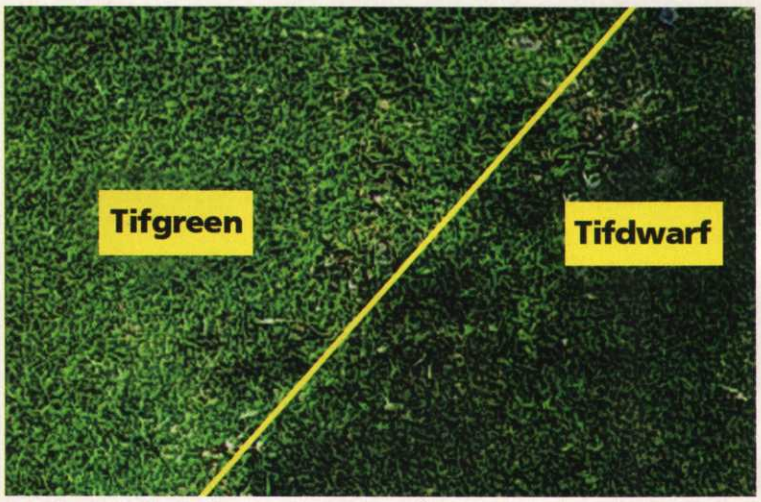
Ultradwarf Bermudagrasses Exhibit Easy Mutation Tendencies

By Patrick McCullough, Bert McCarty, Vance Baird, Haibo Li and Ted Whitwell

Part 2: Do certain pre-emergent herbicides exacerbate this problem?

In Part 1 of this series, the natural genetic instabilities of dwarf-type bermudagrasses were discussed. Golf courses renovating to the new and improved ultradwarf bermudagrass cultivars hope to avoid previous problems of off-types developing five to 10 years after planting. Obviously, one would want to know management practices that could advance or, more importantly, prevent off-type contaminations.

Because DNA replication and chromosome separation are fundamental parts of mitosis, cell-division-inhibiting herbicides and plant growth regulators (PGRs) have the potential to disrupt genetic replication and even damage DNA sequences, exacerbating bermudagrass genetic instabilities and resulting in off-type mutations.



Tifdwarf bermudagrass (right) is probably a vegetative mutant from Tifgreen bermudagrass.

Currently, no herbicides or PGRs are labeled for use on the new ultradwarf bermudagrasses, and concerns exist for incorporating these compounds into management programs.

Turf discoloration and negative rooting responses from herbicides and PGRs may limit their potential for routine ultradwarf bermudagrass maintenance.

Herbicides may cause bermudagrass mutations

Pre-emergent herbicides for summer annual weeds are applied before soil temperatures become favorable for seed germination 54 degrees Fahrenheit (F) to 64 degrees F during spring months. Consequently, timing of spring pre-emergent applications coincide with bermudagrass root regeneration and emergence from winter dormancy.

Safe application of pre-emergent herbicides on dwarf bermudagrass is critical since roots are most vulnerable to herbicide injury during maximum root regeneration in the spring (Engel and Ilnicki, 1969). Additionally, pre-emergent herbicides concentrated in the surface layer of the soil could expose dwarf bermudagrass roots to initial and residual herbicide effects during critical root-growth initiation.

Pre-emergent herbicides affecting mitosis and cell division are commonly used on bermudagrass golf courses.

At one time, approximately one-quarter of all herbicides marketed affected mitosis as a primary mechanism of action (Vaughn and Lehnen, 1991). These herbicides, including dinitroanilines and pyridines, disrupt the nuclear division sequence by interfering with microtubules responsible for chromosome mobilization during mitosis (Ross and Lembi, 1999). Herbicides that prevent mitotic spindle formation by slowing or preventing the assembly of microtubules stunt new cell production, causing the replicated chromosomes to remain unseparated and eventually become enclosed by a nuclear envelope, leaving a polyploidy cell (Ross and Lembi, 1999). This mechanism of action is effective for pre-emergent control of germinating weed seedlings.

However, with the sensitivity of ultradwarf bermudagrasses to herbicides, concerns exist with root-growth inhibition, turf injury and potential genetic alterations from cell division interference.

The dinitroaniline herbicides, oryzalin and

pendamethalin, were recently studied by Goatley et al. (2003) on Champion, TifEagle, Floradwarf, MS-Supreme, Tifdwarf and Tifgreen bermudagrasses. Dinitroanilines are commonly used as pre-emergent herbicides in golf course management for annual grassy weed control. The principle effects of dinitroanilines are mitosis interference and prevention of normal cell wall/plate formation and root development (Callahan, 1994).

