GENETIC DIVERSITY OF KENTUCKY BLUEGRASS GENOTYPES IN MORPHOLOGICAL, AGRONOMIC, AND ABIOTIC STRESS TOLERANCE CHARACTERISTICS

by

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ABSTRACT OF THE DISSERTATION

Genetic Diversity of Kentucky bluegrass in Morphological, Agronomic, and Abiotic Stress Tolerance Characteristics

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Kentucky bluegrass (*Poa pratensis* L.) is a perennial turfgrass species that is widely adapted to many distinct environments. Kentucky bluegrass reproduces through an asexual process called apomixis, resulting in many unique and distinctly different cultivars with specific niche uses. Therefore it is important to quantify and classify the broad range of adaptations within this species. The objectives of this study were to: i. Classify Kentucky bluegrass genotypes based on morphological and agronomic traits and determine their inheritance patterns, ii. Evaluate the range of variability Kentucky bluegrass cultivars and selections exhibit in response to a novel annual bluegrass control, bispyribac-sodium herbicide, iii. Determine the effects of fertilizer on the response of Kentucky bluegrass cultivars and selections to bispyribac-sodium herbicide, and iv. Evaluate the diversity of Kentucky bluegrass cultivars and selections in their rooting ability under heat stress. To do this a number of experiments were designed between the summers of 2004 and 2008 at the Rutgers University Plant Science Research Farms in Adelphia and New Brunswick, NJ. There is variation in morphological and agronomic

traits in Kentucky bluegrass leading to a 12 group classification system based on the cultivars and selections evaluated. The traits that define these types are highly heritable. There is a differential response among Kentucky bluegrass cultivars and selections to bispyribac-sodium herbicide with some exhibiting almost complete tolerance and others complete susceptibility. Fertilizer can offset the injury associated with bispyribac-sodium herbicide on some cultivars, but actually increases the injury seen on others. There is variation in the rooting ability of Kentucky bluegrass cultivars under heat stress and based on the work of others it should be possible to select for and improve upon this trait. Kentucky bluegrass is a high quality turfgrass species with many distinct uses and based on these results the selection and management of Kentucky bluegrass cultivars is greatly simplified leading to proper cultivar use and long-term persistence.

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Introduction

The "living green carpet" we call turf has many inherent benefits (Burton, 1992). Their benefits include functional, recreational, and aesthetic components such as erosion control, protection from personal impact injuries, and enhanced beauty and attractiveness (Beard and Green, 1994). Within the last century the art of turfgrass culture has become a true science, when Drs. C.V. Piper and R.A. Oakly initiated the campaign in 1906 and later founded the United States Golf Association in 1920 (Horrell, 1980). Rutgers University realized both the importance and value of the turfgrasses since their formal conception. Here at Rutgers, Dr. H.B. Sprague began improving turf species prior to World War II, which led to the development and release of some of the first improved grasses including 'Raritan' velvet bentgrass (*Agrostis canina* L.), 'Merion' Kentucky bluegrass (*Poa pratensis* L.), and 'Meyer' zoysiagrass (*Zoysia japonica* Steud.) (Funk and Meyer, 2001). Together with the help of Drs. G. Burton, C.R. Funk, R. Skogley, R. Engel, and H. Indyk, over the course of 70 plus years began investigating all aspects of turfgrass culture from genetic improvement and management practices to biotic and abiotic interactions (Funk and Meyer, 2001) making Rutgers University "The Center for Turfgrass Science" that we know today.

Currently, the maintenance of these grasses provide a substantial number of jobs and contribute $45 billion annually to the United States economy (Beard and Green, 1993). Currently we see an increase in the intensity of turfgrass use and management with skyrocketing budgets for fine and sports turf maintenance. As well as, a constant release of new and improved cultivars, domestication of new and possibly better species for turf applications, reduced ecological impacts through the development of best management practices, and social acceptance of turfgrass for environmental improvement and carbon sequestration. Making this group of grasses one of the most utilized and durable, non-food plants mankind has ever seen.

Kentucky Bluegrass

Kentucky bluegrass is a perennial turfgrass species that is widely adapted to many distinct environments. The extensive rhizome system of Kentucky bluegrass gives it the ability to tolerate and recover from many environmental stresses more quickly than bunch-type species (Bonos and Murphy, 1999; Meyer and Funk, 1989). This also makes it the staple species for sod production and contributes to its nickname the "Final Triumph of Nature" (Funk, 2000).

Kentucky bluegrass reproduces through an asexual process called apomixis. Apomixis allows for the development of true-to-type seed from the maternal plant (Bashaw and Funk, 1987; Funk, 2000; Turgeon, 1999). It reduces out-crossing, which serves to prevent recombination and fix hybrid vigor (Bicknell and Koltunow, 2004). This results in many unique genotypes of Kentucky bluegrass. This is beneficial because once superior genotypes are identified they can be preserved through apomixis and produce uniform, stable cultivars (Bashaw and Funk, 1987; Funk, 2000).

Breeding for improved qualities in Kentucky bluegrass initially proceeded slowly, with cultivars being selections from well adapted, highly apomictic ecotypes. The discovery of an intraspecific hybridization method within this facultative apomict showed that bluegrasses could be hybridized and that those hybrids could also be highly apomictic (Funk and Han, 1967; Pepin and Funk, 1971, 1974). Additional work by Hintzen and van Wijk (1985) and Riordan et al. (1988) increased the functional understanding of this unique species and gave breeders the opportunity to shuffle traits throughout the population. Since these discoveries hundreds of improved bluegrasses have been released commercially (Bonos, 2007).

Kentucky bluegrass can exhibit poor performance during summer months, especially in the transition zone due to heat and drought stress combined with insect and disease pressure (Bonos and Murphy, 1999). Texas bluegrass *(P. arachnifera* Torr.), native to Texas and parts of Oklahoma, is a strongly rhizomatous, dioecious species that is more tolerant of heat and drought stress than Kentucky bluegrasses (Gould, 1975; Hitchcock, 1950). Texas x Kentucky bluegrass crosses were made as early as 1908 by George H. Oliver who noticed a wide variation in first generation hybrids including plants that were more heat and drought tolerant and more productive than Kentucky bluegrass (Vinall and Hein, 1937). The breeding strategy of crossing female Texas bluegrass plants with Kentucky bluegrass is helping to expand the adaptation of Kentucky bluegrass into transition zone areas where better heat and drought tolerance is always needed. Presently, refined methods of interspecific hybridization has led to introgression of this native bluegrass species into elite Kentucky bluegrass backgrounds to increase performance. This may also function to reduce water and other costly inputs that are associated with intensive turfgrass management and provide consumers with more environmentally-friendly, sustainable systems.

Kentucky Bluegrass Classification

The apomictic reproductive behavior of Kentucky bluegrass has resulted in many distinctly different cultivars each with their own unique strengths and weaknesses so the need for a way to classify them quickly became evident. Initial efforts to classify Kentucky bluegrass were typically time consuming. When visual appearance fell short many approaches were implemented to simplify cultivar selection and identification. In 1972, Wilkinson and Beard used protein extracts and electrophoresis to separate cultivars. They evaluated 10 cultivars and selections (‘Merion’, ‘Fylking’, ‘Cougar’, ‘Prato’, ‘Windsor’, ‘Belturf’, ‘Nugget’, ‘NJE-P27’, ‘Park’, and ‘Kenblue’) using protein extraction and polyacrylamide gel disc electrophoresis. Their study found that band number between cultivars ranged from 14 – 24 which identified ‘Merion’ and ‘Fylking’ as a group, ‘Cougar’ and ‘Prato’ as a group, ‘Windsor’ and ‘Belturf’ as a group, ‘Nugget’ independently, and NJE-P27 independently, based on distinct and unique bands. They did not find any consistent banding patterns with ‘Park’ or ‘Kenblue’ and based on these complications concluded that both banding patterns and morphological differences should be used to identify cultivars.

Wehner et al. (1976) also used a polyacrylamide gel electrophoresis on isoenzymes extracted from the seedling leaf tissue of 15 Kentucky bluegrass cultivars and selections. In this study, the cultivars were initially separated into three groups, those containing two, three, and four bands, respectively. Then by measuring the intensity of each band they were able to identify 11 cultivars individually, and the remaining four into two pairs.

Interestingly, they also showed the uniqueness of ‘Nugget’, as well as the distinct differences in banding patterns between ‘Windsor’ and either ‘Merion’ or ‘Fylking’ (which showed similar banding patterns). They also showed that four off-type plants of ‘Pennstar’ had similar banding patterns to ‘Pennstar’ and that this method would not be able to differentiate between the cultivar and off-types, further reinforcing the need to use multiple characters or methods to characterize Kentucky bluegrass cultivars.

Spoor and Hay (1979) used esterase and peroxidase banding patterns from seed extracts in an attempt to classify 16 Kentucky bluegrass lines and 14 ecotypes, representing various cultivars and selections. They showed that there was variability in banding patterns within the lines, furthermore, the lines themselves showed variability for this variation with ‘Merion Blue’ showing almost identical banding in all of the 40 seeds evaluated and HV140 showing more variation within its banding patterns. Based on these results Spoor and Hay (1979) concluded that it was possible to identify cultivars using the banding patterns produced from their enzyme systems, but this technique should be used in conjunction with both seed and seedling characteristics.

Wu et al. (1984) once again used electrophoresis, but this time using four enzyme systems in both seed and seedling tissue of Kentucky bluegrass cultivars and selections. They also investigated the differences between seeds and seedlings of the same cultivars as well as differences between the same cultivars from different seed lots. Their study was able to separate 22 of the 24 cultivars and selections evaluated. Interestingly, their method was unable to separate the cultivars ‘Baron’ and ‘Victa’ leading them to believe that those two entries were genetically similar. They also found distinct differences in banding patterns between seed and seedling tissues as well as differences in band variability between seed lots of the same cultivars grown in different environments. Wu et al. (1984) concluded that this technique was useful in separating most Kentucky bluegrass cultivars and selections but unable to separate the cultivars that were genetically very similar.

Weeden and Emmo (1985) evaluated 22 Kentucky bluegrass cultivars with electrophoresis using six isoenzyme systems and also found polymorphisms between cultivars in each system. The results of their study were generally similar to Wu et al. (1984), who also grouped ‘Baron’ and ‘Victa’ together. Although these phenotypic screenings cannot be used to identify an unknown cultivar, they can be used to rule out what cultivar the sample is not and could prove useful in that respect.

Freeman and Yoder (1991) used similar techniques to evaluate 20 Kentucky bluegrass cultivars, however their objective was to identify the composition of Kentucky bluegrass blends. Unlike many of the studies before them, Freeman and Yoder (1991) were able to produce consistent and repeatable banding patterns in different runs from different seed lots and harvest years. They were able to identify blends of two Kentucky bluegrass cultivars, but when three, four, or five cultivars were used in the blend the phenotypic bands increased in complexity, respectively, and were not helpful in cultivar identification, but could still be used to detect some cultivar substitutions. They also showed that some Kentucky bluegrass cultivars were more related then others. Freeman and Yoder (1991) were able to conclude that different blends had different banding patterns and that identical blends from different sources had identical banding patterns indicating this could be used as a rapid tool in seed certification. However, they too reiterated the importance of long term field screenings.

Bell et al. (1995) took this technique to the next level incorporating what Freedman and Yoder did in 1991 with computer imaging to read phenotypic band intensity. They only used the Kentucky bluegrass cultivars 'Glade' and 'NuStar' in a two cultivar blend, but that varied the percentage of each in 10% increments ranging from 10% to 90%. This study was able to differentiate between blends within 8.6% of either cultivar 95% of the time. Providing seed companies and seed certification agencies with a fast and reliable method to ensure that what's on the tag is actually in the bag and based on the increased resolution due to computer imaging this technique may be able to differentiate between blends with more cultivars or unlabeled cultivar substitutions although this was not evaluated here.

Later in an effort to improve cultivar purity evaluations a morphological approach was employed. Nittler and Kenny (1976) used the morphological characteristics of crowns, tillers, and rhizomes to identify growth habit phenotypes of Kentucky bluegrass grown hydroponically. In their study evaluating 26 cultivars and selections for crown spread, tiller number, and rhizome number they observed two crown phenotypes (spreading and compact) and significant differences in rhizome to tiller ratios. It was shown that 'Bonnieblue', 'Fylking', and 'Pennstar' had spreading crowns with tillers that first grew horizontally before turning vertical, whereas, 'Fjord', 'Park' and 'Troy' had tillers that immediately grew upward. They also found that 'Prato' and 'Kenblue' had a low percentage of tillers that became rhizomes (below 10%) however 'Adelphi' and 'Sodco' had 39% and 41%, respectively, of the tillers develop into rhizomes. They concluded that it would be possible to differentiate between off-types and contaminants in a seed lot when specific cultivars are questioned thus helping to ensure that they are properly labeled, but based on the seemingly continuous distribution of traits it would be difficult to use this as successful means of cultivar identification; especially with the cultivars and selections exhibiting intermediate phenotypes. Nittler and Kenny (1976) also concluded that correct cultivar identification must be based on multiple methods of characterization.

More recently morphological and agronomic traits were used to develop a classification system to characterize the large number of Kentucky bluegrass cultivars that had been developed (Bara et al., 1993; Bonos et al., 2000; Murphy et al., 1997). These classification systems helped define the similarities and differences between cultivars and selections and were used as a guide to help turfgrass managers increase diversity and uniformity when developing blends of Kentucky bluegrass.

In 1993 a compilation of turf plot performance in the transition zone was reported in a proceedings article from the New Jersey Experiment Station (Bara et al., 1993) where visual observations from Rutgers University helped place Kentucky bluegrass cultivars and selections into useful groups.. They devised a seven group classification system consisting of Aggressive Types, BVMG Types, Common Types, Mid-Atlantic Types, Compact Types, Bellevue Types, and Other Types. This research showed that Aggressive Types showed vigorous lateral spread, BVMG Types (named after the first commercially released cultivars of this type, 'Baron', 'Victa', 'Merit', and 'Gnome') showed very high seed yields, susceptibility to stripe smut disease (caused by the fungus *Ustilago striiformis*), and a tendency to become stemy in the spring due to seed head production even when mowed. Common Type cultivars and selections were characterized as having erect growth, narrow leaves, and ability to survive hot, dry summers in the dormant state. Mid-Atlantic Type cultivars and selections showed deep, extensive rhizome systems, Compact Type cultivar and selections had a compact growth habit, resistance to leaf spot disease (caused by the fungus *Drechslera poae*), and poor winter color. Whereas Bellevue types showed early spring green-up and excellent winter color (Bara et al, 1993).

Then in 1997, Murphy et al. further refined this classification system and published the grouping system based on the following agronomic traits winter dormancy, winter color, resistance to stripe smut disease, resistance to leaf spot disease, and recovery from summer stress evaluated in turf trials at two locations in the transition zone over five years. This was the first study of its size and included all of the entries from the 1990 National Kentucky Bluegrass Test sponsored by the National Turfgrass Evaluation Program (NTEP). Evaluation of these characteristics grouped the Kentucky bluegrass cultivars and selections into seven categories: Compact Type, Bellevue Type, Mid-Atlantic Type, BVMG Type, Common Type, Aggressive Type, and Other Type (Murphy et al., 1997).

The Compact Type includes cultivars and selections with compact growth habits and excellent resistance to leaf spot and stripe smut. However, they have poor winter color with late green-up in the spring. Compact Type cultivars were variable in the other traits examined (Murphy et al, 1997). The variability between cultivars and selections for the other traits evaluated is similar to the continuous range of biochemical phenotypes showed by other researchers (Wehner et al., 1976; Spoor and Hay; 1979; Wu et al., 1984; Weeden and Emmo, 1985; Freeman and Yoder, 1991; Bell et al., 1995), and also shows that thorough evaluation using multiple traits or screening methods is essential to correctly classify cultivars and selections.

Bellevue Type cultivars and selections are distinctly different in their winter performance than the other cultivars and selections evaluated. Bellevue types have excellent winter color, high turf quality in the winter, and early spring green-up (Murphy et al., 1997).

In addition they show good resistance to summer stress, leaf spot, and stripe smut. Mid-Atlantic Type cultivars and selections similarly shows good summer stress tolerance, but exhibits only moderate winter performance. Mid-Atlantic Types are unique in their ability to develop a very deep and extensive rhizome system which helps these cultivars to quickly recover from damage. However, many of these cultivars and selections are susceptible to leaf spot (Murphy et al., 1997).

Some of the older cultivars that were developed exhibited similar performance in some of the traits evaluated. Murphy et al. (1997) grouped these cultivars into the BVMG Type. This group was extremely susceptible to stripe smut disease and generally exhibited poor winter and summer performance. However, plant breeders and seed growers were well aware of the similarities within these cultivars and selections due to the excellent seed yields they produced (Meyer and Funk, 1989). Interestingly, seed yield, disease resistance, and leaf biochemical assay's all placed 'Baron' and 'Victa' in a group, which indicates how genetically similar the two are despite the origin of 'Baron' being in eastern Holland, and 'Victa' originating from old lawns in San Fernando, CA (James and Sharp, 1994). Unfortunately, 'Merit' and 'Gnome' were not characterized for isoenzyme phenotypes but based on the similarity of the agronomic traits evaluated here they should also share similar protein fingerprints.

Common Type Kentucky bluegrass cultivars and selections are some of the original Kentucky bluegrass cultivars first used for turfgrass, they originated from naturalized ecotypes from old pastures (Meyer and Funk, 1989). These cultivars and selections are extensively damaged from leaf spot disease, show poor winter color and often tolerate summer stress by inducing dormancy. Common type cultivars and selections are best suited for conservation purposes (Murphy et al., 1997).

A variable group proposed in this research is the Aggressive Type. These cultivars and selections were grouped together based on high shoot density and aggressive lateral growth under mowed conditions (Murphy et al., 1997), however, many of the other traits evaluated within this group in their study were quite variable. Lastly, the remainder of the cultivars and selections showed intermediate agronomic phenotypes in this study and were accordingly named the Other Type. This reoccurring theme in all Kentucky bluegrass research is often observed when a number of cultivars or selections are evaluated and is due to the apomictic mode of reproduction within this species resulting in a large number of cultivars. Further screening methods are needed to achieve greater resolution into what is responsible for the differences observed which may help to identify more useful groups.

The Kentucky bluegrass classification system was further redefined physiologically through evapotranspiration rates (Ebdon and Petrovic, 1998) in an effort to identify water conserving Kentucky bluegrasses. Ebdon and Petrovic, (1998), evaluated 61 Kentucky bluegrass cultivars and selections in a greenhouse to determine the relationship between the morphological and growth characteristics as they relate to comparative water use. Through this research they were able to identify both low and high water use cultivars. They concluded that low water use cultivars had a more horizontal leaf orientation, narrower leaf blades, more lateral shoots per plant, slower vertical leaf extension rates, more leaves per shoot, and shorter leaf blades and sheaths than high water use cultivars. These characteristics when combined with Murphy et al. (1997) would indicate that Compact Type and Bellevue Type cultivars should use less water then Mid-Atlantic Type cultivars.

Interestingly, Ebdon and Petrovic (1998) presented a dendrogram based on evapotranspiration rates where the BVMG Type cultivars evaluated (‘Baron’, ‘Gnome’, ‘Merit’, and ‘Kelly’ all came from the same branch. Similarly, this was seen with some of the Mid-Atlantic Types evaluated (‘Eagleton’ and ‘Preakness’), as well as some Compact Type cultivars (‘Midnight’, ‘Blacksburg’, ‘Able-1’, ‘Glade’, and others).

Bonos et al. (2000a) continued in an effort to classify the Kentucky bluegrasses by selecting 43 cultivars and selections representing all of the classification types proposed by Murphy et al. (1997). In this study, the entries were grown in an un-mowed, spaced-plant nursery over two years in the transition zone. In addition to the characteristics evaluated by Murphy et al. (1997), the plants in this study were also evaluated for morphological characteristics similar to those used by the U.S. Dept. of Agriculture (USDA) on Plant Variety Protection (PVP) Applications, the addition of morphological characters were an important step given their importance to both PVP and water use (Ebdon and Petrovic, 1998). These measurements included: plant height, panicle length, flag leaf height, flag and subtending leaf blade length and width, and rhizome length.

Bonos et al. (2000a) determined that the BVMG and Compact Type cultivars and selections had the shortest plant and leaf heights with some Aggressive Type cultivars also showing a compact growth habit. They also indicated that Mid-Atlantic and Bellevue Type cultivars have a similar growth habit. Mid-Atlantic Type cultivars and selections exhibited an extensive rhizome spread, indicated by their long rhizome lengths. Of all the groups evaluated, Common Type cultivars and selections exhibited the most erect plant morphology and consistently produced the tallest plants in the study, they also showed that these cultivars and selections had the finest leaf texture. Furthermore, Bonos and coworkers

(2000a) used principal component analysis to visualize the variation between and among groups on a graph. This analysis supported previous research which found differences in the variability within each group. They concluded that BVMG Types were morphologically similar to Compact Types; Bellevue Types were morphologically similar to Mid-Atlantic Types; and that both Aggressive and Other Type cultivars did not form a distinct group with the parameters evaluated in their study (Bonos et al., 2000a).

