of DNA termed the *bar* gene. This gene conferred resistance to an herbicide then known as bialophos (now termed glufosinate). We know it as the active ingredient in the herbicide Finale.

The O.M. Scotts Co. in Marysville, OH, could arguably be called the "godfather" of the turf



## GAZING IN THE GRASS

seed and fertilizer industry in the U.S. About the time Dr. Lee was learning how to transform bentgrasses, O.M. Scotts was negotiating with Monsanto Corporation for rights to use the gene for Roundup resistance in turfgrasses. While eventually successful, they also needed the ways and means to transform the plants. They needed the gene gun.

Sanford Scientific owned the patent rights for the gene gun. Additional negotiations and a partnership with Sanford Scientific provided O.M. Scotts Co. with exclusive access to use the gene gun for turfgrass transformation. They now had the gene and the means to transform the grass. One final piece of the puzzle remained, or so it seemed.

To put the final piece of the puzzle together, O.M. Scotts needed someone who knew how to use the gene gun and how to regenerate turfgrass plants using tissue culture. They were able to hire Dr. Lee, and for a moment the sun shined brightly. Transformations went well. O.M. Scotts will likely have Roundup-ready creeping bentgrass for sale by 2001. Roundup-ready Kentucky bluegrass could be here within the next three to five years. But since then a few clouds have rolled onto the horizon. Government regulations require compliance. Part of the problem is that since genetic transformation of plants is a relatively new social and economic force, the regulations are still evolving. Government agencies are still winnowing and sifting through their territories, attempting to define who has what jurisdiction. and when. Public antipathy has reared its head, with vandals striking at groups who conduct research attempting to address the safety of genetically modified organisms. Other issues such as the potential development of herbicide resistance in *P. annua* populations are starting to surface. Rumors abound about other transformations: 1) "Scotts has transformed turfgrasses with the luciferase (firefly) gene so turfgrasses can glow in the dark, allowing golf to be played at night", or 2) "Scotts will soon be marketing turfgrasses to homeowners in designer colors-red, orange, blue, etc.". These rumors are false. In order to deal with public misunderstanding, all of us must be prepared and knowledgeable to utilize this new technology of the 21st century.

Authors note: The idea for this article came to me as I watched a class of turf students attending a lecture on biotechnology in turf. Many of the students, top students for the most part, struggled to keep pace with the lecture. Some seemed to have difficulty comprehending the basic subject matter (many college students, especially potential legislators in the arts and sciences, do not take a single genetics course). This bothered me as it is often up to golf course superintendents to speak up for turf management issues in the public eye. The availability and use of genetically modified turfgrasses will be no exception and may even be more contentious than environmental issues. *Clearly an understanding of the* technology is needed. Our department teaches a "Survey of Horticulture" course for nonmajors and attracts many nonscience students. The curriculum now includes content to help them appreciate the aspects of genetic engineering. Will it be too little, too late?

*Next issue:* The promises, pitfalls, and ethics of genetic transformation of turfgrasses.

## Literature Cited

Lee, L., C.L. Laramore, P.R. Day, and N.E. Turner. 1996. Transformation and regeneration of creeping bentgrass (*Agrostis palustris* Huds.) protoplasts.

Lee, L. and P. Day. 1998. Herbicide-resistant transgenic creeping bentgrass. *In* M.B. Sticklenand M.P. Kenna (eds.) Turfgrass biotechnology: Cell and molecular genetic approachesto turfgrass improvement. Ann Arbor Press, Chelsea, MI.