Because somatic mutations occur during cell division, the scientists analyzed phenotypic stabilities of these six bermudagrass cultivars during repeated cycles of grow-in with and without chronic exposure to dinitroaniline herbicides. From five studies, four off-type grasses were found in Champion bermudagrass treated with both herbicides. DNA testing revealed the off-types were distinct from Champion, while flow cytometry tests indicated genetic changes at the genome level were likely responsible. No off-types were observed in the untreated bermudagrass.

This experiment shows surprising new possibilities of exposing hybrid bermudagrass to cell division inhibiting herbicides and resulting in somatic mutations and genetic instabilities. Somatic mutations include single gene changes, aneuploidy (the addition or loss of a chromosome) and polyploidy (Wan et al., 1991).

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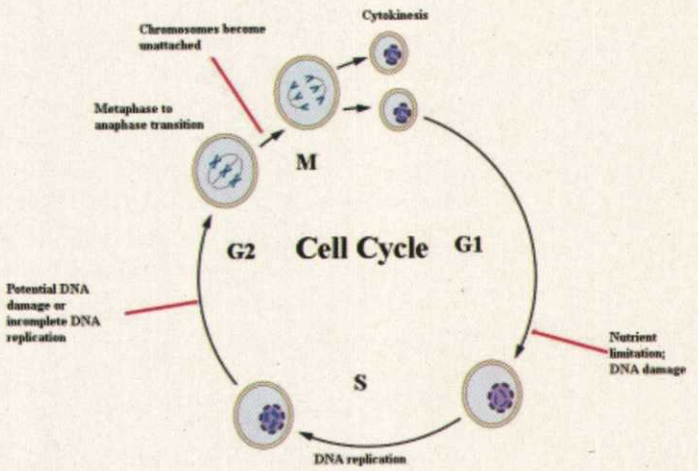
DNA replication and chromosome segregation are fundamental processes required for cell division and are strictly controlled during the cell cycle (Buchanan et al., 2000). Cell cycle disruption from herbicide exposure could therefore alter bermudagrass chromosome segregation and arrangement in DNA sequencing and result in off-type mutations (Figure 1).

As turfgrass cells divide, spreading of genetic information to new cells increases the likelihood of species survival (Buchanan et al, 2000). Mitotic inhibiting herbicides, however, disrupt these events. Cell division inhibitors not affecting DNA synthesis may still affect bermudagrass genetics by increasing the amount of DNA per cell (Devine et al., 1993).

FIGURE 1



Exposure to herbicides with prevalent soil persistence and potential residual effects may have deleterious effects on ultradwarfs.

Exposure to these herbicides with prevalent soil persistence and potential residual effects may have deleterious effects on ultradwarf bermudagrass, as exemplified by the study of Goatley et al. (2003). While the researchers examined dinitroanaline herbicides, many other herbicides commonly used in golf course management inhibit mitosis, microtubule assembly, RNA biosynthesis and protein biosynthesis.

Cell division inhibitors are effective for preemergent control of *Poa annua* and summer annual weeds, but will likely not be suitable for ultradwarf bermudagrass putting greens. Dithiopyr has a similar mode of action to the dinitiroanalines and has shown bermudagrass injury (Fagerness et al., 2002; Ferrell et al., 2003). Bensulide, a thiocarbamate, has shown minimal to no foliar injury during spring transition and summer growth (Callahan, 1976). However, root mass of actively growing Tifgreen bermudagrass was reduced after 60 days following exposure to bensulide at 8.4 and 16.8 kilograms of active ingredient per hectare (kg a.i. per hectare) (Bingham, 1967), and root mass restrictions of zoysiagrass and Kentucky

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bluegrass have also been reported (Engel and Callahan, 1967; Fry et al., 1986).

Amide herbicides, such as napropamide, provide effective pre-emergent control of *Eleusine indica* (Dernoeden et al., 1984). These herbicides will likely not be applicable for ultradwarf bermudagrass management because of their inhibitory effects on root growth and DNA synthesis. DiTomaso et al. (1988) found napropamide reduced root DNA synthesis after 24 hours by up to 89 percent. In addition, it was determined that the protein synthesis necessary during mitosis was also depressed. The researchers noted the majority of cells exposed to napropamide were arrested in the G_1 phase of the cell cycle.