Bonos et al. 2000b, took the classification system one strep forward and redefined the seven groups from their previous work into 12 groups. This was done by separating the Compact Type into three types: Compact Type, Compact-Midnight Type, and Compact-America Type. Compact-Midnight Types exhibit similar characteristics to the cultivar 'Midnight', Compact-America Types exhibit characteristics similar to the cultivar 'America'. They also added three new groups: Shamrock Type, Julia Type, and CELA type (Bonos et al., 2000b).

In their study (Bonos et al., 2000b, 2000c), Compact-Midnight Types were defined as having long winter dormancy, late spring green-up, and no resistance to powdery mildew disease (caused by the fungus *Erysiphe sp.*) especially when grown in the shade. Compact-America Types have finer leaf textures, higher densities, and better powdery mildew resistance when compared to the other Compact Types. Shamrock Types were characterized as having phenotypes similar to that of the cultivar 'Shamrock', which is mainly characterized as having high seed yield production (comparable to that of BVMG Types) with good resistance to strip smut disease. Julia Types typically have similar characteristics to the cultivar 'Julia', which commonly exhibits high turf quality but poor tolerance to Dollar Spot disease (caused by the fungus *Sclerotinia homeocarpa*) and Brown

Patch disease (caused by the fungus *Rhizoctonia solani*). CELA Types were named after the cultivars 'Challenger', 'Eclipse', 'Liberty', and 'Adelphi' and behave relatively similar to Bellevue Types without the unsightly seed head formation under mowed conditions (Bonos et al., 2000b, 2000c).

Given the increasing popularity of applied molecular biology in turfgrasses Curley and Jung (2004) came up with the idea to analyze this complicated species with random amplified polymorphic DNA (RAPD) markers . In their study of 123 Kentucky bluegrass cultivars and selections representing the range of Kentucky bluegrass groups proposed by Bara et al., 1993; Bonos et al, 2000a, 2000b, 2000c; and Murphy et al., 1997, were chosen for further analysis with RAPD markers to determine the genetic similarities between cultivars and selections both within and between groups. They determined that plant introductions and interspecific hybrids of Texas x Kentucky bluegrasses were genetically divergent from Kentucky bluegrass which indicates their role in plant breeding and cultivar development. They also found that Compact-Midnight cultivars and selections grouped tightly together indicating the similarity of the plants within that group. Not surprisingly, this finding agrees with the dendrogram produced by Edbon and Petrovic (1998). In addition BVMG Types were found to form two groups, one near the Common Types and another near a tight cluster of experimental selections. Other than these three groups Curley and Jung did find differences between classification types but identified no clustering which is probably due to the variation between cultivars and selections within a group in combination with variation between seedling replicates of each entry. This also indicated that not all cultivars within a group are necessarily related and based on this important finding it may be possible to incorporate certain cultivars of the same group into blends (Curley and Jung, 2004).

Many new Kentucky bluegrasses have been released since the previous classification based on spaced-plants was completed and other older cultivars have been discontinued (Bonos, 2007). Therefore the agronomic and morphologic traits of currently available Kentucky bluegrass cultivars and selections should be evaluated. Shortell et al. (2009) studied 173 Kentucky bluegrass cultivars and selections evaluated in the 2000 National Kentucky Bluegrass Test sponsored by NTEP. Their study measured morphological traits such as plant height, panicle length, flag leaf height, length, and width, and rhizome spread in a spaced-plant nursery in the transition zone over two years. Based on their study the Aggressive Type was renamed to the High Density Type, due to the fact that under un-mowed conditions these cultivars and selections did not exhibit aggressive behavior and in fact had a small rhizome spread when compared to the other types. In addition a Texas x Kentucky bluegrass Hybrid Type was added, but it should be noted that this type performed similarly to the Mid-Atlantic Types for the characteristics evaluated here.

Heritability in Kentucky Bluegrass

Given the apomictic nature of Kentucky bluegrass successful intraspecific hybridization can only be expected when a large number of seedlings are screened (Funk, 2000; Pepin and Funk, 1971). Certain morphological and agronomic characteristics are utilized by breeders in the development of improved cultivars. Plant height and spread are useful characteristics because a low growing, aggressive spreading cultivar should be able to tolerate lower heights of cut, recover quickly and fill in damaged areas (Meyer and Funk, 1989). Leaf texture is also an important characteristic used in the development of new turfgrass cultivars. Cultivars with finer leaves typically have higher shoot density in mowed turf situations (Bashaw and Funk, 1987; Turgeon, 1999). Additionally, early vs. late seed maturity is also important especially for Kentucky bluegrass cultivars grown in non-irrigated seed production areas (Bashaw and Funk, 1987; Burt and Christians, 1990) where the ability to set seed before drought is of utmost importance. Therefore, the heritability of such characteristics would be helpful to turfgrass breeders attempting to develop improved cultivars for specific environments.

Broad-sense heritability estimates the total genetic effects affecting a trait including additive, dominance and epistatic effects (Nyquist, 1991; Poehlman and Sleper, 1995). Narrow-sense heritability only estimates additive variance (Poehlman and Sleper, 1995), which is more useful to plant breeders working in cross-pollinated species because the additive gene effects can predict progeny performance better than dominant or epistatic gene effects (Poehlman and Sleper, 1995). However, when working with apomictic or asexually propagated crops, where both additive and non-additive gene action can be fixed, estimates of broad-sense heritability are more appropriate (Poehlman and Sleper, 1995). Plant characteristics have different heritability estimates (Berry et al., 1969; De Araujo and Coulman, 2002; Kneebone, 1958). Characteristics that are highly affected by the environment typically have low heritability, and characteristics that are less affected by the environment have high heritability. Therefore, in Kentucky bluegrass broad-sense heritability would be a good measure of environmental stability for the morphological and agronomic traits that define the bluegrass classification system.

Turfgrass breeders have improved many traits other than overall quality within Kentucky bluegrass. Seed yield is also an important trait that makes the cultivar profitable but is often unacceptable in high quality cultivars (Bashaw and Funk, 1987). An example of this can be seen in the cultivar ‘Midnight’ which has excellent turf quality for multiple characters over different environments throughout the U.S (Morris, 2006), however it is a low seed yielder. However, the production of ‘Midnight’ is still possible because its high quality and popularity offset the costs of minimal seed production. The opposite is seen in the cultivars ‘Baron’ and ‘Kenblue’ which exhibit poor quality, but produce so much seed that it is still economically feasible to grow.

In the field, from a practical stand point, some selections with excellent performance can be immediately rouged due to limited seed heads. Nguyen and Sleper (1983) studied the variability of seed yield in tall fescue (*Festuca arundinacea* Schreb.) and found seed yield heritable. In their study they found narrow sense heritability estimates (the amount of additive genetic variation within a population for the given trait) for maturity (h = 0.86), panicle number (h = 0.92), panicle length (h = 0.90), seed yield (h = 0.67) to be high. They also showed that the effect of panicle number contributed 81% to overall seed yield. Based on these results in tall fescue (Nguyen and Sleper, 1983) it should be possible to increase the seed yield of high quality Kentucky bluegrass cultivars.

Berry et al. (1969) conducted a study to determine the variation of some of the quantitative turf characteristics in Kentucky bluegrass. They choose the six characteristics of leaf width and angle, growth habit and spread, and rust resistance and found them to be highly correlated and heritable. They estimated broad sense heritability to be 0.98 for leaf width, 0.95 for growth habit, 0.94 for leaf angle, 0.96 for rust resistance, and 0.95 for total sod spread. They also showed growth habit to be significantly negatively correlated to leaf angle (-0.85, $p = 0.01$) and positively correlated to sod spread (0.68, $p = 0.01$). These results showed that it was possible to improve certain morphological characteristic in Kentucky bluegrass and that selection for some characteristics was strongly connected to other characteristics (Berry et al., 1969) which has led to the release of many denser turfs with finer leaves and an aggressive nature.

Shortell et al. (2009) also investigated Kentucky bluegrass morphological characteristics and found high broad-sense heritability estimates for plant height (H = 0.84), panicle length (H = 0.88), flag leaf height (H = 0.85), and rhizome spread (H = 0.85). This work corroborates the work of Berry et al. (1969) and based on the ability to fix hybrid vigor through apomixis these characteristics that help to define the morphological classification system should remain constant over multiple environments.

In other crops morphological characteristics have also been shown to have high heritability. In wheat (*Triticum aestivum* L.) Johnson et al. (1966) estimated the narrow sense heritability for plant height to range from 0.45 – 0.65. In oats (*Avena sativa* L.) Petr and Frey (1966) studied the genetic variation in number of panicles per plant, grain yield, panicle length, plant height, number of spikelets per panicle, and heading date which had narrow-sense heritability estimates of 0.33, 0.53, 0.54, 0.61, 0.74, and 0.87, respectively. Johnson et al. (1986) showed plant height to have high broad-sense heritability in corn (*Zea mays* L.) (H = 0.84) based on a two year study at three locations in Mexico. Hanks et al. (2005) studied an experimental population of crested wheatgrass (*Agropyron cristatum* L.)

and concluded that this grass had a broad-sense heritability estimate of 0.76 for rhizome spread. Research has shown across a diverse set of grasses that the genes involved in controlling the morphological and agronomic characteristics are all heritable. Therefore it should be possible to select and improve upon these traits in Kentucky bluegrass. Furthermore, when heritable morphological traits are associated with specific characteristics, such as yield or abiotic stress tolerance, selection for one trait can indirectly improve the other.

Another ongoing objective in Kentucky bluegrass breeding is increased disease resistance. Bonos et al., 2006 reported that leaf spot disease, stem rust disease (caused by the fungus *Puccinia graminis*), and stripe smut disease were three important problems in Kentucky bluegrass. They pointed out multiple examples of control through incorporation of genetic resistance. With leaf spot disease, the experimental selection RSP which shows good characteristics including deep, extensive rhizomes and excellent summer stress tolerance, however it is very susceptible to leaf spot disease. Through intraspecific hybridization the beneficial rhizome traits and summer stress tolerance was combined with tolerance to leaf spot disease which resulted in the cultivar 'Cabernet' that exhibited combined tolerance to both problems. Further complicating the breeding of Kentucky bluegrass for increased disease resistance are the pathogens themselves. Many of them have been shown in the past to change races and vary the tolerance of formerly resistant genotypes, as was the case with stem rust and stripe smut disease (Bonos et al., 2006). Therefore the task of improving disease resistance goes hand in hand with maintaining resistance.

Summer stress tolerance is variable within Kentucky bluegrass (Bonos and Murphy, 1999) and given its importance in the overall persistence of Kentucky bluegrass turf in the transition zone this is also a major breeding objective. Bonos and Murphy (1999) evaluated 10 Kentucky bluegrass genotypes in a field study over two years at a site built to maximize heat stress. They found that stress tolerant entries (as indicated by high turf quality during the experiment) also showed lower stomatal resistance and less change in canopy temperature then did intolerant entries. They also found that canopy temperature depressions were highly negatively correlated ($r = -0.90$, $p = 0.05$) to summer stress tolerance which indicates the possibility of using and infrared thermometer to screen Kentucky bluegrass for tolerance to summer stress. Therefore it should be possible to increase the summer stress tolerance in Kentucky bluegrass cultivars and selections.

The first step to improving any weakness in Kentucky bluegrass is the identification of genetic variability for the given trait in the population. Then through incorporation of plants with the improved characteristics into elite genetic backgrounds of currently successful cultivars it is possible to increase the performance of the species in that environment. This is the backbone of plant improvement and is partially responsible for the many adaptations of Kentucky bluegrass as well as its numerous niche uses.

Chemical Control of Annual Bluegrass in Kentucky Bluegrass

Annual bluegrass (*P. annua* L.) is a persistent weed which reduces overall turfgrass utility, due to its light green color, high reproductive capacity, and shallow root system (Lush, 1989; Sprague and Burton, 1937). Annual bluegrass tolerates close mowing and germinates rapidly allowing it to out-compete desirable turfgrass species. This is particularly pronounced in Kentucky bluegrass due to its slow establishment rate. It can also encroach into stressed Kentucky bluegrass turfs mown at low cutting heights. The poor disease, drought, and wear tolerance of annual bluegrass compared to Kentucky bluegrass (Beard et al., 1978; Lush, 1989), contribute to its decline in quality. Consequently, Kentucky bluegrass infested with annual bluegrass requires more water and fungicides, especially in the summer months. The difficulties associated with annual bluegrass have even forced some breeders to take the opposite approach and start to select for improved traits in this difficult weed (Huff, 2000).

Chemical control options for annual bluegrass in Kentucky bluegrass are limited. Use of preemergence herbicides prior to the annual bluegrass germination cycle can limit infestations from encroaching into Kentucky bluegrass swards, but typically have no effect on established annual bluegrass populations. Ethofumesate is a postemergence herbicide that is labeled for annual bluegrass control, but often provides inconsistent control with unacceptable Kentucky bluegrass injury (Adams, 1989; Dernoeden and Turner, 1988).

Aside from ethofumesate there are currently no selective herbicides labeled for control of established annual bluegrass in Kentucky bluegrass. Bispyribac-sodium is a pyrimidinyl carboxy herbicide that controls weeds by inhibiting acetolactate synthase (Shimizu et al., 2002). Bispyribac-sodium has been used for selective postemergence control of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and other weeds in rice (*Oryza sativa* L.) (Schmidt et al., 1999; Webster et al., 1999; Williams, 1999) and was recently registered for turf (Anonymous, 2004).

Mesotrione is a unique active ingredient with pre- and postemergence activity that has been shown safe on Kentucky bluegrass and effective on control of annual bluegrass (Hart and McCullough 2007; Hart et. al. 2008). This product however, is currently only labeled for Kentucky bluegrass grown for seed production or when grown on golf courses and sod farms, and its utility for controlling established annual bluegrass is still under investigation (Anonymous 2008a, 2008b). Aside from the herbicides mentioned above there are currently no widely labeled herbicides for control of established annual bluegrass in Kentucky bluegrass turf.

Research with bispyribac-sodium indicates applications of 60 to 148 g/ha can reduce populations of annual bluegrass without injuring creeping bentgrass (*A. stolonifera* L.) (Askew et al., 2004; Borger and Watschke, 2005; Dernoeden et al., 2004; Lycan et al., 2003; Lycan and Hart, 2005). Additionally, research has found that two applications are needed for complete annual bluegrass control (Lycan and Hart, 2006a; McCullough and Hart, 2006a; Park et al., 2002). Limited research with Kentucky bluegrass found a single application of 296 g/ha on 'Baron' Kentucky bluegrass resulted in 28% injury five weeks after initial treatment (WAIT) (Lycan and Hart, 2005). However, more comprehensive research emphasizing the response of Kentucky bluegrass to multiple applications of bispyribac-sodium is needed.

The selectivity of bispyribac-sodium on cool-season grasses could be due to a number of different effects. Lycan and Hart (2006b) determined that both creeping bentgrass and annual bluegrass absorbed greater than 90% of the foliar applied bispyribac-sodium into the leaves, however annual bluegrass translocated a greater proportion of the chemical into their crowns where plant meristems could have been damaged. However, in other acetolactate synthase inhibiting herbicides selectivity was shown to be caused by differential herbicide metabolism. Halosulfuron, for example, was shown to be safe on corn and wheat due to rapid metabolism of the parent molecule, however soybean showed reduced metabolism and subsequent injury (Dubelman et al., 1997). King et al. (1997) showed that AE-F130060-03 could selectively control Italian ryegrass in wheat and barley due to a combination of decreased herbicide absorption and increased herbicide metabolism. Therefore, the selectivity of bispyribac-sodium in the cool season grasses could be due to an combination of differential translocation and metabolism inside the plant.

Several herbicides have been shown to interact with management programs to influence herbicide efficacy or crop injury. Nitrogen applications have been generally shown to reduce turfgrass chlorosis in response to many herbicides and plant growth regulators (Dernoeden et al. 1993; Johnson 1984, 1990; Johnson and Burns 1985). It has also been shown to reduce weed control when used in conjunction with some herbicides including atrazine, mesotrione, nicosulfuron, glufosinate, and glyphosate (Cathcart et al. 2004). The opposite trend was observed in wheat, when sulfonylurea herbicides, mixed with urea ammonium nitrate, increased injury when compared to the sulfonylurea without fertilizer (Olson et al. 2000). Research on tolerant species, including both creeping bentgrass and perennial ryegrass (*Lolium perenn*e L.), found that applications of nitrogen and iron prior to bispyribac-sodium treatments reduced the initial injury, and increased the speed of recovery without reducing annual bluegrass control (McCullough and Hart 2006b; McDonald et al. 2006). A subsequent study by McCullough and Hart (2009) found that nitrogen generally increased annual bluegrass tolerance to bispyribac-sodium applied at 74g/ha, but not at 148g/ha. These results suggest that the evaluation of bispyribac-sodium and nitrogen fertilizer interactions for the potential to decrease turfgrass injury while maintaining herbicide efficacy warrant further investigation.

Summer Stress in Kentucky Bluegrass

Summer stress is the combined effects of both heat and drought in combination with biotic pests that lead to the deterioration of the turfgrass sward. If significant progress can be made in summer stress tolerance it would minimize other problems within the system, such as annual bluegrass encroachment. For informational purposes these stresses need to sometimes be separated to fully understand the contributions of each to the detrimental effects they cause. The difficulties associated with separating these stresses in the field has led a number of researchers to study their combined effects (Bonos and Murphy, 1999; Jiang and Huang, 2000; Jiang and Huang, 2001a, 2001c; Wang and Huang, 2004; and Su et al. 2007). Bonos and Murphy (1999) found that Kentucky bluegrass intolerant of summer stress had a dramatic increase in stomatal resistance, leading to increased canopy temperatures. They also found that the tolerant entries evaluated had a higher percentage of roots in the deeper profiles of the root zones.

Jiang and Huang (2000, 2001c) under more controlled conditions in a series of growth chamber evaluations attempted to separate heat and drought stress in Kentucky bluegrass (2000), tall fescue (*Festuca arundinacea* Schreb.), and perennial ryegrass (2001c). Their findings proved that the combined effects were more detrimental than either stress alone; and markedly reduced both canopy photosynthetic rate and photochemical efficiency. Jiang and Huang (2001a) evaluated tall fescue and Kentucky bluegrass under similar environmental conditions and confirmed the effects of combined heat and drought using relative water content and chlorophyll content measurements. However, it was shown that the effects of heat and drought stress on root dry weight was not significantly different then the effects of heat stress alone. Interestingly, when root viability was examined the combined stresses of heat and drought caused a dramatic decrease in root viability compared to either stress alone, reinforcing the importance of root viability in maintenance of physiological functions and overall stress tolerance (Jiang and Huang, 2001c).

Differences in Kentucky bluegrass recovery to drought and heat stress are also variable, but equally important. Rapid recovery from stress is one of the traits that helps turfgrass persist in unfavorable environments where stress events are often sporadic. Wang and Huang (2004) showed differences in heat and drought tolerance between ‘Midnight’, stress tolerant, and ‘Brilliant’, stress sensitive, Kentucky bluegrass cultivars. The focus of this study was to evaluate the recovery potential of the two under different environmental conditions. Re-watering and cooling were essential for the rapid recovery of the cultivars, but even then, the stress sensitive cultivar ‘Brilliant’ recovered slower than the stress tolerant cultivar ‘Midnight’. A similar study by Su et al. (2007) evaluating Kentucky bluegrass, Texas x Kentucky bluegrass hybrids, and tall fescue under high temperatures and deficit irrigation showed the combined stresses to be more damaging than either stress alone. More importantly they showed that hybrid bluegrasses were more tolerant to heat stress than Kentucky bluegrass or tall fescue based on the traits they evaluated.

The Effects of Heat Stress on Plants

High temperature causes a number of detrimental effects on plants. Depending on the plant species and the plant organs evaluated there are different optimum temperature ranges. The effects of heat stress are directly related to the energy balance of the plant. When energy intake through both ambient high temperature and dissipation of excess light exceeds the amount of energy that can be handled through heat avoidance mechanisms, heat stress begins (Nilsen and Orcutt, 1996). Enzymes are very sensitive to heat stress and are easily denatured by temperatures that exceed their optimum range. Many heat sensitive enzymes are involved in photosynthesis therefore the chloroplasts are one of the first organs to show signs of heat stress. Under heat stress photochemical processes and electron transport initially continue, however photochemical processes gradually decline due to leakage of the thykaloid membranes. This allows the light harvesting complexes of photosystem II to dislodge from the membrane which reduces energy yield. Concurrently, enzymes responsible for generating electrons through the Hill Reaction are also being damaged, thus decreasing the flow of electrons that are used to drive photosynthesis (Nilsen and Orcutt, 1996). In addition plant respiration is less affected by high temperature; therefore we see an increase in respiration relative to photosynthesis which results in a net decrease in photosynthesis as temperatures increase.

The Analysis of Heat Tolerance

Heat stress is a major limitation of all cool-season turfgrasses grown in warm climactic regions. Physiologically, there are various ways to study turfgrass tolerance to heat stress. Including, but not limited to, shoot dry weights, vertical shoot extension rates, photochemical efficiency, photosynthetic rates, canopy temperature depressions, root mortality rates, and electrolyte leakage (Aldous and Kaufmann, 1979; Bonos and Murphy, 1999; Liu et al., 2002; Xu and Huang, 2001a; Zhang et al., 2003). However, many of these measurements are destructive and time consuming which limits the amount of samples that can be reasonably processed or the number of selections that can be efficiently screened (Marcum, 1998). These difficulties result in the breeder's inability to screen all of the available germplasm and could lead to the rejection of otherwise superior genotypes. Often times non-destructive, efficient measures of quantifying heat stress are adapted and used, but they are not always directly correlated to the stress or applicable in the given environment.

Photochemical efficiency is a measure of the efficiency in photosystem II, which is often decreased under heat stress due to inefficient electron transport (Papageorgio, 1975) most likely caused by enzymatic failure and thykaloid membrane leakage. This is easily measured with a standard fluorometer and a good sign of plant tolerance to heat stress (Bolhar-Nordenkampf and Oquist, 1993; Miles, 1990; Smillie and Hetherington, 1983; and Zhang and Schmidt, 2000).

Infrared thermometers can also be used to measure the temperature of the turf canopy, then differences between the canopy and the air can be quantified and related to plant health. The canopy temperature differential is an indirect measurement of stomatal resistance through transpirational cooling (Balota et al., 2007; Ervin and Koski, 1998).

Throssell et al. (1987) even used this technique to schedule irrigation in Kentucky bluegrass turf.

Cell membrane stability is an important measure of heat tolerance in plants. The cell membranes are the first to show damage when heat stress is imposed. Cell membrane stability is measured through leakage of cell solutes (electrolytes) out of the cell. This can then be quantified through ultra-violet fluorescence at 280 nm (Navari-Izzo et al., 1989; Reyes and Jennings, 1994) or through changes in electrical conductivity in solution (Blum and Ebercon, 1981; Huang et al., 2001; Marcum, 1998). Given the importance of the membranes in heat tolerance, it has been proposed that high cytoplasmic and membrane protein content may contribute to heat tolerance (He and Huang, 2007). These proteins can be extracted (Shimoni et al., 1997) and quantified (Bradford, 1976) and then used to differentiate cultivar tolerance.

Root viability reductions caused by heat stress can be quantified by measuring root dehydrogenase activity using triphenyltetrazolium chloride reduction techniques (Knievel, 1973). Carbohydrate loss through root respiration can separate heat tolerant from heat sensitive cultivars (Rachmilevitch et al., 2006). Chlorophyll content in the leaves can be used to measure plant heat tolerance, in this case, chlorophyll is extracted with an organic solvent and then measured on a spectrophotometer (Arnon, 1949). Leaf relative water content (Barrs and Weatherley, 1962), and osmotic adjustment or the difference in leaf osmotic potentials (Blum and Sullivan, 1986; Blum, 1989) are two commonly used techniques to measure turgor maintenance under heat stress and overall plant tolerance to heat stress. Both are important in heat tolerance due to their role in evaporative cooling of leaf tissue and are also commonly used to quantify drought.

Heat Tolerance of Kentucky Bluegrass

A major limitation of all cool-season grasses grown in warm climatic zones is high temperature stress (Xu and Huang, 2001a). The optimum temperature range for shoot and root growth of cool-season grasses are 10 to 24°C (Beard, 1973). Typical symptoms of heat stress include wet wilt and leaf firing which cause an elevation in cellular temperatures, resulting from reduced evapotranspiration, as well as, reductions in photosynthesis from a physical reduction in leaf tissue (Marcum, 1998). This limits the regions where Kentucky bluegrass can be grown and complicates turf management in the transition zone where sporadic heat waves cause damage to turf stands. Similarly, damage is also experienced in the below ground parts of the plant system, especially in the roots where it is more difficult to quantify. Plant roots are the primary sites of cytokinin synthesis and water acquisition, both essential to the growth and development of the plant (Liu et al., 2002). Therefore, plants that are able to grow and maintain root systems under heat stress should be capable of maintaining physiological function and persist until more favorable environmental conditions return for active growth to resume.

Heat stress is a problem for cool-season grasses grown in the transition zone during July and August. There is genetic variability in heat tolerance within Kentucky bluegrass. Evaluation of the decline of growth rates when entries are shifted from low to high temperature is a simple method to quickly study the relative tolerance of Kentucky bluegrass cultivars to heat stress (Watschke et al., 1970, 1972). In these studies, the most heat tolerant bluegrasses had the highest photosynthetic rates. Watschke et al. (1970)

evaluated five Kentucky bluegrass genotypes under three temperature regimes. They concluded that high temperature increased respiration rates and that the plants with the highest carbohydrate levels in the leaves were least affected by the high temperature treatments. They also pointed out that cultivars 'Nugget', from Alaska, and 'Pennstar', from Pennsylvania, had significantly less heat tolerance then two experimental selections from Virginia and contributed these differences to elevated carbohydrate concentrations in the leaf tissue of the southern ecotypes adapted to high temperatures.

Then in 1972, Watschke and coworkers hypothesized that you could minimize the effects of heat stress through management practices that conserved carbohydrates, such as increasing the mowing height and reducing nitrogen fertility. They designed an experiment with 10 Kentucky bluegrass genotypes under high temperature stress in a growth chamber. During heat stress they measured both photosynthesis and respiration with and without oxygen and carbon dioxide in the growth chamber, analyzed the effects of each treatment per cultivar or selection and correlated the results to above and below ground growth. They attributed the difference between entries to genetic variability in carbon use and fixation. Their work identified 'Merion', 'Belturf', and Ba6124 as heat tolerant and 'Delta' and 'Nugget' as heat sensitive and stated that breeding techniques to increase heat tolerance should focus on improving the efficiency of carbon fixation (Watskche et al., 1972).

Jiang and Huang (2001d) used drought preconditioning on 'Mystic' Kentucky bluegrass to increase heat tolerance. There two treatments consisted of preconditioning Kentucky bluegrass to heat through two cycles of drying and rewatering and then compared these plants under heat stress to non-preconditioned controls. They found up to a 21% increase in turf quality, 10% higher levels of relative water content, 48% higher osmotic adjustment, 44% more soluble carbohydrate content and significantly higher root dry weights in the 40 – 60 cm soil profile in preconditioned plants after 21 days of heat stress. They concluded that the increase in tolerance in drought preconditioned plants was due to elevated osmotic adjustment combined with increases in root weights at the 20 – 40 cm layer (Jiang and Huang, 2001d). They also suggested that management techniques like deficit irrigation could be used to increase the heat tolerance of Kentucky bluegrass cultivars.

The Importance of Roots

Roots are important plant organs that function for water and nutrient uptake, hormone production and translocation, and plant anchorage (Nilsen and Orcutt, 1996). Not only is it beneficial for plant root systems to have increased weights, lengths, and branches, but it is also essential for the roots to be viable in order to maintain their physiological functions (Huang et al., 1997). Given their role in water uptake many researches have focused on the correlation between deep extensive root systems and drought tolerance in the field. Increased root weights are positively correlated to field performance under drought in zoysiagrass (Marcum et al, 1995; Huang et al, 1997), tall fescue (Carrow, 1996a), bermudagrass (*Cynodon dactylon* L.), centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.), seashore paspalum (*Paspalum vaginatum* Sw.) (Carrow, 1996b; Huang et al, 1997), and Kentucky bluegrass (Bonos and Murphy, 1999, Lehman and Engelke, 1993).

The Analysis of Root Systems

To date studies investigating root systems in Kentucky bluegrass under a range of environmental conditions are more limited than studies investigating shoot growth (Jiang and Huang, 2000; Jiang and Huang, 2001c; Su et al., 2007). This is probably due to the technical difficulties associated with rooting studies (Biswell and Weaver, 1933), where we see either an in-depth physiological analysis of plants in relation to root growth or large scale screenings that show variability in root growth without conclusive proof of the mechanisms involved. Furthermore, based on the variable phenotypes and diversity among cultivars and selections, large-scale cultivar screenings on Kentucky bluegrass are most likely needed.

For example, Jiang and Huang (2000) evaluated Kentucky bluegrass under drought alone, heat alone, and heat and drought together. They concluded that the decline in photosynthesis was more pronounced under heat stress and that declines in photochemical efficiency were more pronounced under drought stress. They also showed that all stresses reduced root dry weights and that the combined stress treatment was more detrimental than either treatment alone. This level of detail is important when trying to understand the underlying mechanisms that the plant uses to resist stress. However, Kentucky bluegrass is a diverse species that has variable phenotypes, therefore the question arises whether or not the one cultivar evaluated ‘Mystic’ represents the entire population.

Alternatively, Richardson et al. (2008) evaluated 50 Kentucky bluegrass cultivars and selections representing the range of Kentucky bluegrass types. This large study evaluated both the field drought tolerance and the greenhouse rooting ability. Although valuable information was generated in the process, the researchers were unable to link the field performance to root growth. This study provides us with an idea of how the population is responding, but because we lacked physiological detail it is difficult to conclusively say why it is going on.

The complications with balancing detail with broad population responses often results in contradictory information in the literature. In a study by Su et al. (2007) they evaluated a Texas x Kentucky bluegrass hybrid, a Kentucky bluegrass, and a tall fescue for drought and heat tolerance in the growth chamber. They determined that the Texas x Kentucky bluegrass hybrid was more heat resistant then either Kentucky bluegrass or tall fescue in terms of turf quality, photosynthesis, electrolyte leakage, and dry matter production. Su et al. (2007) did not find differences in drought tolerance between the hybrid bluegrasses and Kentucky bluegrass. However a larger study evaluating a number of Kentucky bluegrass and Texas x Kentucky bluegrass cultivars and selections under deficit irrigation (Abraham et al., 2004), indicated that there was genetic variability in the drought tolerance of Texas x Kentucky bluegrass hybrids and that the parents of the cross had contributed to the observed effects. Based on these results it would be interesting to look at the results of Su et al. (2007) if a different Texas x Kentucky bluegrass exemplar was chosen.

It would also be interesting to know if certain visual ratings and other non-destructive physiological measurements could be assessed on above ground plant parts and correlated to below ground measures of rooting ability such as root initiation and overall root weights. This could prove to be a useful and efficient way for turfgrass breeders to screen for increased root growth and produce new cultivars with increased rooting. Such cultivars should be more competitive and ultimately better for sod production.

In order to make this possible, the study of root systems needs to be simplified into repeatable measurements that we can collect data from. Although unseen their growth and development dictate overall canopy performance and given their location, the study of the root system can be a complex process. Furthermore, differences in soil characteristics over the range of root studies makes direct comparison difficult and the need for destructive sampling techniques limits the amount of information that can be gathered.

Based on these hurdles scientists throughout history have developed ingenious ways to make the study of root systems possible. Biswell and Weaver (1933) studied the growth of roots of native prairie grasses using galvanized steel troughs. After a predetermined grow-in, plants were removed from the troughs and gently washed over a fine screen and later analyzed for both root and shoot weights. They determined that frequent removal of top growth reduced root growth in seven grass species. They also pointed out the care that was necessary when evaluating the rooting traits of these species due to the high proportion of fine, fibrous roots and the difficulties associated with cleaning them off.

Sprague (1933) had an iron tool custom manufactured to facilitate root sampling in the field, and is one of the first published reports of creating and using a soil probe for root studies. Through this study Sprague (1933) determined that the effects of frequent clipping had more of an effect on the three bentgrass species evaluated than it did on Kentucky bluegrass. In addition this work also showed that slow release nitrogenous fertilizers permitted greater root development than soluble fertilizer at the same rates. Sprague (1933) also notice the effects of variable soil characteristics even within the same field and attributed a lot of the variation in rooting to this effect alone.

Rivers and Faubin (1961) evaluated the roots sesame (*Sesamum indicum* L.) in the field. The difficulties associated with the evaluation of rooting traits led these researchers to rent what they had called a "Tree Ogger"; from the pictures this machine resembles a modern day tree transplanter; however it was powered by hand instead of hydraulically. In any case, this machine removed the whole plant with uniform root balls, which were then wrapped in burlap and taken to the lab for analysis. The only drawback was that this machine could only sample to a depth of 35 cm, which would not account for deep root production. Through this method root length, angle, number, area, volume, and weight of the root system in the 0 – 35 cm profile could all be ascertained. Rivers and Faubin (1961) also noticed that reliable results could only be obtained by careful removal of the soil from the roots.

A novel method of root study in the field was developed by Peterson et al. (1979). Here they placed pre-weighed amounts of ammonium nitrate in plastic bags at varying depths and put a pin hole in each bag. Kentucky bluegrass was then sodded on the surface and root depth was measured when the fertilizer response became evident in the shoots. Through this method they were able to ascertain that Kentucky bluegrass roots penetrated to 15 cm shortly after sodding and continued to access nitrogen at 30 cm, 37.5

cm, and 45 cm depths by the third, fourth, and fifth growing seasons, respectively. Interestingly this method was used to repudiate the work of Sprague (1933) who stated that the majority of Kentucky bluegrass roots generally occupy the top 15 cm of soil, however these differences could have been due to the fact that Sprague used dry weight to quantify root growth whereas Peterson et al. (1979) only harvested fresh white roots.

On a larger scale soybean breeders Pantalone et al. (1996) took advantage of a tractor-drawn peanut (*Arachis hypogaea* L.) inverter to uniformly unearth a soybean cultivar trial for root evaluation. Although a destructive technique, it allowed for the large scale evaluation of rooting phenotypes. In addition they took a visual root score on a 0 – 10 scale, with 10 = 100% fibrous roots, as well as root weights, root surface area and root nodule numbers. They found that their visual root rating highly correlated with root surface area, root nodule number and root dry weight (r = 0.74, 0.83, and 0.80, respectively). Indicating that it may be adventitious to use a visual root rating rather then a time consuming root weight.

Many researchers prefer to study root systems under more controlled conditions in glasshouses and growth chambers. To facilitate analysis plants are often grown in sand-culture or hydroponics (Kim et al., 1999), which have the benefit of ease of processing, but sacrifice the effects of root penetration ability. Kim et al. (1999) evaluated 16 tall fescue cultivars and selections of four growth types (dwarf, turf, intermediate, and forage) in sand in 75 cm deep poly-vinyl chloride pipes. They determined that dwarf and turf-type cultivars and selections showed the best total root production. However differences between this study and others (Carrow, 1996a) that suggest dwarf type tall fescues have decreased rooting could be do to differences in the rooting environment (Kim et al., 1999).

Evans (1970) studied the root growth of ryegrass seedlings in 100% sand in glass-sided containers and only focused on seedlings so that the effects of root viability would be negligible. Although this is an efficient screening procedure for seedling rooting ability it would only be applicable to seedling establishment and have little value in predicting the response of mature root systems. In addition these results would be difficult to extrapolate to the field based on the large differences in the root environments.

Taylor and Klepper (1974) were some of the first researches to develop and use a rudimentary rhizotron and rainout shelter to study root development in cotton (*Gossypium hirsutum* L.). Here they used glass-sided containers to view rooting in combination with makeshift shelters to stop unwanted rain. Then by using different irrigation regimes were able to study the root responses, non-destructively, to drought. Through this work Taylor and Klepper were able to correlate decreases in root growth with decreases in plant water potential and soil water content. This technique proved to be so successful that it led Ohio State University to build a rhizotron facility (Karnok and Kucharski, 1982). In their case they constructed a completely underground facility with different rhizotron-lysimeter stations, in combination with a high-tech weather station to measure and record all environmental variables. This site was used to evaluate the root growth of several cool-season grasses.

The combined work of Taylor and Klepper (1974) and Karnok and Kucharski (1982) led Upchurch and Ritchie (1983) to develop a mini-rhizotron (what we would think of as a modern day rhizotron). They used clear piping buried at angles in the turf system and used regular video recording equipment to monitor and evaluate the growth and development of the root system from above ground. This was very influential to the study of root systems and today almost all root field work is done in this manner.

Roots grow downward in the soil profile because of heavy amyloplasts in the root tips that direct growth based on gravity (Nilsen and Orcutt, 1996). Taylor et al. (1978) took advantage of this fact and evaluated soybean root growth in tubes held on an angle so that the longest root was always touching the wall of the tube and could be visually accessed in translucent piping. This technique was further developed by Lonkerd and Ritchie (1979) who added a partition to the system thus creating a split root observation system, consisting of glass walls and root subsections that enabled the evaluation of different root treatments on the same plant. These methods of root observation have been further refined and are now the standard in root analysis.

A popular method developed by Lehman and Engelke (1991) used flexible, sand-filled polyethylene tubes to screen creeping bentgrass germplasm for rooting phenotypes. This method was so effective it allowed them to calculate heritability estimates for rooting traits in creeping bentgrass. One of the reasons it is so powerful is because it enables researchers to investigate root systems non-destructively. Marcum et al. (1995) also used this method to successfully study zoysiagrass rooting characteristics.

This method was later modified by Bonos et al. (2004) which incorporated the work of Taylor et al. (1978) using a frame to hold the flexible tubes on a 30° angle, so the longest root could be visually identified and measured non-destructively throughout the course of the study. This method allowed for the selection of tall fescue and perennial ryegrass plants that had consistently more roots in the deeper profile combined with limited shoot growth in an effort to increase the drought avoidance of both species. This technique was very successful and resulted in significant gains from selection in the traits selected for in just a few generations.

Huang et al. (1997) used polyvinyl chloride tubes split down the middle and reattached to help make root harvesting more efficient. This increased the speed at which root harvesting took place and allowed for the efficient screening of time sensitive root traits like root viability to take place. Palazzo and Brar (1997) also had good results with acrylic piping grown on a 30° angle when evaluating fine fescues for sandy soil and indicated that this was a reliable method to study root length over time in these grass species.

In an effort to make root studies more realistic a number of researchers started to incorporate field soil into the sand based root zones with the hopes of making these studies more comparable to what is seen in the field. Huang and Gao (2000) incorporated two parts v/v of sterilized Chase silt loam into their root zones making the data more comparable while still facilitating root harvest, processing, and analysis. Rice breeders even attempted to separate rooting ability from root penetration using wax-petroleum layers at different depths within the root zone (Yu et al., 1995). The first screening was able to find germplasm with increased rooting and the second independent screening was used to evaluate the better rooters for penetration ability into hard or compacted layers. This method led to the identification of QTLs for rooting ability in rice (Zheng et al., 2000).

Marcum et al. (1995) evaluated 25 zoysiagrass cultivars and selections for root growth in a greenhouse under optimum conditions, then used correlation analysis to relate greenhouse rooting data to field performance of 11 zoysiagrass cultivars and selections that were determined previously in another study (White et al., 1993) under three levels of irrigation (no irrigation, 35% evapotranspiration, and 100% evapotranspiration). Cultivars ‘El Toro’ and ‘Meyer’ along with five experimental selections showed increased root weights in the 30 – 50 cm profile when compared to the other zoysiagrasses evaluated. When the data from Marcum et al. (1995) was analyzed with the data from White et al. (1993) percent green cover was correlated to average maximum root depth ($r = 0.71$, $p= 0.01$), root weight ($r = 0.72$, $p = 0.01$), root number in the 10 – 20 cm profile ($r = 0.67$, $p = 0.01$), root number in the 20 – 30 cm profile ($r = 0.78$, $p = 0.01$), and root number in the 30 – 40 cm profile ($r = 0.72$, $p = 0.01$). Based on the variation in rooting traits among cultivars and selections, the tight correlation of increased root weight and number on percent green cover under deficit irrigation, and the high heritability of rooting traits in turfgrasses (Lehman and Engelke, 1991), it should be possible to improve rooting characteristics while increasing field drought performance in zoysiagrass and other turfgrass species.

Carrow (1996a) evaluated six tall fescue cultivars and selections (‘Rebel II’, ‘Rebel Jr’, ‘Kentucky-31’, ‘Bonsai’, GA-5-EI, and GA5-EF) for turf quality, leaf firing, evapotranspiration rates, and root length density under field dry down in Griffin, Georgia. He concluded that drought tolerance based on the characteristics he evaluated ranged from best to worst in the following order: ‘Rebel II’ > ‘Rebel Jr.’ = ‘Kentucky-31’ = GA-5-EF = GA-5-EI > ‘Bonsai’. He also used multiple regression analysis to determine that root length density in the 20 – 60 cm depth was associated with less leaf firing and wilt; and that high root length density in the 3 – 10 cm depth was associated with more leaf firing and wilt (Carrow, 1996a).