It's reasonable to consider a potentially safe pre-emergent herbicide for dwarf bermudgrass that would not inhibit root-cell division or disrupt mitosis.

DNA synthesis occurs during interphase between the G_1 and G_2 phases of the cell cycle. DNA synthesis in G_2 and early mitosis would lead to a change in ploidy, DNA content and genome copy number, and interfere with chromosome segregation (Buchanan et al., 2000). However, assembly of protein complexes that mediate initiation of DNA synthesis is promoted during the phase preceding DNA synthesis. Protein kinases and regulation subunits, potential targets for herbicides, control major cell cycle transitions (Buchanan et al., 2000).

Herbicides, like pronamide, may increase DNA, RNA, and cell cycle proteins and are commonly used in golf course management. Auxin-type postemergent herbicides, such as the phenoxyalkanoic acids, cause phytotoxic symptoms such as epinasty, swelling, twisting and bending of treated plant parts, eventually causing cell division to cease (Rao, 2000).

The postemergence herbicide, 2,4-D, affects all types of RNA, as well as DNA and ribonuclease, while asulam appears to inhibit cell division and expansion of plant meristems by disrupting microtubule assembly (Rao, 2000). Interference with RNA and protein synthesis has also been observed with asulam (Veerasekharan et al., 1977).

Potentially safe pre-emergents for ultradwarfs

Since pre-emergent herbicides are applied months before weed-seed germination ceases, the herbicide must strongly adsorb to soil particles and remain in sufficient concentrations to provide effective season-long weed control (Branham, 1994). Thus, persistence of herbicide exposure is crucial for these herbicides to be effective.

It is reasonable to consider a potentially safe pre-emergent herbicide for dwarf bermudagrass that would not inhibit root cell division or disrupt mitosis.

Turf scientists have regarded a protoporphyrin inhibitor, oxadiazon, as one of the safest pre-emergent herbicide for high-quality turfgrasses (McCarty and Murphy, 1994). This contention is based on the mode of action of this compound, inhibiting shoot emergence of susceptible weeds without preventing root cellular division.

Oxadiazon, an oxadiazole herbicide, has a similar mode of action to diphenyl ethers. These herbicides are potent inhibitors of the enzyme protoporphyrinogen oxidase, commonly referred to as protox, key to chlorophyll and cytochrome syntheses (Rao, 2000). Protox oxidizes protoporphyrinogen (PPGIX) to protoporphyrin IX (PPIX). When protox is inhibited by oxadiazon, an uncontrollable accumulation of PPIX occurs in the thylakoid membrane, where chlorophyll harvests light energy for photosynthesis.

An oxidation of molecular oxygen to PPIX occurs causing an abstraction of hydrogen from fatty acids (Rao, 2000). Lipid radicals and lipid peroxidations cause a loss of chlorophyll and carotenoids and eventually leaky membranes leads to cellular disintegration.

On Tifgreen bermudagrass, oxadiazon provided 100 percent control of *Digitaria sanguinalis* in three consecutive years (Callahan and High, 1990). Oxadiazon applied 60 days before overseeding at 2.2 kg per hectare provided ≥90 percent annual bluegrass control in overseeded bermudagrass putting greens (Toler et al., 2003). Tifway bermudagrass treated with

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single applications of oxadiazon at 1.12 and 2.24 kg per hectare provided 70 percent pre-emergent control of *Kyllinga squamulata* 18 weeks after initial treatments (Bunnell et al., 2001). Johnson (1980) observed that oxadiazon at 4.5 and 13.4 kg per hectare did not affect Tifdwarf or Tifway bermudagrass rooting.

From these studies, oxadiazon appears to give exceptional control of summer and winter annual weeds in bermudagrass turf. Because mitosis is unaffected in root cellular division, this mode of action will likely be most suitable for ultradwarf bermudagrass management.

As dwarf-type bermudagrasses continue to expand on Southern putting greens, superintendents depend heavily on new research regarding best management practices. Pre-emergent herbicide applications are the basis of turf chemical weed control programs and ideally should not restrict rooting of bermudagrass during spring root regeneration (McCarty et al., 2001).

Currently, turf managers require information regarding herbicide safety on ultradwarf bermudagrass as there are none labeled for use on these grasses.

Superintendents managing ultradwarf bermudagrass should be aware of the potential harmful effects of pre-emergent herbicides, especially with cell division inhibitors. Since cell division and mitosis inhibitors are commonly used in golf course management, turf managers will likely need to explore other herbicide options for successful long-term ultradwarf bermudagrass culture.

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