Carrow (1996b) evaluated six popular warm-season grasses and two tall fescue cultivars. He determined that rooting in the 20 – 60 cm soil profile was variable for the species evaluated, ranking the seven grasses from best to worst as follows: 'Tifway' bermudagrass > 'Rebel Jr.' tall fescue = common bermudagrass > 'Kentucky-31' tall fescue = 'Raleigh' St. Augustinegrass > common centipedegrass > 'Meyer' zoysiagrass. Of the cultivars evaluated the entries with higher root length densities in the 20 – 60 cm profile also showed higher turf quality during drought. However, Carrow (1996b) also observed significant variation from his rooting data and the rooting data of others and suggested that differences in soil type could have been responsible.

Huang et al. (1997) studied the effects of soil dry down and rewatering on rooting in bermudagrass, centipedegrass, seashore paspalum, and zoysiagrass in sectioned poly-vinyl chloride tubes with four soil moisture treatments. They determined that drought resistance was associated with enhanced root growth, rapid root water uptake at deeper soil layers, maintenance of root viability in the drier profiles, and rapid root regeneration after watering. They also indicated that the multiple accessions of seashore paspalum exhibited variation in these characteristics; therefore it should be possible to select for and improve the rooting traits associated with increased drought resistance in other grasses.

Bonos and Murphy (1999) evaluated 10 Kentucky bluegrass cultivars under summer stress. Canopy characteristics, root and shoot responses, and water depletion patterns were all evaluated. The tolerant cultivars and selections evaluated (A82-1182, A84-563, 'Baltimore City', Bel 21, and RSP) exhibited 19% more roots in the 15 – 30 cm profile, and 65% more roots in the 30 – 45 cm profile than intolerant entries ('Nutop', 'Washington', 'Nublue', 'Amazon', and H86-697). This research indicates that increased rooting in the deeper soil profiles where water is still available is responsible for the increased physiological performance of the tolerant entries (Bonos and Murphy, 1999)

Karcher et al. (2008) showed a positive correlation between tall fescue populations selected in greenhouse screenings with high root to shoot ratio's and good drought tolerance in the field, however, Richardson et al. (2008) could not do the same in Kentucky bluegrass, thus reinforcing the complicated nature of root evaluation and indicating that other mechanisms must be in place to protect plants from drought, as well as, the need for further research.

White et al. (1993) evaluated four tall fescue cultivars ('Arid', 'Bonsai', 'Rebel Jr.', and 'Kentucky-31') for their rooting characteristics and water relations in a greenhouse study. They found that tall fescue cultivars vary in both drought avoidance characteristics (as measured by root length density) and drought tolerance characteristics (as measured by maintenance of relative water content under drought). Arid has better drought avoidance characteristics then 'Bonsai' based on root length density. They found no significant differences in relative water content between the cultivars, but this study was not conducted under stress. White et al. (1993) suggested that drought tolerance and drought avoidance were independent.

Roots have also been implicated in response to plant heat tolerance. The plant roots are very sensitive to abiotic stresses. It has even been shown that high soil temperatures are more detrimental to plant function then high air temperatures when evaluated separately (Kuroyanagi and Paulsen, 1988; Udompraset et al., 1995; Xu and Huang, 2000a,b, 2001b). Given this complication the effects of summer stress function to limit water availability in the soil while high temperatures reduce root growth and increase root death which exacerbates the problem and hastens the detrimental effects on the plant exponentially. Therefore, the development of cultivars with broad stress tolerance is necessary to improve turf growth and persistence in the field during the summer.

Kuroyanagi and Paulsen (1988) evaluated the effects of differential air/soil temperatures in *Triticum aestivum* during reproductive growth. They found that high root temperatures increased the activity protease and RNase enzymes leading to degradation of chlorophyll, protein, and RNA from shoots. Whereas high shoot temperature disrupted the export of cytokinins and reduced leaf protease activity which resulted in the senescence of reproductive growth. They concluded that both high air or soil temperatures hastened senescence and reduced reproductive growth (Kuroyanagi and Paulsen, 1988).

Udompraset et al. (1995) studied the effects of root zone temperatures on leaf gas exchange in two bean species (*Phaseolus acutifolius* L. and *P. vulgaris* L.) differing in heat tolerance. They determined that high soil temperatures reduced carbon exchange rates and subsequent growth. They also found that lowering soil temperatures to that of the control restored carbon exchange rates to that of the control. Since there was no difference in leaf temperature they concluded that the reduced carbon exchange rates, decreased stomatal conductance, and reduced growth was due to increases in root zone temperatures (Udompraset et al., 1995).

Xu and Huang (2000a) evaluated creeping bentgrass response to changes in air and soil temperatures. Creeping bentgrass plants were exposed to four air/soil temperature regimes. They found that the combined effects of high air and soil temperature were more detrimental than high air or soil temperature alone. They also determined that root growth, canopy photosynthesis, photochemical efficiency, and turf quality could be increased by decreasing the soil temperature alone. This shows the importance of root growth and viability in the tolerance to long term heat stress and also suggests management practices that can cool the root zone could be a viable way to manage heat stress events in plants (Xu and Huang, 2000a). The results reported here are in accordance with results on other crops indicating that elevated soil temperatures are more detrimental to plant growth then ambient temperatures (Udompraset et al., 1995).

Xu and Huang (2000b) evaluated ‘L-93’ and ‘Penncross’ under differential air and soil temperatures to determine the effects of either on carbohydrate metabolism. Interestingly in all of the high temperature treatments respiration increased with the largest increases from high temperature treatments in the root zone. In any case, depending on cultivar or treatment evaluated carbon consumption exceeded carbon production by 2 – 12 times, and also reduced total nonstructural carbohydrates in both the leaves and roots. They concluded that high soil temperatures cause an imbalance between photosynthesis and respiration, resulting in decreased carbohydrate content and canopy decline (Xu and Huang, 2000b), which is in total agreement with Nilsen and Orcutt (1996).

They took this idea one step further and compared the same creeping bentgrass cultivars to determine the minimum temperature reduction in the root system necessary to alleviate the signs of heat stress. They found that the positive affects of lower soil temperatures was more pronounced on the heat sensitive cultivar, 'Penncross'. In conclusion they determined that a 3°C drop in root zone temperature was effective in offsetting the effects of 35°C ambient temperatures (Xu and Huang, 2001b).

Xu and Huang (2001a) looked at two creeping bentgrass cultivars ('L-93' and 'Penncross') on the basis of photosynthetic capacity, tiller, and root growth under heat stress. They showed 'L-93' to be heat tolerant and 'Penncross' to be heat sensitive based on canopy photosynthesis. 'L-93' also showed better plant density, higher root to tiller ratios, and more fine roots than 'Penncross' under heat stress. They went on to conclude that breeders could select for narrow leaves, small plants, dense tillers, big root systems, and high root to shoot ratios to develop heat tolerant cultivars (Xu and Huang, 2001a).

Root morphology is a variable trait that exhibits intraspecific diversity. Rooting characteristics were shown to be quite variable in Kentucky bluegrass (Bonos and Murphy, 1999; Lehman and Engelke, 1993; Richardson et al., 2008), perennial ryegrass and tall fescue (Bonos et al., 2004; Karcher et al., 2008), and creeping bentgrass (Lehman and Engelke, 1991). These characteristics were also shown to have high heritability estimates. Lehman and Engelke (1991) showed creeping bentgrass root weight at the 41 – 50 cm depth to have a narrow-sense heritability of 0.82. Indicating that progress increasing drought avoidance characteristics through rooting could be made, however they also pointed out that there was no significant correlation to the above ground characteristics they studied (tiller number or shoot weight) indicating that selection procedures must be carried out on the root system. Their method of root screening using flexible tubes should work well in evaluating other grasses for increased drought avoidance characteristics.

Bonos and colleagues (2004) performed a similar study on perennial ryegrass and tall fescue populations. By selecting for two traits simultaneously: increased rooting in the 30 – 60 cm profile and decreased shoot weight, they were able to show gains from selection for increased root mass in the 30 – 60 cm zone after two cycles of selection ranging from 81 and 130% in tall fescue and perennial ryegrass, respectively. These results suggest that not only can we find segregants capable of increased root growth but we can stably pass these characteristics on to future generations.

Molecular Approaches to Evaluate Plants for Increased Root Growth

Given the difficulties evaluating rooting ability in plants the selection of plants with increased rooting ability is difficult. Either limited amounts of germplasm can be thoroughly studied or large amounts of germplasm can be superficially evaluated, in either case, the breeder is unable to screen all of the available germplasm and good plants may be discarded. This makes rooting traits prime candidates for marker assisted selection. Although not yet utilized in turfgrass species this technique is commonly used in a number of grass crops.

Given the importance of rooting to plant growth and development as well as their interaction with all types of abiotic stress tolerance and the difficulties associated with their evaluation and analysis, and assuming a genetic map of the species can be obtained, rooting ability would be a good candidate for marker assisted selection (MAS) in turfgrass. If the genes for improved rooting can be identified through genotypic analysis and markers can be found to hybridize with these areas then the presence or absence of the marker can be used to quickly tell if the plant is capable of increased rooting. Furthermore, these traits should be identifiable even at the seed or seedling stage and could provide a efficient method of selecting germplasm for further field evaluation. These techniques are currently used on various crops including corn, wheat, and rice therefore it should be possible to adapt these technologies to turfgrass.

Price and Tomos (1997) used restriction fragment length polymorphisms (RFLP) to identify gene segments on chromosome 11 in rice that consistently correlated to the plants with the longest root lengths in a hydroponic screen. Through this work quantitative trait loci (QTL) for root length in rice have been developed and can be used to identify rice seedlings with the section of chromosome 11 that confers long root length. However the application of this technology will be based on how tightly linked the marker is to the QTL and how this trait is expressed in the field.

Zheng et al. (2000) evaluated rice for root-penetration ability through a wax petroleum layer system and overall root thickness. They identified four QTLs for root penetration ability, four QTLs for root thickness, two QTLs for penetrated root number, and two QTLs for total root number. Collectively the QTLs identified accounted for 16.4% of the phenotypic variation. After comparative testing across two populations and different environments the four QTLs for root penetration ability were found to be consistent. This indicates that markers associated with the four root penetration ability

QTLs could be used in MAS to help select rice with increased root penetrating ability (Zheng et al., 2000).

Shen et al. (2001) identified QTLs for rooting parameters from an evaluation of a cross between IR64 and ‘Axucena’, a drought susceptible by drought resistant variety, respectively. They then started a MAS backcross program to introgress the beneficial genes for rooting parameters into IR64. The selection of the backcross progeny was based solely on the presence or absence of the marker. 29 backcross lines were then compared under multiple environments to IR64 for rooting traits. One of the near isogenic lines showed significantly improved rooting ability when compared to IR64, which suggests it is possible to use MAS to increase rooting traits in rice.

Steele et al. (2006) identified QTLs affecting root depth in rice by studying a doubled haploid population from a cross between a drought susceptible and drought resistant rice line (‘Kalinga III’ x ‘Axucena’, respectively). They then used these QTLs in a MAS backcross program to select plants containing the insert in eight successive backcross generations using 3000 marker assays in 323 near isogenic lines. 22 of which were field screened under both optimum and drought stressed conditions for rooting traits. They identified a segment on chromosome 9 that significantly increased root length under all of the environments evaluated. Markers associated with this section could be used to select for increased root growth in rice (Steele et al., 2006).

Huff (1997) used random amplified polymorphic DNA (RAPD) markers to survey the variation both within and between perennial ryegrass populations as well as to characterize perennial ryegrass cultivars. Huff (1997) showed that RAPD marker frequency strongly associated with the breeding history of any given selection. He also showed that it was difficult to separate genetically similar populations using this technique. Based on his results Huff (1997) concluded that RAPD characterization could be useful in supplementing morphological and agronomic data used in plant variety protection.

Kubik et al. (2001) similarly screened perennial ryegrass cultivars for genetic diversity, however, they used simple sequence repeats (SSRs). Their analysis showed that all of the cultivars showed high levels of genetic diversity. Furthermore, the SSRs used in this study were able to identify an additional 210 individuals to their respective cultivar. Kubik et al. (2001) using SSRs was more successful at cultivar characterization then Huff (1997) using RAPDs, indicating that SSRs might be a better alternative when evaluating ryegrass. Guthridge et al. (2001) used amplified fragment length polymorphisms (AFLPs) to analyze the genetic diversity of perennial ryegrass populations. They found significant differences in within population diversity and were also able to identify plants to their respective cultivars. They concluded that AFLPs could be used to detect and quantify genetic variation in perennial ryegrass.

Yamada et al. (2004) used QTL analysis to study the morphological and agronomic traits in perennial ryegrass. They evaluated plants for winter hardiness, frost tolerance, heading date, height, tiller size, leaf length and width, plant type, spikelet number and spike length. They found QTLs associated with frost tolerance and heading date and concluded that these markers could be used in MAS to help improve these qualities.

Although the progress of MAS in turfgrass is clearly lagging behind that of rice it should be possible to further develop this technology and identify more QTLs that are more applicable to turf-type characteristics. The development of segregating mapping populations for traits like drought tolerance or root growth would enable molecular biologist to identify QTLs associated with beneficial rooting traits. Then incorporation of MAS techniques into traditional breeding systems could help to increase root growth in the cool-season grasses, without the guesswork associated with root analysis, leading to an increase in the benefits associated with larger and more productive root systems, like its associated drought and heat resistance. These techniques have the ability to revolutionize the way we screen cultivars and selections for rooting ability and could vastly increase our ability to improve rooting characteristics.

Objectives

Therefore the objectives of this research are to i. Quantify the extent of variability within Kentucky bluegrass for morphological and agronomic traits, simplify the variability of the current Kentucky bluegrass cultivars and selections into useful groups, and determine the genetic transmissibility of the characteristics evaluated. ii. Quantify the range of response of a diverse set of Kentucky bluegrass cultivars and selections to bispyribac-sodium herbicide, a novel annual bluegrass control method. iii. Determine if fertility could be used as a management practice to reduce Kentucky bluegrass chlorosis in response to bispyribac-sodium herbicide and make it more attractive to the Kentucky bluegrass manager. iv. Determine the extent of variability in rooting morphology of Kentucky bluegrass cultivars and selections under heat stress and to investigate any correlations that may exist between rooting and summer performance in the field.

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CHAPTER 1

Classification and Inheritance of Morphological and Agronomic Characteristics in Kentucky Bluegrass

Abstract. The apomictic breeding behavior of Kentucky bluegrass (*Poa pratensis* L.) results in many unique cultivars. A classification system was previously developed to characterize the large number of Kentucky bluegrass cultivars into different types. However many new cultivars have been released since the last refinement of the classification system. The objectives of this study were to determine differences in morphological and agronomic characteristics among select Kentucky bluegrass cultivars representing the major classification types and to determine broad-sense heritability estimates for important morphological (plant height, panicle length, flag leaf height, and flag leaf length and width) and agronomic (rhizome spread) traits in Kentucky bluegrass. A spaced-plant nursery trial was established in the spring of 2003 at Adelphia, NJ. One-hundred seventy-three cultivars and selections were planted in a randomized complete block design with three replications. The morphological and agronomic traits listed above were measured on spaced-plants. High Density type cultivars (formerly the Aggressive type cultivars) had the most prostrate growth habit with plant heights of 33 and 43 cm in 2004 and 2005, respectively. Mid-Atlantic and Texas x Kentucky bluegrass hybrids had the widest rhizome spread (Mid-Atlantic = 73 and 121 cm; Texas x Kentucky bluegrass hybrids = 72 and 122 cm) in 2004 and 2005, respectively. Broad-sense

heritability estimates were high for plant height (H = 0.84), panicle length (H = 0.88), flag leaf height (H = 0.85), and rhizome spread (H = 0.85), moderate for flag leaf length (H = 0.71) and low for flag leaf width (H = 0.11). This study characterizes new cultivars into respective groups and identifies the genetic inheritance of important morphological and agronomic traits in Kentucky bluegrass.

Kentucky bluegrass (*Poa pratensis* L.) is a perennial turfgrass species that is widely adapted to many distinct environments. The extensive rhizome system of Kentucky bluegrass gives it the ability to tolerate and recover from many environmental stresses more quickly than bunch-type species (Bonos and Murphy, 1999; Meyer and Funk, 1989).

Kentucky bluegrass reproduces through an asexual process called apomixis. Apomixis allows for the development of true-to-type seed from the maternal plant (Bashaw and Funk, 1987; Funk, 2000; Turgeon, 1999). It reduces out-crossing, which serves to prevent recombination and fix hybrid vigor (Bicknell and Koltunow, 2004). This results in many unique genotypes of Kentucky bluegrass. This is beneficial because once superior genotypes are identified they can be preserved through apomixis and produce uniform, stable cultivars (Bashaw and Funk, 1987; Funk, 2000).

A classification system was previously developed to characterize the large number of Kentucky bluegrass cultivars based on common growth and performance characteristics (Bara et al., 1993; Bonos et al., 2000; Murphy et al., 1997). This classification system was developed to characterize the similarities and differences between cultivars and as a guide to help turfgrass managers increase diversity and uniformity when developing blends of Kentucky bluegrass. The description of the classification types has been previously reported (Bonos et al., 2000; Murphy et al., 1997). Many new Kentucky bluegrasses have been developed since the previous classification based on spaced-plants was completed and other older cultivars have been discontinued (Bonos et al., 2000; Bonos, 2007). Therefore, we felt it was important to characterize recently-developed Kentucky bluegrass cultivars that are or will become commercially available.

Morphological and agronomic characteristics are utilized by breeders in the development of improved cultivars and by managers for specific cultivar selection. Plant height and rhizome spread are useful characteristics because a low growing, aggressive spreading cultivar should be able to tolerate lower heights of cut, recover quickly and fill in damaged areas (Meyer and Funk, 1989). Leaf texture (measured here as leaf width) is also an important characteristic used in the development of new turfgrass cultivars. Fine leaf texture is typically correlated with higher shoot density in mowed turf situations (Bashaw and Funk, 1987; Turgeon, 1999). Turfgrass breeders have been selecting for higher shoot density for decades (Meyer and Funk, 1989) because it results in improved turf quality and better weed competition.

Since these traits are widely used to predict cultivar performance in a number of different environments, it would be helpful to turfgrass breeders to know the heritability of these morphological traits in Kentucky bluegrass. Broad-sense heritability estimates the total genetic effects influencing a trait and includes additive, dominance and epistatic effects (Nyquist, 1991; Poehlman and Sleper, 1995). In cross-pollinated species, narrow-sense heritability is typically more useful to plant breeders because it measures the additive gene effects, which are passed on to the progeny more predictably than dominant or epistatic gene effects (Poehlman and Sleper, 1995). However, when working with apomictic or asexually propagated crops, where hybrid vigor and both additive and non-additive gene action are fixed, estimates of broad-sense heritability are more appropriate (Poehlman and Sleper, 1995). This technique has been utilized by Berry et al. (1969) to calculate heritability of several agronomic traits in Kentucky bluegrass. Heritability partitions the phenotypic variation into genetic and environmental components so that the effect of the environment on a specific trait can be determined. Characters with low heritability are typically highly affected by the environment, and characters with high heritability are less affected by the environment. Therefore, characters with high heritability have little environmental influence and should be consistent in multiple environments.

Materials and Methods

One-hundred seventy-three Kentucky bluegrass cultivars and selections were evaluated in this study (Table 1). These cultivars and selections represented all of the entries included in the 2000 National Kentucky bluegrass test sponsored by the National Turfgrass Evaluation Program (NTEP) (Morris, 2000). Single seedlings were transplanted into 48-cell flats (90cm x 45cm), and allowed to establish in the greenhouse. The plants were screened for apomixis in the greenhouse and all off-types were discarded. The plants were then established in a spaced-plant nursery at the Rutgers University Plant Biology and Pathology Research and Extension Farm in Adelphia, NJ, in April of 2003, on a well-drained Freehold sandy loam (fine-loamy, mixed, mesic, Typic Hapludult). The experiment was established in a randomized complete block design with three replications. Four plants of each entry were planted per replicate for a total of twelve plants per entry. The field received 98 kg N ha^{-1} $year^{-1}$ using a 3.5 - 4.4 - 8.3 fertilizer (elemental N, P, and K, respectively), applied half in the spring and half in the fall. The field was mowed at a height of 20 cm twice per year, once after seed maturity, and once before the onset of winter. Labeled pre- (DCPA and dithiopyr) and post-emergence (dicamba, and halosulfuron) herbicides were used to control broadleaf weeds, sedges, and *P. annua*. The field was not irrigated and no severe disease outbreaks were observed.

The agronomic and morphological characteristics were measured on spaced-plants in 2004 and 2005 following the experimental methods required for Plant Variety Protection (PVP) applications (USDA, 2005). Plant morphological measurements (plant height, panicle length, flag leaf height, width, and length) were taken approximately two weeks after anthesis, when all plant panicles were fully expanded (late-May to mid-July in both years). Plant height was measured from the soil surface to the average height of the majority of the panicles. Rhizome spread was measured, after mowing, in two directions perpendicular to each other and represented the longest extending rhizomes in those two directions. Rhizome spread measurements were used to characterize cultivars and selections that have been described as having extensive, spreading rhizome systems. Rhizome spread, and plant height were taken on an individual plant basis, with each plant receiving one observation per plant. Data was collected on each of the four plants per replicate and averaged to obtain one value per replication. Panicle length, flag leaf height, width and length measurements were calculated as the average of three separate flowering culms from each of the four plants per replicate. Panicle length was measured from the bottom node to the tip of the longest panicle. Flag leaf height was measured as the distance between the soil surface and the collar of the flag leaf. Flag leaf blade width was measured at the widest point on the flag leaf. Flag leaf blade length was measured from the collar to the tip of the blade. Flag leaf length and width measurements were used to characterize those types with distinct leaf texture differences, such as narrow leaf blades. Due to the large number of entries and the hot temperatures of late spring/early summer, plants were measured in order of maturity; which helped to minimize differences in the plants response to desiccation caused by seed maturity.

Statistical analysis – means separation of types. In order to determine differences in morphological and agronomic characteristics between the Kentucky bluegrass classification types data from cultivars and selections within types were combined and subjected to analysis of variance. Means were separated using Fisher's protected least significant difference (LSD) at the 0.05 probability level (Table 2). Due to space restrictions the data on individual cultivar responses was not included in this manuscript. It was previously reported by Shortell et al. (2006).

Statistical analysis – broad-sense heritability. All characteristics were subjected to analysis of variance to determine broad-sense heritability estimates on an entry-mean basis. Broad-sense heritability estimates were determined from restricted maximum likelihood (REML) variance and covariance components using the random model of Proc MIXED (Bonos et al., 2004)(SAS Institute, Cary, NC). Ninety-five percent confidence intervals for heritability estimates were determined according to Steel et al. (1997). This technique (or a variation thereof) has previously been used to calculate broad-sense heritability from clonally replicated turfgrass plants (Berry et al., 1969; Bonos et al., 2003; Burton and Devane, 1953).

Statistical analysis – principal component analysis. The relationships between cultivars and selections based on all morphological characteristics were tested using multivariate principal component analysis (PCA) PROC PRINCOM (SAS Institute, 2001) as used by Bonos et al. (2000) (Figures 1 and 2). One-hundred seventy-three cultivars and selections and seven morphological variables were included in the data set analyzed. However, the addition of the 'Other' group of cultivars (66 cultivars or selections which possess characteristics intermediate between the two groups) in the principal component analysis graph made it difficult to see relationships between cultivars and selections within the major groups. Therefore, cultivars and selections in the 'Other' group were omitted from the principal component analysis graph (Figures 1 and 2, Table 1).

Results and Discussion

Significant differences between types were observed for all morphological measurements except flag leaf length and width in both years of the study (Table 2). Measurements in both 2004 and 2005, under spaced-plant conditions, indicated that the High Density (formerly Aggressive type), BVMG, and Compact (including Compact-Midnight and Compact-America) type cultivars evaluated in this study had the shortest plant heights. Common type cultivars and selections were the tallest entries in both years of the study. This is consistent with previous research (Bonos et al., 2000; Burt and Christians, 1990; and Ebdon et al., 1998). Mid-Atlantic type and Texas x Kentucky bluegrass hybrids were intermediate in height. Compact and Compact-Midnight type entries had similar morphological traits in both years. Compact-America type cultivars and selections exhibited similar morphological traits to the BVMG types.

High broad-sense heritability estimates were observed for traits affecting plant stature (plant height: H = 0.84; panicle length: H = 0.88; and flag leaf height: H = 0.85 [Table 3]). High heritability estimates have also been observed for plant height measurements in Kentucky bluegrass (Pepin and Funk, 1974) and maize (*Zea mays* L.) (Soleri and Smith, 2002).

Low growth habit is a trait often selected for in turfgrass breeding (Meyer and Funk, 1989). Plants with lower growth habits can typically tolerate closer mowing due to the lower placement of the crown relative to the soil surface and the slower rate of leaf elongation (Meyer and Funk, 1989; Turgeon, 1999). The high broad-sense heritability estimates observed for these traits indicate that they are under strong genetic control and not strongly affected by the environment. This indicates that selected plants should maintain a low growth habit across differing environments.

Rhizome spread is an indication of rhizome elongation and vigorous lateral growth. This is a beneficial characteristic in Kentucky bluegrass because plants with more lateral spread should be able to recover more quickly from stresses including mowing or traffic. Mid-Atlantic type cultivars, Texas x Kentucky bluegrass hybrids, and Common type cultivars exhibited the most extensive rhizome spread (Table 2). This is a defining attribute of these types (Bonos et al., 2000; Murphy et al., 1997; Shortell et al., 2005), so it is not surprising that they exhibited the farthest spreading capability. Cultivars within the High Density and Compact-America types exhibited the shortest rhizome spread (Table 2) when evaluated as spaced-plants.

The results for the High Density (formerly Aggressive) type cultivars are somewhat contradictory since this type exhibited aggressive rhizome growth in a previous study (Bonos et al., 2000). However, the only cultivar replicated in both trials was 'Limousine' and when grown as a spaced-plant in NJ, Limousine is very decumbent and slow growing (personal observation). Previous studies have suggested that this type may dominate blends or mixtures at lawn type cutting heights (Bonos et al., 2000; Murphy et al., 1997, Park et al., 2005). However, at fairway cutting heights this group did not exhibit improved wear tolerance (Shortell et al., 2005; Park et al., 2005), which would be expected from a cultivar with aggressive spreading capabilities. Because of these contradictory results, this group has been renamed the High Density type (Shortell et al., 2006) because they may not exhibit aggressive spreading characteristics in all environments.

High broad-sense heritability estimates were observed for rhizome spread measurements (Table 3) which supports previous data collected on rhizome spread in Kentucky bluegrass (Berry et. al., 1969; Pepin and Funk, 1974) and sand bluestem (*Andropogon hallii* Hack.) (Kneebone, 1958). However selection for this trait when grown as a spaced-plant is not necessarily correlated to aggressive lateral rhizome spread when maintained under frequently mowed (Brosnan et al., 2005) or worn turf conditions (Park et al., 2005).

There were no significant differences between Kentucky bluegrass classification types for flag leaf length or width, indicating that these measurements were not useful in differentiating the classification types. Additionally flag leaf length was moderately heritable (H = 0.71) and flag leaf width was not heritable (H = 0.11) (data not shown). These results indicate that selection for fine or wide leaf texture may be difficult and that the phenotype may not be the same across environments. This agrees with the data reported by Simon (1999) on wheat (*Triticum aestivum* L.). However, earlier research on Kentucky bluegrass (Berry et al., 1969), perennial ryegrass (*Lolium perenne* L.) (Rogers, 1989), and bermudagrass (*Cynodon dactylon* [L.] Pers.) (Wofford and Baltensperger, 1985) has shown this trait to be more heritable.

Not surprisingly, leaf texture (or leaf width) has been shown to be correlated with plant and/or tiller density (Turgeon, 1999). Thereby, plants that have a high tiller density also have finer leaves and plants with low tiller density have wider leaves (Nilsen and Orcutt, 1996). This can be partially attributed to the space available for the plants to grow within the turfgrass community. If plants are not competing for space, leaves have the opportunity to elongate and widen to maximize photosynthesis (Nilsen and Orcutt, 1996). Additionally, as plants mature, flowering culms begin to desiccate and flag leaves start to senesce. Due to the considerable number of plants to measure and a short time frame for collecting this data in the field flag leaf width measurements may have been partly influenced by plant desiccation. Flag leaf width is a standard measurement required for Plant Variety Protection (PVP) applications. Claims are often made regarding variety performance compared to other cultivars. The low heritability of this trait indicates that the flag leaf width measurement may not be as reproducible as once perceived.

Principal component analysis is used to take a large number of variables and convert them into a smaller number of informative factors that account for more variation then any one variable alone. These factors, or principal components, can then be visualized to determine whether or not any correlations exist. Principal components are created through matrix algebra using SAS. The residuals from a least squares estimation of the morphological parameters are used to construct a matrix. Principal components 1 – 6 are generated via a singular value decomposition of this matrix (Saxton, 2004). The resulting components are then ordered and the variation can be graphed.

Principal component 1 (PC1) and Principal component 2 (PC2) in 2004 accounted for 68.7% and 22.5% of the variation, respectively, or 91.2% of the total variation (Table 4). Principal component 3 (PC3) raised the total variation to 95.6% but did not change the overall pattern of the graph, and therefore is not presented. PC1 and PC2 in 2005 accounted for 73.3% and 21.6% of the variation, respectively, or 94.9% of the total variation (Table 4). PC3 raised the total variation to 99.8% but did not change the overall pattern and therefore is not presented. Rhizome spread had the strongest influence on

PC1 and plant height had the strongest influence on PC2 in both 2004 and 2005. Certain cultivars and selections formed distinct clusters including the Common, Shamrock, Compact-America, Compact-Midnight, and BVMG types in both 2004 and 2005 (Figs. 1 and 2). However, Compact-America types were more widely dispersed than the other groups in 2005.

Compact-America and Compact-Midnight cultivars did segregate, but the general Compact type was dispersed across both Compact-Midnight and Compact-America types. Mid-Atlantic, Texas x Kentucky bluegrass hybrids, and to some degree the Shamrock types were morphologically similar based on the traits studied in this experiment. This could be due to the high correlation of PC1 to rhizome spread measurements. Julia types tended to weakly cluster within the Compact type grouping in 2004 (Fig. 1).

Similar clustering results were reported by Curley and Jung (2004) who evaluated a set of random amplified polymorphic DNA markers among Kentucky bluegrass cultivars and selections. They also found that Compact-Midnight type cultivars formed a distinct cluster separate from the other types evaluated in their study (Curley and Jung, 2004). In our study, Compact-Midnight types also formed a tight cluster but it was within the general Compact type. Curley and Jung's genetic analysis with molecular markers also agrees with our morphological classification of the Common and BVMG types. This concordance between morphological and molecular based analysis helps support the Kentucky bluegrass classification system and further work using a combined approach could help add cultivars and selections from the Other type into more defined groups. This combination of approaches could also help clarify existing classification groups, and help discover new groups with unique traits and uses.

We found significant differences in morphological and agronomic traits between the classification types of Kentucky bluegrass, which is consistent with previous research. However, unlike previous studies which are now outdated, this project reports on new, recently-developed Kentucky bluegrass cultivars. High density, BVMG, and Compact (including Compact-Midnight and Compact-America) type cultivars has the shortest plant heights. Mid-Atlantic, Texas x Kentucky bluegrass hybrids, and Common type cultivars exhibited the most extensive rhizome spread. Principal component analysis grouped Compact-Midnight and Compact-America types as clusters within the general Compact type. Common types were consistently grouped separately from the other groups, while Mid-Atlantic, Texas x Kentucky bluegrass, and to some extent Shamrock types were grouped in close proximity to each other indicating that these groups have similar morphological characteristics. Principal component analysis results based on morphological measurements were similar to results based on molecular markers indicating that the similarity between groups is consistent with both marker types and should both be useful to help classify Kentucky bluegrass further.

Based on the data reported here it should be possible to select Kentucky bluegrass genotypes with certain morphological and agronomic traits including plant height, rhizome spread, panicle height, flag leaf height and length that behave consistently across environments. This information will help breeders be more successful in developing cultivars with important morphological and agronomic characteristics. It will also be useful to turfgrass managers in selecting blends of Kentucky bluegrass cultivars with complementary characteristics.

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Figure 1. Principal component analysis of Kentucky bluegrass cultivars and selections based on morphological characteristics in 2004. PC1 accounted for 68.7% of the variation and was strongly influenced by rhizome spread. PC2 accounted for 22.5% of the variation and was strongly influenced by plant height.

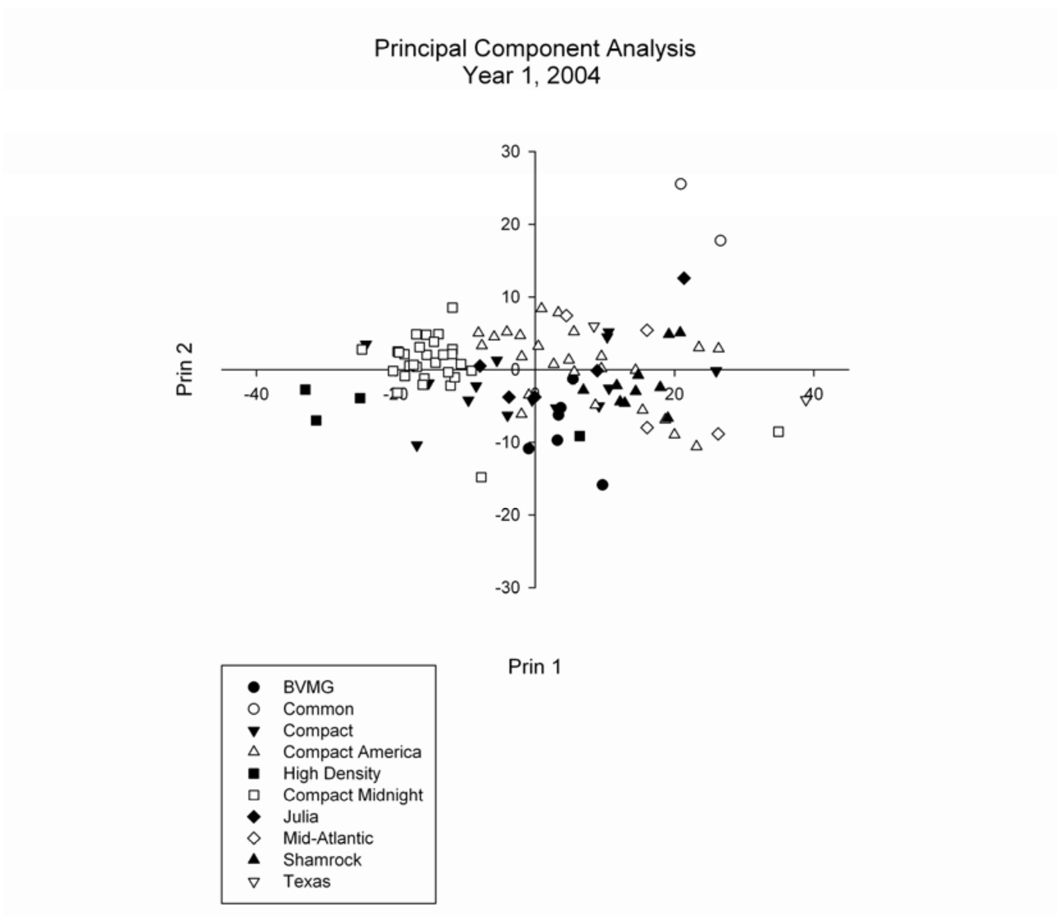

Figure 2. Principal component analysis of Kentucky bluegrass cultivars and selections based on morphological characteristics in 2005. PC1 accounted for 73.3% of the variation and was strongly influenced by rhizome spread. PC2 accounted for 21.6% of the variation and was strongly influenced by plant height.

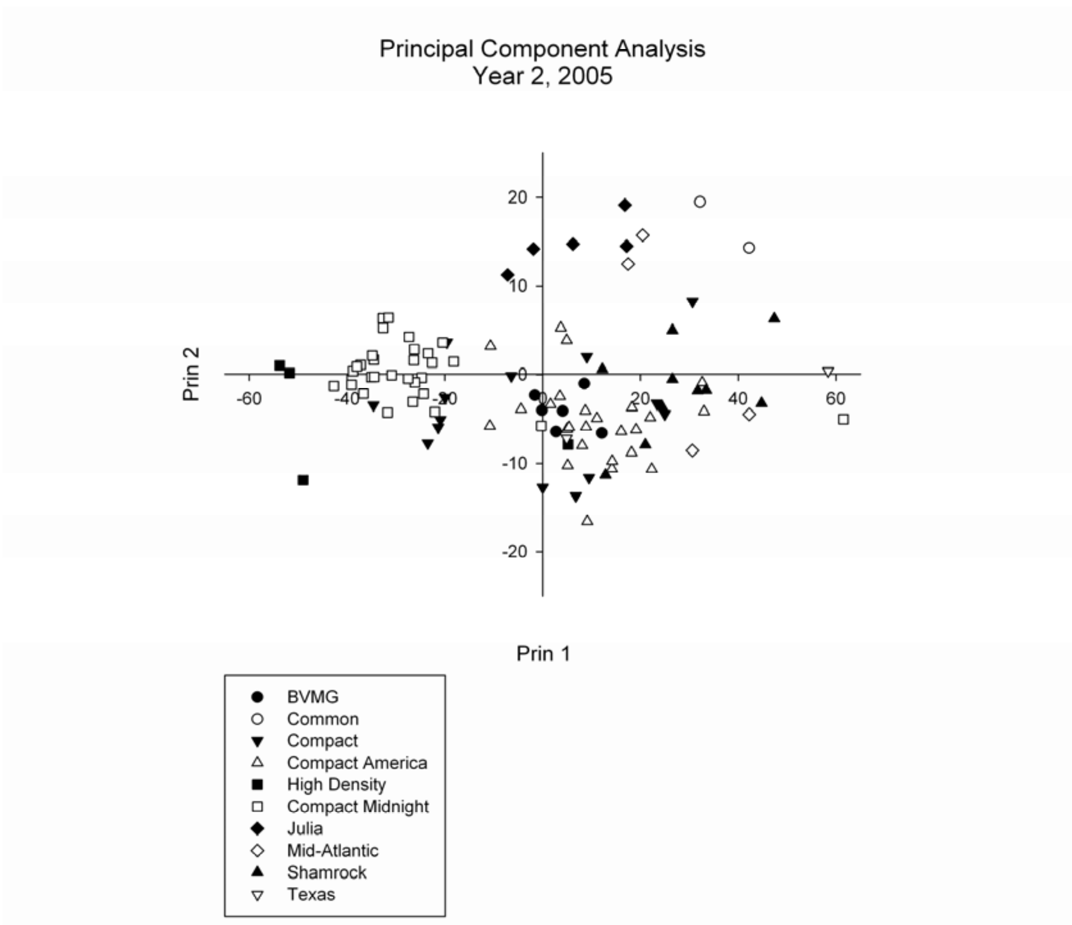

Table 1. Kentucky bluegrass (*Poa pratensis* L.) cultivars and selections evaluated in a spaced-plant nursery established in May of 2003.

Classification Type	Cultivar or Selection
High Density	HV 140[z], Limousine, Julius, North Star, Bariris
BVMG	Baron, Envicta, Goldrush, Abbey, Raven, Marquis
Common	Wellington, Kenblue
Compact	Wildwood, Hallmark, PST-B5-125[z], Blue-tastic, PST-B4-246[z], Alpine, Ascot, Ba 82-288[z], Skye, Diva, Goldstar, Princeton P-105, Chicago II, Moon Shadow, Moonlight, Blackstone
Compact-America	Bedazzled, Royale, Glenmont, PST-604[z], Brilliant, PST-222[z], Mallard, Apollo, Delight, Arrow, Dynamo, Casablanca, PST-H6-150[z], Valor, Boutique, Showcase, Kingfisher, Sonoma, Bordeaux, SR 2284, Langara, PST-B3-170[z], Baroness, Barnique, BAR Pp 0566[z], BAR Pp 0573[z], Unique
Compact-Midnight	Midnight, Midnight II, Quantum Leap, Arcadia, Unknown, Impact, Total Eclipse, Odyssey, NuGlade, Perfection, Tsunami, Ginney, Courtyard, Alexa, J-2885[z], Blue Velvet, Everest, Awesome, Excursion, Freedom III, EverGlade, Nu Destiny, Barrister, Beyond, Rugby II, Award, Freedom II, Liberator, Bluestone
CELA	Jefferson[y]
Julia	Avalanche, Pick 453[z], Rampart, H92-558[z], Julia
Mid–Atlantic	Eagleton, PST-161[z], Cabernet, Appalachian
Shamrock	Shamrock, Lakeshore, Moonshine, Katie, Brooklawn, Champagne, Durham, Mongoose, A98-1028[z], Champlain
Texas x Kentucky bluegrass Hybrid	Thermal Blue, Longhorn
Other	Lily, Limerick, Bodacious, Boomerang, Cheetah, Yvette, Pp H 7929[z], Ulysses, Pp H 7907[z], Monte Carlo, Coventry, PST-108-79[z], Voyager II, Bluemax, PST-York Harbor 4, Blacksburg II, Blue Ridge, HV 238[z], Mercury, B5-43[z], B5-45[z], Markham, B5-144[z], Misty, Fairfax, Baronette, Ba 83-113[z], Ba 84-140[z], Chateau, , CVB-20631[z], Chelsea, A97-1409[z], Allure, Blue Sapphire, NA-K992[z], SRX 26351[z], SRX 27921[z], Jewel, Blue Knight, DLF-76-9032[z], DLF-76-9034[z], DLF-76-9036[z], DLF-76-9037[z], SI A96-386[z], SRX 2114[z], SRX QG-245[z], 99AN-53[z], A98-407[z], A98-183[z], Royce, A98-365[z], Rambo, A96-739[z], PST-H5-35[z], B4-128A[z], Washington, A96-742[z], A97-857[z], Bartitia, Baritone, Barzan, Baronie, Serene, Rita

[z]Denotes experimental selection
[y]Not included in principle component analysis or Table 3 due to the limited number of cultivars representing this group

Table 2. Morphological and agronomic characteristics of Kentucky bluegrass classification types established in a field trial in May 2003.

	Plant Height		Panicle Length		Flag Leaf Height		Rhizome Spread		Flag Leaf Length		Flag Leaf Width	
Type	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
	cm											
Common	69	71	8	9	36	45	65	116	0.27	0.31	4.1	3.4
Julia	46	67	8	9	27	39	61	102	0.40	0.32	3.7	2.6
Mid-Atlantic	50	61	9	10	26	34	73	121	0.34	0.31	4.2	3.5
Shamrock	50	58	8	8	26	32	69	120	0.35	0.30	3.8	2.8
Other	45	55	8	8	24	32	58	101	0.33	0.29	3.7	3.1
TB x KB Hybrids[z]	51	53	8	8	34	39	72	122	0.35	0.33	5.1	3.6
Compact	47	53	8	8	25	29	62	108	0.31	0.28	3.7	3.0
BVMG	40	51	7	8	21	30	64	103	0.37	0.31	3.5	3.3
Compact-Midnight	43	50	8	8	23	29	60	101	0.31	0.28	4.1	3.3
Compact-America	38	47	7	7	23	30	47	79	0.31	0.29	4.2	3.3
High Density	33	43	5	6	15	23	47	74	0.31	0.26	3.1	2.5
$LSD_{0.05}$	5	6	1	2	3	5	8	6	NS	NS	NS	NS
CV%[y]	22	24	17	19	20	21	23	24	NS	NS	NS	NS

[z]Texas x Kentucky bluegrass hybrids

[y]The cv presented is the total cv for the analysis of variance

Table 3. Analysis of variance and broad-sense heritability estimates (H) of plant height, panicle length, flag leaf height, and rhizome spread of 173 Kentucky bluegrass cultivars and selections (includes all entries of the 2000 National Kentucky bluegrass test sponsored by NTEP) evaluated in 2004 and 2005 in a spaced-plant nursery established in Adelphia, NJ, in 2003.

	Plant Height				**Panicle Length**			
	MS	F value	P value	Var. Comp.[z]	MS	F value	P value	Var. Comp.
Year	71674.9	1242.7	0.0008		58.5	67.8	0.0144	
Rep[y]	6211.1	107.7	< 0.0001		1.9	2.2	0.1162	
Year x Rep	906.6	15.7	< 0.0001		14.3	16.5	< 0.0001	
Cult[x]	1274.1	22.1	< 0.0001	177.4	27.7	32.2	< 0.0001	4.1
Cult x Year	209.8	3.6	< 0.0001	19.1	3.3	3.8	< 0.0001	0.2
Cult x Rep x Year	95.1			15.9	2.0			0.3
	H = 0.835[w]				**H = 0.881**			

95% confidence interval for 3-replicate mean heritability= 0.88-0.79 (Plant Height)

95% confidence interval for 3-replicate mean heritability= 0.93-0.84 (Panicle Length)

	Flag Leaf Height				**Rhizome Spread**			
	MS	F value	P value	Var. Comp.	MS	F value	P value	Var. Comp.
Year	43923.7	2585.2	0.0004		1507579.2	14682.0	0.0001	
Rep	389.1	22.9	< 0.0001		4787.8	46.6	< 0.0001	
Year x Rep	545.2	32.1	< 0.0001		1899.5	18.5	< 0.0001	
Cult	517.5	30.5	< 0.0001	72.9	4588.4	44.7	< 0.0001	687.2
Cult x Year	79.9	4.7	< 0.0001	6.7	465.1	4.5	< 0.0001	34.9
Cult x Rep x Year	39.6			6.6	255.4			42.6
	H = 0.846				**H = 0.846**			

95% confidence interval for 3-replicate mean heritability= 0.89-0.80 (Flag Leaf Height)

95% confidence interval for 3-replicate mean heritability= 0.88-0.79 (Rhizome Spread)

[z]Variance components were determined using Restricted Maximum Likelihood Estimation (REML) using the random model of Proc Mixed Procedure in SAS (Cary, NC).
[y]Rep = replication.
[x]Cult = cultivar.
[w]Heritability was determined from variance components using the following equation = σ^2_C / σ^2_P, where $\sigma^2_P = \sigma^2_C + \sigma^2_{CY} + \sigma^2_{CRY}$, where P = phenotype, C = cultivar, R = replication and Y = year.

Table 4. Eigenvectors of the principal component axes (PC) from principal component analysis of Kentucky bluegrass types. Eigenvalues and their contribution to total variation are listed at the bottom of columns.

	PC1		PC2		PC3		PC4		PC5		PC6	
Character	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
	Eigenvectors											
Plant Height	0.41	0.27	0.70	0.92	-0.58	-0.30	0.04	-0.01	-0.05	0.00	0.00	0.00
Panicle Length	0.04	0.03	0.08	0.02	0.06	0.06	0.21	0.80	0.97	-0.60	-0.01	-0.01
Flag Leaf Height	0.21	0.12	0.54	0.27	0.81	0.95	-0.10	-0.06	-0.08	0.04	0.00	0.00
Flag Leaf Length	0.00	0.00	0.01	0.00	0.09	0.00	0.97	0.60	-0.21	0.80	-0.03	-0.01
Flag Leaf Width	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.01	0.00	1.00	1.00
Rhizome Spread	0.89	0.95	-0.46	-0.30	0.08	-0.03	0.00	-0.02	-0.01	0.01	0.00	0.00
Eigenvalue	249.98	553.14	81.72	162.95	16.08	36.73	14.97	1.40	0.90	0.44	0.00	0.00
Percentage of Total Variation	68.7	73.3	91.2	94.9	95.6	99.8	99.8	99.9	100.0	100.0	100.0	100.0

CHAPTER 2

Response of Kentucky Bluegrass Cultivars and Selections to Bispyribac-Sodium Herbicide

Abstract. Annual bluegrass (*Poa annua* L.) is a problematic weed in Kentucky bluegrass (*Poa pratensis* L.). Bispyribac-sodium herbicide can effectively control established annual bluegrass in other cool-season turfgrasses but unacceptable injury to Kentucky bluegrass has been reported. However, only a few Kentucky bluegrass cultivars have been evaluated. The objective of this study was to determine the extent of intra-specific variability among Kentucky bluegrass cultivars and selections to sequential applications of bispyribac-sodium herbicide. Field experiments were conducted in 2004 and 2005 in New Jersey to determine the response of fifty-five Kentucky bluegrass cultivars and selections to bispyribac-sodium. The herbicide was applied at 188 g/ha followed three weeks later by a second application of 281 g/ha. Kentucky bluegrass injury ranged from eight to 93% eight weeks after initial treatment (WAIT). 'Blackstone', 'Serene', and A98-962 were the most tolerant to bispyribac-sodium; exhibiting less than 20% injury eight WAIT. Conversely, 'Washington', 95AN-10, and 'Avalanche' were the most susceptible with up to 93% injury eight WAIT. The range in tolerance to bispyribac-sodium within Kentucky bluegrass indicates the potential for the identification and development of cultivars with improved tolerance to bispyribac-sodium herbicide.

Kentucky bluegrass (*Poa pratensis* L.) is a popular turfgrass species throughout its adaptive range. It typically produces a dense stand of turf with dark green color, high overall turf quality, and a wide range of disease tolerance. This makes it a premier species for lawns, sports fields, and golf courses (Beard, 1973). However, Kentucky bluegrass often becomes infested with annual bluegrass (*P. annua* L.), especially when maintained at low mowing heights and other stressful environments.

Annual bluegrass is a persistent weed which reduces overall turfgrass utility, due to its light green color, high reproductive capacity, and shallow root system (Lush, 1989; Sprague and Burton, 1937). Annual bluegrass tolerates close mowing and germinates rapidly allowing it to out-compete desirable turfgrass species. This is particularly pronounced in Kentucky bluegrass due to its slow establishment rate. It can also encroach into stressed Kentucky bluegrass turfs mown at low cutting heights. The poor disease, drought, and wear tolerance of annual bluegrass compared to Kentucky bluegrass (Beard et al., 1978; Lush, 1989), contribute to its decline in quality. Consequently, Kentucky bluegrass infested with annual bluegrass requires more water and fungicides, especially in the summer months.

Chemical control options for annual bluegrass in Kentucky bluegrass are limited. Use of preemergent herbicides prior to the annual bluegrass germination cycle can limit infestations from encroaching into Kentucky bluegrass swards, but typically have no effect on established annual bluegrass populations. Ethofumesate is a postemergence herbicide that is labeled for annual bluegrass control, but often provides inconsistent control with unacceptable Kentucky bluegrass injury (Adams, 1989; Dernoeden and Turner, 1988).

Aside from ethofumesate there are currently no selective herbicides labeled for control of established annual bluegrass in Kentucky bluegrass. Bispyribac-sodium is a pyrimidinyl carboxy herbicide that controls weeds by inhibiting acetolactate synthase (Shimizu et al., 2002). Bispyribac-sodium has been used for selective postemergence control of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and other weeds in rice (*Oryza sativa* L.) (Schmidt et al., 1999; Webster et al., 1999; Williams, 1999) and was recently registered for turf (Anonymous, 2004). Research with bispyribac-sodium indicates applications of 60 to 148 g/ha can reduce populations of annual bluegrass without injuring creeping bentgrass (*Agrostis stolonifera* L.) (Askew et al., 2004; Borger and Watschke, 2005; Dernoeden et al., 2004; Lycan et al., 2003; Lycan and Hart, 2005). Additionally, research has found that two applications are needed for complete annual bluegrass control (Lycan and Hart, 2006; McCullough and Hart, 2006; Park et al., 2002). Limited research with Kentucky bluegrass found a single application of 296 g/ha on 'Baron' Kentucky bluegrass resulted in 28% injury five weeks after initial treatment (WAIT) (Lycan and Hart, 2005). However, more comprehensive research emphasizing the response of Kentucky bluegrass to multiple applications of bispyribac-sodium is needed.

A significant amount of genetic diversity exists within Kentucky bluegrass. This is due in part to the variable ploidy levels and the unique apomictic breeding behavior of the species (Raggi et al., 2006). This reproductive breeding behavior results in many distinct phenotypes and preserves genomic complexity through the inhibition of out-crossing. Many examples of this complexity can be found in the literature, including differences in agronomic and morphological traits (Bonos et al., 2000; Shortell et al., 2006), summer stress tolerance (Bonos and Murphy, 1999), both drought and heat tolerance (Wang and Huang, 2004), and disease tolerance (Bonos et al., 2000; Bonos et al., 2006; Czembor et al., 2001; Czembor, 2003). The objective of this study was to determine the amount of intra-specific variability of Kentucky bluegrass cultivars and experimental selections to sequential applications of bispyribac-sodium herbicide.

Materials and Methods

This experiment was conducted in 2004 and 2005 at the Rutgers Plant Biology and Pathology Research and Extension Farm in Adelphia, New Jersey. The soil type was a Freehold sandy-loam (fine-loamy, mixed, active, mesic Aquic Hapludult) with a pH of 6.4 and 2.0% organic matter. Separate Kentucky bluegrass cultivar trials were established in the fall of 2000 and 2001. Entries in each test were sown by hand using a maximum of 15 g of seed per 0.9 X 1.5 m plot (10 g / m^2). An unplanted 15 cm border was left around each plot. Kentucky bluegrass cultivars and selections were arranged in a randomized complete block design with three replications in each trial. Fifty-five cultivars or experimental selections were replicated in both trials. These entries represent a broad range of diversity within Kentucky bluegrass and contain cultivars within the major classification types (Bonos et al., 2000). The trials were maintained at 3.8 cm and mowed twice weekly with a reel mower (Toro, Bloomington, MN) with clippings returned. The turf trials received 98 kg N/ha/yr applied in four applications of 25 kg N/ha. The plots were irrigated only to prevent drought stress.

Bispyribac-sodium treatments (Velocity®, 80WP herbicide, Valent U.S.A. Corp. PO Box 8025, Walnut Creek, CA 94596) were applied in the early summer of 2004 and 2005 to mimic the conditions of highest annual bluegrass activity seen in previous studies (Lycan and Hart, 2006). One half of each plot was treated with bispyribac-sodium and the other half was left untreated as a control. The Kentucky bluegrass trial seeded in 2001 was treated with 188 g/ha on 14 June 2004 followed by 281 g/ha on 7 July 2004 (three WAIT). The Kentucky bluegrass trial seeded in 2000 was treated with 188 g/ha on

9 June 2005 followed by 281 g/ha on 30 June 2005 (three WAIT). Even though lower rates do provide adequate annual bluegrass control, higher rates were used in this study to help achieve maximum cultivar differentiation to bispyribac-sodium and to determine maximum cultivar susceptibilities. Treatments were applied with a single-nozzle CO_2 backpack sprayer equipped with a 9504E nozzle tip (Tee Jet® flat fan nozzles, Spraying Systems Co. Wheaton, IL 60189-7900) which delivered 374 L/ha of spray solution at 221 kPa. Turfgrass injury was visually assessed as the amount of injury observed on the treated half of each plot directly compared to its adjacent untreated control and reported on a 0-100% scale with 0 representing no injury and 100 representing complete necrosis. Cultivars and selections were evaluated at two, four, and eight WAIT.

Data were tested for normality using the univariate procedure of the Statistical Analysis System (SAS) V.8.2 (SAS Institute, Cary, NC). These data did not show significant deviation from normality and were therefore, directly subjected to the Analysis of Variance (ANOVA) for a randomized complete block design. Differences in mean visual injury between cultivars were determined using Fisher's protected least significant difference test at the 0.05 probability level.

Results and Discussion

The analysis of variance for injury ratings taken at two, four, and eight WAIT are presented in Table 1. There was no significant cultivar by year interaction for any of the rating dates, therefore results from 2004 and 2005 were combined (Table 2). The fact that there was no interaction between years and relatively similar results were observed for each cultivar in each year indicates that this trait is under strong genetic control and not highly affected by the fluctuating environmental conditions. Significant differences in injury to bispyribac-sodium between cultivars and selections were observed at four and eight WAIT (Table 1).

Bispyribac-sodium typically causes a transitory chlorosis that starts in tolerant grasses such as creeping bentgrass (*Agrostis stolonifera* L.) and perennial ryegrass (*Lolium perenne* L.) one WAIT and lasts for approximately three weeks (Anonymous, 2004). This transitory chlorosis is represented in the first rating taken two WAIT. All Kentucky bluegrass cultivars and selections evaluated in this study were initially injured by bispyribac-sodium and there were no significant differences between entries two WAIT (Tables 1 and 2).

Following the second application of bispyribac-sodium made three WAIT in 2004 and 2005, visual injury increased (Table 2). This response is represented by the visual injury rating taken four WAIT. The injury rating at this time represented the initial transitory chlorosis from the second application of bispyribac-sodium and permanent damage caused by the first application. ‘Blackstone’, ‘Lakeshore’, ‘Kingfisher’, and A98-877 exhibited the least injury with 42, 43, 45, and 45% visual injury, respectively, four WAIT. While, 95AN-10, ‘SR 2100’, NB67-6, ‘North Star’, ‘Champagne’, and ‘Avalanche’ exhibited injury greater than 70% four WAIT (Table 2).

Overall this injury is higher than previous reports within Kentucky bluegrass. Lycan and Hart (2005) found 28% injury on Baron Kentucky bluegrass following a single application of bispyribac-sodium at 296 g/ha five WAIT. In the current study, Baron was injured 63% after the sequential application of 188 g/ha followed by 281 g/ha of bispyribac-sodium. The differences between the two studies were likely due to the increased efficacy (and subsequent injury) of bispyribac-sodium when applied sequentially (Lycan and Hart, 2006), compared to a single application.

The most striking cultivar differences in visual injury were observed eight WAIT. This injury rating represented cumulative and permanent effects of the two bispyribac-sodium applications. Injury at this time was primarily in the form of necrosis of the leaf tissue and stand reduction. Blackstone exhibited the least injury (8%) followed by Serene (15%), A98-962 (18%), A98-1025 (20%), A98-877 (25%), ‘Cabernet’ (25%), and ‘Unique’ (25%). Avalanche exhibited the most injury (93%). 95AN-10 (88%), Washington (78%), North Star (77%), SR 2100 (73%), and NB67-6 (73%) were statistically similar to Avalanche and exhibited high levels of injury to bispyribac-sodium (Table 2). These cultivars and selections did not recover from bispyribac-sodium applications.

The majority of the cultivars evaluated in this study displayed intermediate tolerance to bispyribac-sodium with injury levels ranging from 30 to 70%, eight WAIT. Kentucky bluegrass cultivars ‘Livingston’ and ‘Midnight’ are good examples of this level of tolerance with Livingston exhibiting 52% injury, eight WAIT, and Midnight showing

35% injury eight WAIT. Other cultivars exhibiting an intermediate level of injury, included ‘SR 2284’ and ‘Liberator’ with 35 and 38% injury eight WAIT, respectively.

In both years, Kentucky bluegrass cultivars responded to bispyribac-sodium in three relatively distinct consistent patterns. Although all of the cultivars were initially injured, some such as Blackstone and Serene recovered rapidly from initial chlorosis and suffered little to no decline in turfgrass stand. The majority of the cultivars such as Midnight and ‘Livingston’ did not recover rapidly from initial chlorosis, exhibited some necrosis of the leaf tissue and slight thinning of the turfgrass stand. Lastly, several cultivars such as Avalanche, Washington, North Star, and Champagne did not recover from chlorosis, exhibited significant desiccation of the leaf tissue, and suffered nearly complete turfgrass stand loss at eight WAIT.

Bispyribac-sodium herbicide has been shown to be highly effective at removing populations of annual bluegrass from agricultural commodities, including rice (*Oryza sativa*), and some cool-season turfgrasses (Lycan and Hart, 2006; Schmidt et al., 1999; Webster et al., 1999; Williams, 1999). As hypothesized, a wide range of injury in response to bispyribac-sodium was observed in this study. Although injury levels observed in this study on most Kentucky bluegrass cultivars may be considered unacceptable to turfgrass managers, some tolerant Kentucky bluegrass cultivars and experimental selections had the ability to recover from multiple applications of bispyribac-sodium at higher than labeled use rates for annual bluegrass control, and showed acceptable turf quality within eight weeks. Thus tolerant cultivars should be evaluated for tolerance to bispyribac-sodium applied at lower rates that are effective for annual bluegrass control. The consistent results between years and large variation between entries provide evidence that this trait is genetically controlled. This indicates that the relative response of these cultivars and selections to bispyribac-sodium should be consistent over multiple locations with different environments (Poehlman and Sleper, 1995). These results indicate the potential to identify current cultivars and develop new cultivars of Kentucky bluegrass with acceptable tolerance to bispyribac-sodium.

In conclusion, high rates of bispyribac-sodium were able to significantly differentiate Kentucky bluegrass cultivars and selections for tolerance to bispyribac-sodium. Kentucky bluegrass cultivars and selections were identified that exhibited very little injury in response to bispyribac-sodium. The apomictic breeding behavior of Kentucky bluegrass allows for the ability to fix traits within a cultivar and provides stable inheritance through successive generations. This suggests that the identification and development of Kentucky bluegrass cultivars with improved tolerance to bispyribac-sodium is possible. Within these tolerant cultivars and selections, bispyribac-sodium could be used as a new tool for annual bluegrass control, provided a period of aesthetic turfgrass injury can be tolerated.

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Table 1. Calculated mean squares of percent injury ratings at two, four, and eight weeks after initial treatment from analysis of variance of bispyribac-sodium tolerance of fifty-five Kentucky bluegrass cultivars and selections. Bispyribac-sodium treatments were applied as 188 g/ha on either 14 June 2004 or 9 June 2005 followed by a sequential application of 281 g/ha three weeks after the initial treatment (7 July 2004 or 30 June 2005, respectively).

Source of Variance	df	Week 2	Week 4	Week 8
	---------------------- Percent Injury ----------------------			
Year	1	1905*	5424***	13822**
Cultivar	54	586	473**	1885*
Rep (Year)	4	700	1544**	3938*
Cultivar x Year	54	232	141	615
Cultivar x Rep	108	119	86	283
Error	216	101	72	280

*, **, *** Significant F tests at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 2. Percent injury of fifty-five Kentucky bluegrass cultivars and selections evaluated in 2004 and 2005 at Adelphia, NJ. Entries are ranked by order of tolerance to bispyribac-sodium[z] herbicide eight weeks after initial treatment.

	Cultivar or Selection	Visual Injury 2 WAIT[y]	4 WAIT	8 WAIT
		--------------------------	%	--------------------------
1	Blackstone	17	42	8
2	Serene	22	47	15
3	A98-962	32	53	18
4	A98-1025	33	48	20
5	A98-877	28	45	25
6	Cabernet	35	52	25
7	Unique	53	58	25
8	A98-363	38	55	27
9	PST-C-74	48	52	27
10	Rita	32	50	28
11	A97-1560	50	52	30
12	A98-283	40	53	30
13	Showcase	53	57	30
14	Lakeshore	25	43	32
15	Sonoma	35	53	32
16	Kingfisher	28	45	35
17	Midnight	30	53	35
18	SR 2284	38	50	35
19	A98-344	57	50	37
20	Arcadia	30	60	37

Table 2 (continued).

	Cultivar or Selection	Visual Injury		
		2 WAIT[y]	4 WAIT	8 WAIT
		------------------------- % -------------------------		
21	Arrow	45	58	37
22	A98-290	35	57	38
23	Award	30	55	38
24	Bariris	33	60	38
25	Baronette	23	53	38
26	Liberator	37	60	38
27	A96-1201	48	68	42
28	Fairfax	35	52	43
29	NuGlade	23	58	43
30	Eagleton	37	63	45
31	A98-516	33	60	47
32	America	52	60	48
33	Baronie	30	58	48
34	Freedom II	43	63	50
35	Livingston	33	58	52
36	Rambo	43	65	52
37	A98-890	32	48	53
38	Baritone	45	63	53
39	Parade	40	62	53
40	Odyssey	40	62	55

Table 2 (continued).

	Cultivar or Selection	Visual Injury 2 WAIT[y]	4 WAIT	8 WAIT
		-------------------------	%	-------------------------
41	RSP	48	65	57
42	SRX 27753	45	67	57
43	Boutique	30	55	60
44	Canon	55	70	62
45	Cynthia	37	70	62
46	Bordeaux	47	57	63
47	F-124	45	67	63
48	Baron	47	63	67
49	Champagne	55	78	67
50	NB67-6	50	73	73
51	SR 2100	48	75	73
52	North Star	22	72	77
53	Washington	42	68	78
54	95AN-10	45	73	88
55	Avalanche	48	77	93
	LSD (0.05)[x]	NS	17.5	23.6

[z]Bispyribac-sodium was applied at 188 followed by 281 g/ha three WAIT. Application dates were 14 June and 7 July, 2004 and 9 June and 30 June, 2005.

[y]WAIT = weeks after initial treatment.

[x]LSD (0.05) = Fisher’s Protected Least Significant Difference at a 5 % probability level.

CHAPTER 3

The Effects of Fertilizer on the Tolerance of Kentucky Bluegrass Cultivars to Bispyribac-Sodium Herbicide

Abstract. Previous research has demonstrated that Kentucky bluegrass cultivars vary widely in their tolerance to bispyribac-sodium herbicide. The effect of fertilizer and bispyribac-sodium rate on the tolerance of six Kentucky bluegrass cultivars to bispyribac-sodium herbicide was evaluated in 2005 and 2006 at the Rutgers University Plant Biology and Pathology Research Center in Adelphia, NJ. The trial was arranged in a split-split plot design with three replications. Kentucky bluegrass cultivars were the main plots and bispyribac-sodium rate and fertility were the sub plots. The Kentucky bluegrass cultivars evaluated were: 'Avalanche' and 'Washington' representing sensitive cultivars, 'Midnight' and 'Boutique' representing moderately tolerant cultivars, and 'Lakeshore' and 'SR 2284' representing tolerant cultivars. Bispyribac-sodium was applied at 75 g ai/ha followed by (fb) 75 g ai/ha 21 days later or 150 g ai/ha fb 150 g ai/ha 21 days later. Nitrogenous fertilizer was applied at 0 or 50 kg N/ha in early June. Turfgrass injury was rated on a weekly basis. Based on the results of this study, fertilizer treatments reduced injury of tolerant cultivars by approximately 4%, did not significantly affect injury in moderate cultivars, and increased injury in susceptible cultivars by approximately 10%.

Kentucky bluegrass (*Poa pratensis* L.) is a popular and widely used turfgrass species in lawns, parks, sports fields, and golf courses in the northern United States and Canada (Huff 2003). Kentucky bluegrass has the ability to develop dense stands of dark green turf with high turf quality in a wide range of soils and climates (Shortell et al. 2007). It has an extensive rhizome system that provides sod strength and an ability to recover after stressful periods. However, when Kentucky bluegrass is maintained at low cutting heights in stressful environments, the turf stand often can become infested with annual bluegrass (*P. annua* L.).

Annual bluegrass is a persistent weed in many desirable turfgrass species. It reduces overall turfgrass utility, due to its poor color, high reproductive capacity, and shallow root system (Lush 1989; Sprague and Burton 1937). Annual bluegrass tolerates close mowing and germinates rapidly allowing it to out-compete desirable turfgrass species. This is particularly pronounced in Kentucky bluegrass due to its slow establishment rate. It can also encroach over time when Kentucky bluegrass is maintained in a stressful environment for long periods of time (Beard 1973). The poor disease, drought, and wear tolerance of annual bluegrass when compared to Kentucky bluegrass (Beard et al. 1978; Lush 1989), contribute to the overall decline in turfgrass quality. Consequently, Kentucky bluegrass infested with annual bluegrass requires more water, fungicides, and intensive management, especially in the summer months.

Chemical control options for annual bluegrass in Kentucky bluegrass are limited. Use of preemergence herbicides prior to the annual bluegrass germination cycle can limit infestations from encroaching into Kentucky bluegrass swards, but typically have no effect on established annual bluegrass populations. Ethofumesate is a postemergence herbicide that is labeled for annual bluegrass control but often provides inconsistent control with unacceptable Kentucky bluegrass injury (Adams 1989; Dernoeden and Turner 1988). Mesotrione is a unique active ingredient with pre- and postemergence activity that has been shown to be safe on Kentucky bluegrass and effective on control of annual bluegrass (Hart and McCullough 2007; Hart et. al. 2008). This product however, is currently only labeled for Kentucky bluegrass grown for seed production or when grown on golf courses and sod farms, and its utility for controlling established annual bluegrass is still under investigation (Anonymous 2008a, 2008b). Aside from the herbicide mentioned above there are currently no widely labeled herbicides for control of established annual bluegrass in Kentucky bluegrass turf.

Bispyribac-sodium is a pyrimidinyl carboxy herbicide that controls weeds by inhibiting acetolactate synthase (Shimizu et al. 2002). Bispyribac-sodium has been used for selective postemergence control of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and other weeds in rice (*Oryza sativa* L.) (Schmidt et al. 1999; Webster et al. 1999; Williams 1999) and was recently registered for turf (Anonymous 2004). Research with bispyribac-sodium indicates applications at 60 to 148 g/ha can reduce populations of annual bluegrass without injuring creeping bentgrass (*Agrostis stolonifera* L.) (Askew et al. 2004; Borger and Watschke 2005; Dernoeden et al. 2004; Lycan et al. 2003; Lycan and Hart 2005; Lycan and Hart 2006; McCullough and Hart 2006a; Park et al. 2002). Although bispyribac-sodium has the potential to control annual bluegrass, Lycan and Hart (2005) found single applications of 296 g/ha on ‘Baron’ Kentucky bluegrass resulted in 28% injury five weeks after initial treatment (WAIT). It is now known that two applications are needed for optimum annual bluegrass control (Askew et al. 2004; Borger and Watschke 2005; Dernoeden et al. 2004; Lycan et al. 2003; Lycan and Hart 2006). A subsequent study by Shortell et al. (2008) found that two applications of bispyribac-sodium (188 g/ha followed by 281 g/ha 21 days later) resulted in Kentucky bluegrass injury levels ranging from eight to 93% eight WAIT.

Nitrogen applications have been generally shown to reduce turfgrass chlorosis in response to many herbicides and plant growth regulators (Dernoeden et al. 1993; Johnson 1984, 1990; Johnson and Burns 1985). It has also been shown to reduce weed control when used in conjunction with some herbicides including atrazine, mesotrione, nicosulfuron, glufosinate, and glyphosate (Cathcart et al. 2004). The opposite trend was observed in wheat (*Triticum aestivum* L.), when sulfonylurea herbicides, mixed with urea ammonium-nitrate, increased injury when compared to the sulfonylurea without fertilizer (Olson et al. 2000). Research on tolerant species, including both creeping bentgrass and perennial ryegrass (*Lolium perenne* L.), found that applications of nitrogen and iron prior to bispyribac-sodium treatments reduced the initial injury, and increased the speed of recovery without reducing annual bluegrass control (McCullough and Hart 2006b; McDonald et al. 2006). These results suggest that the evaluation of bispyribac-sodium and nitrogen fertilizer interactions for the potential to decrease turfgrass injury while maintaining herbicide efficacy warrant further investigation.

Therefore, the objective of this study was to determine the influence of fertilizer on the response of previously identified sensitive, moderately tolerant, and tolerant Kentucky bluegrass cultivars to labeled rates of bispyribac-sodium herbicide.

Materials and Methods

This experiment was conducted at the Rutgers Plant Biology and Pathology Research and Extension Farm in Adelphia, New Jersey. The soil type was a Freehold sandy-loam (fine-loamy, mixed, active, mesic Aquic Hapludult) with a pH of 6.4 and 2% OM. The experiment was established in the fall of 2004 and 2005 and arranged in a split-split plot design with three replications. Kentucky bluegrass cultivar was the main plot with six treatments. 'Avalanche' and 'Washington' represented sensitive cultivars, 'Midnight' and 'Boutique' represented moderately tolerant cultivars, and 'Lakeshore' and 'SR 2284' represented tolerant cultivars (Shortell et al. 2008). Cultivars were seeded in 2.7 x 3.7m plots with a Lesco drop spreader[1] at a rate of 108 kg/ha. At seeding, turf received 25 kg N/ha using a 10-4-8 (elemental N-P-K, respectively) formulation[2] and was irrigated to prevent drought stress.

Bispyribac-sodium treatments[3] were sub plots. The plots were treated with either 0 g/ha, 75 g/ha, or 150 g/ha on June 11 and June 28 (three WAIT) in 2005 (on the trial established in the fall of 2004) and on June 15 and July 6 (three WAIT) in 2006 (on the trial established in 2005), respectively. These dates were chosen because previous research has demonstrated that annual bluegrass control with bispyribac-sodium is greatest in late spring/summer relative to early spring or fall applications (Lycan and Hart 2006). Bispyribac-sodium at 75 g/ha is the labeled use rate for creeping bentgrass and perennial ryegrass (Anonymous 2004). Treatments were applied with a single-nozzle CO_2 backpack sprayer equipped with a 9504E nozzle tip[4] which delivered 374 L/ha of spray solution at 221 kPa.

Fertility treatments were sub-sub plots. They were randomized within the main cultivar plot so that all cultivars at each bispyribac-sodium rate received either 0 or 50 kg N/ha. Fertilizer treatments were applied using a Lesco drop spreader[1]. The fertilizer source used was an ammonium based 16-2-7 (elemental N-P-K, respectively) homogenous formulation[5] applied four days before the initial herbicide treatment and immediately followed by 1.3 cm of overhead irrigation. The trials were maintained at 3.8 cm and mowed weekly with a reel mower[6] with clippings returned.

Turfgrass injury was visually assessed on a zero to 100 scale with zero representing no injury and 100 representing complete desiccation. Injury ratings were taken at 1, 3, 4, 6, and 8 weeks, with weeks 1, 3, 4, and 6 representing percent chlorosis/necrosis and week 8 representing stand loss. All data was subjected to ANOVA using the general linear model procedure provided by the Statistical Analysis System (SAS) V.9.2 (SAS Institute, Cary, NC). Differences in mean visual injury between cultivars were determined using Fisher's Protected LSD test at the 0.05 probability level.

Results and Discussion

Although the tolerant, moderate, and susceptible cultivars exhibited characteristics of their respective groups in response to bispyribac-sodium, the overall cultivar rankings changed (Boutique showed tolerance in 2005, but was susceptible in 2006), resulting in a cultivar by year interaction (data not shown); therefore, results from 2005 and 2006 are presented separately (Tables 1-3). There was a significant cultivar effect in all five rating dates in both years (Table 1). In 2005 Kentucky bluegrass injury ranged from 0 to 97%, whereas, in 2006 Kentucky bluegrass injury ranged from 0 to 100% (Tables 2-3). Overall, percent injury was higher in 2006 then 2005.

Kentucky Bluegrass Response. Significant differences in tolerance to bispyribac-sodium were observed between cultivars on every rating date (Table 1). Eight WAIT, Kentucky bluegrass cultivars exhibited three distinct responses to bispyribac-sodium: 1) Tolerant (Lakeshore) which exhibited 3 to 7% injury in 2005 and 0 to 3% injury in 2006 (depending on fertilizer and herbicide rate). 2) Moderate (SR 2284 and Midnight) which exhibited 20 to 43%, and 3 to 40% injury, respectively in 2005, and 7 to 20% and 3 to 23% injury, respectively in 2006 3) Susceptible (Boutique, Avalanche, and Washington) which exhibited 43 to 77%, 63 to 97%, and 80 to 93% injury, respectively, in 2005, and 63 to 100%, 50 to 100%, and 77 to 100% injury respectively in 2006 (Tables 2 – 3).

There were some differences in ranking in this study compared to the previous study conducted on these cultivars (Shortell et al. 2008). SR 2284 was selected as a tolerant cultivar based on previous studies; however, it exhibited only moderate tolerance in both years in this study. Additionally, Boutique was selected to be moderately tolerant based on previous studies but exhibited a high degree of sensitivity in this study (Shortell et al. 2008). Boutique Kentucky bluegrass performed inconsistently between years. In 2005, Boutique behaved like a moderately tolerant cultivar at low rates of bispyribac-sodium, however at high rates exhibited a response more similar to the susceptible cultivars. In 2006, at both rates evaluated Boutique behaved as a susceptible cultivar. This difference in response can be attributed to initial cultivar selection based on an earlier field screening. In a previous study Boutique exhibited 60% injury 8 WAIT in response to bispyribac-sodium (Shortell et al. 2008), this injury level fell in between the moderate and susceptible grouping. Although Boutique was chosen to represent the broad range of response to bispyribac-sodium and was less susceptible then some of the cultivars evaluated; our moderately tolerant threshold should have been more stringent and perhaps ‘Fairfax’ or ‘Kingfisher’ would have been more representative of the moderately tolerant category. Aside from SR 2284 and Boutique, all of the other cultivars in this study behaved similarly to the previous study (Shortell et al. 2008) showing similar trends even at the reduced rates used in this study.

Bispyribac-Sodium Response. There was a significant rate effect across all rating dates in both years (Table 1). In weeks 3 through 8 after the initial treatment higher rates resulted in increased injury (Tables 2 – 3). There was a cultivar by rate interaction during all rating dates in both years, because higher application rates increased injury in some cultivars but not others (Table 1). In the tolerant cultivar, Lakeshore, there was no difference in injury between bispyribac-sodium rates (Tables 2 – 3). In some cultivars like Midnight there was a slight decrease in injury in 2005 (Table 2) and a slight increase in 2006 (Table 3). However, in other cultivars like Washington and Avalanche there was a dramatic increase in injury with increased bispyribac-sodium rates (Tables 2 and 3). In general, the response of tolerant cultivars was not affected by bispyribac-sodium rate, moderately tolerant cultivars varied in response to bispyribac-sodium rate, while injury to susceptible cultivars exhibited increases with increased bispyribac-sodium rates.

Fertilizer Response. The effect of fertilizer on the response of Kentucky bluegrass to bispyribac-sodium was significant in weeks 1, 3, 4, and 6 in 2005 (Table 1). Although the effects of the fertilizer treatments were not as visible in 2006 significant differences were still observed 1, 3, 4, 6, and 8 WAIT (Table 1). This could be due to an overall increase in injury observed in 2006.

There was a significant interaction of fertility treatments depending on the tolerance of cultivars evaluated (Table 1). On tolerant (Lakeshore), and to some extent moderate (SR 2284 and Midnight) Kentucky bluegrass cultivars, fertilizer treatments decreased initial injury or increased the speed of recovery (Tables 2 and 3). However, on more sensitive cultivars (Boutique, Avalanche, and Washington), fertilizer treatments generally increased injury and subsequent stand decline (Tables 2 and 3). For example, in 2006 bispyribac-sodium applied at 75 fb 75 g/ha to the fertilized cultivar Midnight had decreased injury of 14% when compared to its non-fertilized control, whereas, the same treatment applied to Avalanche resulted in a 50% increase in injury when compared to its non-fertilized control leading to complete stand loss (Table 3).

This is the first report of an interaction between fertilizer and the response of Kentucky bluegrass to bispyribac-sodium, and may be explained by a combination of mechanisms. For example, in the moderately tolerant cultivar Midnight the addition of fertilizer may increase plant growth and cause an increase in herbicide metabolism, leading to the breakdown and detoxification of the parent molecule within the plant, thus decreasing injury (McCullough 2009). However, in the susceptible cultivars, like Avalanche, the increased fertilizer could have increased plant succulence (Mungikar et al. 1976) and allowed more herbicide to enter the plant increasing injury; similar to what is seen in winter wheat (*Triticum aestivum*L.) (Stahlman et al. 1997). This is not surprising, Smith and Vanden Born (1992), also demonstrated this concept in wild oats (*Avena fatua* L.) and barley (*Hordeum vulgare* L.) where applications of ammonium sulfate caused an increase in herbicide absorption, without increasing herbicide metabolism. Susceptible Kentucky bluegrass cultivars may be behaving in a similar fashion to annual bluegrass with a decreased ability to metabolize bispyribac-sodium with fertilizer treatments increasing herbicide absorption.

Kentucky bluegrass cultivars exhibit a range of tolerance to bispyribac-sodium (Shortell et al. 2008) with susceptible cultivars (that respond in a similar fashion to annual bluegrass and other susceptible grassy weeds) to cultivars that show almost complete tolerance that is similar to creeping bentgrass, perennial ryegrass, and other labeled species (McCullough and Hart 2009). In this study, the response of Kentucky bluegrass cultivars was similar to a previous study (Shortell et al. 2008) confirming the observation that Kentucky bluegrass cultivars vary widely in their response to bispyribac-sodium, ranging from tolerant to highly susceptible. In general, applications of fertilizer reduced discoloration and subsequent stand loss on tolerant cultivars, had no effect on moderately tolerant cultivars and increased discoloration and subsequent stand loss on susceptible cultivars.

Bispyribac-sodium is a new tool for the selective removal of established annual bluegrass in tolerant cool-season grasses. If the benefits of annual bluegrass control outweigh the initial transient injury then this herbicide could be used on tolerant Kentucky bluegrass cultivars. The results of this study suggest that the use of fertilizer before the application could reduce initial chlorosis and decrease stand loss. However, on moderately tolerant, susceptible, and unknown stands of Kentucky bluegrass the potential for significant injury does exist and applications of bispyribac-sodium could result in complete loss of the turfgrass sward. The application of fertilizer may not be beneficial and could potentially increase injury and subsequent stand decline.

Sources of Materials

[1]John Deere Landscapes, Troy, MI

[2]Balanced Blend®, Reed and Perrine Inc., Tennent, NJ

[3]Velocity®, 80WP herbicide, Valent U.S.A., Walnut Creek, CA

[4]Tee Jet® 9504E flat fan nozzles, Spraying Systems Co. Wheaton, IL

[5]Country Club®, Lebanon Seaboard Corporation, Lebanon, PA

[6]The Toro Company, Bloomington, MN

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Table 1. Analysis of variance of the influence of fertilizer on the tolerance of Kentucky bluegrass to bispyribac-sodium herbicide.

	Mean Squares of Percent Injury				
Year 1, 2005					
Source	1 WAIT	3 WAIT	4 WAIT	6 WAIT	8 WAIT
Rep	29	29	34	12	42
Cultivar	815***	1432***	2066***	4321***	9462***
Rep*Cultivar	50**	58**	119***	52**	72*
Bispyribac-sodium Rate	15004***	26295***	38923***	42539***	29658***
Cultivar*Bispyribac-sodium Rate	225***	388***	528***	1186***	2472***
Cultivar(Rep*Bispyribac-sodium Rate)	28*	56**	35	29	50
Fertility	237***	5779***	3333***	1337***	112
Cultivar*Fertility	74***	890***	649***	995***	521***
Bispyribac-sodium Rate*Fertility	70*	1479***	969***	512***	279***
Cultivar*Bispyribac-sodium Rate*Fertility	25	246***	205***	410***	211***
Year 2, 2006					
Source	1 WAIT	3 WAIT	4 WAIT	6 WAIT	8 WAIT
Rep	25	86	142**	26	18
Cultivar	946***	3738***	3020***	7607***	14470***
Rep*Cultivar	136***	23	56**	17	25
Bispyribac-sodium Rate	29753***	25025***	37255***	36547***	28951***
Cultivar*Bispyribac-sodium Rate	337***	966***	823***	2012***	3685***
Cultivar(Rep*Bispyribac-sodium Rate)	84***	40	31*	9	12
Fertility	1134***	237*	20	89**	890***
Cultivar*Fertility	485***	1624***	970***	694***	625***
Bispyribac-sodium Rate*Fertility	301***	116	5	34*	423***
Cultivar*Bispyribac-sodium Rate*Fertility	152***	420***	261***	251***	262***

*, **, *** = significant to the 0.05, 0.01, and 0.001, respectively.

Table 2. The effects of fertilizer on the response of Kentucky bluegrass to bispyribac-sodium herbicide in 2005.

Kentucky bluegrass Cultivar	Rate[a] (g ai/ha)	Fertility[b] (50kg N/ha)	1 WAIT	3 WAIT	4 WAIT	6 WAIT	8 WAIT
			Injury (%)				
Lakeshore	75 fb 75[c]	-	27	43	47	37	7
	75 fb 75	+	23	3	27	23	7
	150 fb 150	-	30	47	47	43	7
	150 fb 150	+	27	3	27	13	3
SR 2284	75 fb 75	-	43	57	67	60	40
	75 fb 75	+	30	20	33	33	20
	150 fb 150	-	43	60	63	67	40
	150 fb 150	+	37	33	43	63	43
Midnight	75 fb 75	-	57	70	67	67	40
	75 fb 75	+	43	27	20	13	7
	150 fb 150	-	63	70	63	60	33
	150 fb 150	+	50	27	23	20	3
Boutique	75 fb 75	-	37	63	73	67	60
	75 fb 75	+	30	33	43	43	43
	150 fb 150	-	40	50	63	57	60
	150 fb 150	+	40	37	60	73	77
Avalanche	75 fb 75	-	27	63	73	80	78
	75 fb 75	+	30	63	77	93	93
	150 fb 150	-	27	60	77	80	63
	150 fb 150	+	27	63	80	90	97
Washington	75 fb 75	-	30	53	73	80	85
	75 fb 75	+	30	60	77	93	90
	150 fb 150	-	27	57	70	80	80
	150 fb 150	+	30	60	73	90	93
		LSD at 0.05 =	3	3	4	3	4

[a]Rate = bispyribac-sodium, Velocity 80WP, Valent USA Corporation, Walnut Creek, CA.
[b]Fertilizer = ammonium based 16-2-7 (elemental N-P-K, respectively) Country Club brand, Lebanon Seaboard Corporation, PA.
[c]Herbicide applications were made on June 11 and June 28 in 2005.

Table 3. The effects of fertilizer on the response of Kentucky bluegrass to bispyribac-sodium herbicide in 2006.

Kentucky bluegrass Cultivar	Rate[a] (g ai/ha)	Fertility[b] (50kg N/ha)	1 WAIT	3 WAIT	4 WAIT	6 WAIT	8 WAIT
			Injury (%)				
Lakeshore	75 fb 75[c]	-	30	33	40	20	3
	75 fb 75	+	40	15	27	10	0
	150 fb 150	-	33	40	50	27	3
	150 fb 150	+	37	30	40	20	0
SR 2284	75 fb 75	-	40	37	50	37	13
	75 fb 75	+	27	10	37	20	7
	150 fb 150	-	53	57	60	40	20
	150 fb 150	+	47	30	40	17	7
Midnight	75 fb 75	-	40	32	30	33	17
	75 fb 75	+	37	0	17	17	3
	150 fb 150	-	47	37	50	47	23
	150 fb 150	+	50	13	27	40	23
Boutique	75 fb 75	-	63	47	67	60	63
	75 fb 75	+	60	20	50	57	93
	150 fb 150	-	50	57	67	70	80
	150 fb 150	+	63	40	53	93	100
Avalanche	75 fb 75	-	40	50	53	70	50
	75 fb 75	+	67	73	73	98	100
	150 fb 150	-	50	60	70	83	87
	150 fb 150	+	77	92	99	100	100
Washington	75 fb 75	-	43	50	60	70	77
	75 fb 75	+	77	88	90	98	100
	150 fb 150	-	47	60	70	80	93
	150 fb 150	+	73	93	99	100	100
		LSD at 0.05 =	4	4	3	2	3

[a]Rate = bispyribac-sodium, Velocity 80WP, Valent USA Corporation, Walnut Creek, CA.
[b]Fertilizer = ammonium based (16-2-7 elemental N-P-K, respectively) Country Club brand, Lebanon Seaboard Corporation, Lebanon, PA.
[c]Herbicide applications were made on June 15 and July 6 in 2006.

CHAPTER 4

Genetic Variation in Rooting Characteristics in Kentucky Bluegrass Under Heat Stress

Abstract. The identification of Kentucky bluegrass (*Poa pratensis* L.) cultivars with improved rooting ability under heat stress would benefit sod growers and turf managers attempting to establish sod during the summer months. The objectives of this study were to i.) evaluate Kentucky bluegrass cultivars and selections for rooting ability under heat stress, and ii.) determine whether visual quality, leaf firing, and or canopy temperature depression ratings are correlated to rooting ability under heat stress. In the summers of 2007 and 2008, sod plugs (10 x 30 cm) of 25 Kentucky bluegrass cultivars and selections were collected from four-year old turf trials and were planted into pots (10 cm^2 x 36 cm deep) filled with 3:1 sand:soil mixture that were placed in an area with restricted air movement at the Rutgers University Horticulture Farm II in North Brunswick, NJ. Canopy temperature depressions, turfgrass quality, and leaf firing were assessed weekly. Roots were harvested from three replicate pots of each cultivar at two, four, and six week intervals and the soil profile was separated into five 7 cm increments (0-7, 7-14, 14-21, 21-28, and 28-35 cm). The study was repeated on July 1, 2007 and July 1, 2008 to maximize the occurrence of natural heat stress events. Differences in rooting ability under heat stress were observed in Kentucky bluegrass. ‘Hampton’, ‘Bedazzled’, A93-485, and ‘Champagne’ consistently had the highest total root dry weights in 2007 (0.760, 0.636, 0.566, and 0.554 g, respectively) and 2008 (0.380, 0.364, 0.450, and 0.360 g,

respectively). ‘Princeton P-105’ and ‘Chicago II’ consistently had the lowest total root dry weights in 2007 (0.303 and 0.293 g) and 2008 (0.192 and 0.186 g, respectively). Differences in canopy temperature depressions under heat stress were significant in 2007 but not 2008. Hampton and Champagne exhibited the coolest canopies (-2.3 and -2.0°C below ambient temperature, respectively, in 2007) whereas Princeton P-105 and ‘Baron’ exhibited the hottest canopies (0.7 and 1.0°C above ambient temperature, respectively, in 2007). Differences in turfgrass quality were significant in 2007 and 2008. Bedazzled (8 and 6, in 2007 and 2008, respectively), A93-485 (7 and 8, in 2007 and 2008, respectively), and ‘Cabernet’ (8 and 7, in 2007 and 2008, respectively) exhibited the highest turf quality. ‘Fairfax’, Princeton P-105, ‘Liberator’, and Baron (6 and 5 in 2007 and 2008, respectively) exhibited the least turfgrass quality. Differences in leaf firing were significant in 2007 and 2008. ‘Eagleton’ (8 and 6, in 2007 and 2008, respectively), Cabernet (8 and 7, in 2007 and 2008, respectively), ‘Royale’ (7 and 7, in 2007 and 2008, respectively), ‘Monte Carlo’ (8 and 7, in 2007 and 2008, respectively), and A94-703 (7 and 7, in 2007 and 2008, respectively) exhibited the least leaf firing in both years, whereas, Baron, Fairfax, Princeton P-105, and ‘Aura’ (6 and 5, in 2007 and 2008, respectively) showed the most leaf firing in both years. The differences in root weights between cultivars indicate that there is genetic variation in rooting ability in Kentucky bluegrass cultivars during heat stress. Inconsistent correlations between the above ground parameters evaluated here (canopy temperature depressions, turfgrass quality, and leaf firing) and root growth under heat stress could indicate that other variables are also contributing to increased root weights and that these observations should not be used as the only predictor of Kentucky bluegrass root growth under heat stress.

Kentucky bluegrass is the premier cool-season grass grown by sod producers in the United States due to its strongly rhizomatous growth habit. This characteristic allows it to form a dense tightly knit sod (Meyer and Funk, 1989) and to recover from damage and stress quickly. This is especially important in high traffic areas like sports fields and golf courses and stressful areas such as home lawns in the transition zone where resistance to and recovery from damage are important for good turfgrass performance (Meyer and Funk, 1989).

The optimum temperatures for shoot and root growth of cool-season grasses are between 10 to 24°C (Beard, 1973). One of the major limitations of all cool-season grasses grown in warm climatic zones is high temperature stress (Xu and Huang, 2001). This limits the regions where Kentucky bluegrass can be grown and complicates turf management in the transition zone where sporadic heat waves can damage Kentucky bluegrass. Physiologically, there are various ways to study turf tolerance to heat stress including photochemical efficiency, photosynthetic rates, canopy temperature depressions, root mortality rates, shoot dry weights, vertical shoot extension rates, and electrolyte leakage (Aldous and Kaufmann, 1979; Bonos and Murphy, 1999; Liu et al., 2002; Xu and Huang, 2001; Zhang et al., 2003). However, many of these measurements are destructive and time consuming which can limit the amount of samples that can be reasonably processed or the number of selections that can be efficiently screened (Marcum, 1998). This limitation reduces the breeder's ability to screen all of the available germplasm and could lead to the rejection of otherwise superior genotypes.

Heat stress also causes damage to roots which are more difficult to measure and quantify. Plant roots are also primary sites of cytokinin synthesis and water acquisition, both essential to plant growth and development (Liu et al., 2002). Therefore, plants with the ability to grow and maintain root systems under summer stress should be capable of maintaining physiological function and persist until more favorable environmental conditions return. To date, studies investigating root systems in Kentucky bluegrass under a range of environmental conditions are limited (Aldous and Kaufmann, 1979; Bonos and Murphy, 1999; Burt and Christians, 1990; Carrow, 1996; Jiang and Huang, 2000, 2001; King and Beard, 1969; Koski et al., 1988; Peterson et al., 1979; Richardson et al., 2008; Sprague, 1933; Su et al., 2007; Stuckey, 1941; Sullivan et al., 2000). Bonos and Murphy (1999) evaluated 10 Kentucky bluegrass cultivars under summer stress for canopy characteristics, root to shoot responses, and water depletion patterns. They found that tolerant cultivars exhibited 19% more roots in the 15 – 30 cm profile, and 65% more roots in the 30 – 45 cm profile. They also showed that tolerant entries maintained canopy temperatures 5°C cooler than intolerant entries. Carrow (1996) evaluated six tall fescue cultivars (*Festuca arundinacea* Schreb.) for turf quality, leaf firing, evapotranspiration rates, and root length density under a field dry down. He found that root length density in the 20 – 60 cm depth was associated with less leaf firing and wilt.

Furthermore, experiments to evaluate large numbers of cultivars is lacking in Kentucky bluegrass even though there is significant genetic variation between cultivars. It would be interesting to know if certain visual ratings and other non-destructive physiological measurements could be assessed on above-ground plant parts and correlated to below ground measures of rooting ability including root initiation and overall root weights. This could prove to be a useful and efficient way for turfgrass breeders to identify and develop cultivars with increased root growth under heat stress.

Therefore the objectives of this study were to i.) Evaluate a wide range of Kentucky bluegrass cultivars and selections for rooting ability under heat stress, and ii.) Determine whether visual quality, leaf firing, and or canopy temperature depression ratings are correlated to rooting ability under heat stress.

Materials and Methods

Twenty-five Kentucky bluegrass cultivars and selections representing a range of Kentucky bluegrass classification types were chosen for this study (Table 1). The study was initiated on July 1 of both 2007 and 2008 and conducted over six week periods (ending August 15 of both 2007 and 2008). Sod plugs (10 x 30 cm) were removed from four-year old turf trials established at the Rutgers University Department of Plant Biology and Pathology Research Farm in Adelphia, NJ [from a turf trial established in 2003 for run 1 (initiated July 1, 2007) and from a turf trial established in 2004 for run 2 (initiated July 1, 2008)]. Soil and roots were cut from all sod plugs to 3.75 cm below the thatch and sod plugs were then planted into four liter pots (TallOne TreePots®, Stuewe and Sons, Tangent, Oregon) filled with a 3:1 sand:soil mixture to a height of 36 cm (and standard weight of 4.2 kg). The sand was clean topdressing sand (Sure-Play Topdressing Sand®, US Silica, Berkeley Springs, West Virginia) that met the specifications for a USGA putting green. The soil was a Nixon sandy loam (fine-loamy, mixed, semi-active, mesic type Hapludults) obtained from the Rutgers University Plant Biology Research and Extension Farm II in North Brunswick, NJ. The soil was triple screened through a 0.6 cm mesh, and steam sterilized. A 3:1 sand:soil mixture was used to facilitate root sampling. It is realized that roots grow more easily in sand and that a higher soil content may result in slightly different results however, this sacrifice was necessary to obtain intact root samples.

The mix was measured in 19 L buckets, 3 sand to 1 soil; thoroughly mixed with a shovel and allowed to air-dry completely before filling the pots. The mix was created independently for each year and mechanically tested for structure at the Rutgers University Soil Testing Lab in North Brunswick, NJ to ensure consistency between root environments in all of the experiments. The sand:soil mix for 2007 consisted of 79% sand (very fine sand = 4.3%, fine sand = 9.7%, medium sand = 53.6%, course sand = 25.2%, very course sand = 6.8%, sample larger than sand = 2.6%), 13% silt, and 8% clay with a loamy sand texture. The sand:soil mix for 2008 consisted of 80% sand (very fine sand = 2.3%, fine sand = 9.3%, medium sand = 52.8%, course sand = 28.1%, very course sand = 7.5%, sample larger than sand = 2.4%), 14% silt, and 7% clay with a loamy sand texture. These results indicate a uniform sand:soil mix for both runs of the experiment.

Sod plugs were planted in the sand:soil mix in four L pots and then grown at the Rutgers University Plant Biology and Pathology Research Farm in North Brunswick, NJ, in a completely random design with three replications in an area with restricted air movement that maximized heat stress (5 - 36°C in 2007 and 4 - 35°C in 2008, Figure 1) for a total of six weeks. Concrete cinder blocks were used to form a perimeter around the study to create uniform soil temperatures from the edge to the center. Additionally, pots were re-randomized weekly to eliminate positional effects. This allowed soil temperature differentials to be maintained below 2°C (data not shown) between pots. Drought stress was avoided by watering to soil capacity once daily during the study. This was defined when water freely flowed from the bottom of the pots.

The study was trimmed to a height of 4 cm weekly with a scissor. It was fertilized in early evenings with 10-4-8 (elemental N-P-K, respectively) water soluble fertilizer (Peters Professional®, Scotts Professional, Geldermalsun, Netherlands) at 1.5 cm^3 to 4 L water (1/20th strength) weekly with each pot receiving approximately 250 ml.

Pythium blight (*Pythium sp.*) and other diseases were prevented with biweekly applications of mefenoxam and chlorothalonil, respectively, (Subdue Maxx® and Daconil Ultrex®, respectively, Syngenta Professional Products, Wilmington, Delaware) at labeled rates.

Three replicate pots of each cultivar or selection were harvested at two, four, and six weeks after sodding. At harvest, the entire plants were gently removed from each pot, leaving the root systems intact. The root system was separated into seven cm increments (0-7, 7-14, 14-21, 21-28, and 28-35 cm) where the first root layer was measured from the base of the sod plug to 7 cm below the surface, the second root layer was measured from 7 cm below the surface to 14 cm below the surface, the third root layer was measured from 14 cm below the surface to 21 cm below the surface, the fourth root layer was measured from 21 cm below the surface to 28 cm below the surface, and the fifth root layer was measured from 28 cm below the surface to 35 cm below the surface, respectively. Roots were washed free of the sand:soil mixture and stored in a ten percent methanol solution at 4°C until time was available for processing. Root samples were taken to the laboratory one rep at a time and washed over a soil sieve (30 um) to remove any fine particles. Roots were dried at 60°C for 48 hours and then weighed. All entries were assessed for canopy temperature depression, turf quality, and leaf firing at weeks two, four, and six after sodding, of each run. Canopy temperature depression was calculated by using an infrared thermometer (Telatemp Corp., Fullerton, California) in mid-afternoon. Turf quality and leaf firing were visually assessed on a 1-9 scale where 9 equals best turf quality or least leaf firing, respectively.

At the time of sod plug planting it was evident that cultivars accumulated thatch at different rates. Thatch was measured to determine if differential thatch accumulation of individual cultivars was correlated to the differences in rooting observed. Thatch analysis was conducted independently of root analysis and analyzed for compressed thatch thickness at 1kg and percent organic matter through the loss on ignition method (Morris and Shearman, 2006). Three 3.2 cm diameter plugs of twenty-five Kentucky bluegrass entries were sampled from the 2004 Kentucky bluegrass trial at Rutgers University Plant Science Research Station, Adelphia, NJ (same plots used for run 2, 2008 rooting analysis). The plugs were taken randomly from each replication for a total of 3 plugs per entry using a JMC soil probe (30.5 cm long with a 3.2 cm inside diameter, Clements Associates Inc., Newton, Iowa) and they were sampled separately in both July and August of 2008. Samples were prepared for thatch analysis by removing all of the roots and verdure and then frozen at $0^{\circ}C$ to preserve their integrity. Thatch samples were then removed from the freezer one replication at a time and placed at $5^{\circ}C$ for 24 hours to defrost. Defrosted samples were then analyzed for compressed thatch thickness by placing a 1kg weight on each sample and measuring the thickness of the compressed sample in four places with a Starrett® Wisdom Plus micrometer (Cat# F2750-1, Range 4.000 in., Resolution 0.0001 in., S/N 021846605, EDP 65847) as described by Morris and Shearman (2006). Following thickness measurements, the samples were weighed to ensure uniform moisture contents, and then placed in 5.7 cm (inside diameter) crucibles. The crucibles were then placed in an oven at $105^{\circ}C$ for 24 hours and sample dry weights were recorded. The crucibles were then placed in a furnace at $365^{\circ}C$ for 8 hours to oxidize all of the organic matter, allowed to cool in desiccators for 4 hours, and sample mineral weights were recorded to determine the percent organic matter in each sample.

Data was analyzed using the general linear model procedure provided by the SAS Institute, SAS v.9.2 (Cary, NC). Correlation analysis was used to determine associations between above ground measurements, root weights, and thatch accumulation over all runs and timings. Means were then separated using Fischer's protected least significant difference at the 0.05 probability level.

Results and Discussion

Root Weights

There were no significant differences between cultivars in the early periods of the study (weeks 2 and 4) for both years, therefore the data after 6 weeks of sodding are presented. There was a significant interaction between cultivar and year (2007 and 2008), therefore the data are separated and presented by year. In 2007 the samples evaluated were taken from a turf trial that had not received fertilizer for three months (data not shown); however, in 2008 the sampled plots were fertilized monthly and this could have resulted in the observed interaction. Additionally, weather patterns during 2007 and 2008 were different (Figure 1). Although average minimum and maximum temperatures between both years were very similar. During the first week after planting in 2007 maximum temperatures averaged 25°C, whereas in 2008 maximum temperature the first week after planting averaged 30°C (Figure 1). Cooler temperatures in 2007 immediately after planting likely resulted in the observed increase in root weight and could have caused the cultivar by year interaction. There were significant differences in rooting ability among the Kentucky bluegrass cultivars and selections within each year (Table 2) under heat stress. Similar trends in overall rooting ability under heat stress were observed.

0 – 7 cm Root Layer

There were no significant differences at week 2 or 4 but, there were significant differences in root weights at week six in 2007 and 2008 in the 0 – 7 cm soil layer (Table

2) (Figure 2). In 2007, ‘Hampton’ produced the most roots by weight (0.26 g). ‘Aura’, A94-703, ‘Bedazzled’, A93-485, ‘Champagne’, and ‘Eagleton’ also exhibited good root growth in the 0 – 7 cm depth under heat stress in 2007 with root weights of 0.20 g or more. ‘Baron’ and ‘Princeton P-105’ produced the least roots by weight in 2007 (0.10 and 0.12 g, respectively) (Figure 2). In 2008, A94-703 and A93-485 had significantly greater root weights (0.31 and 0.29 g) in the 0 – 7 cm profile than the other entries. ‘Mercury’, ‘Mallard’, Champagne, Hampton, ‘Langara’, ‘Royale’, ‘Monte Carlo’, and Aura also exhibited good root production under heat stress in 2008 with root weights of 0.20 g or more. ‘Liberator’, ‘Midnight’, and ‘Lakeshore’ produced the least roots by weight (0.10, 0.09, and 0.08 g, respectively) in 2008 (Figure 2).

Hampton, A94-703, A93-485, Aura, and Champagne consistently produced roots in the 0 – 7 cm layer in both years. A94-703, A93-485, and Aura were developed through intraspecific hybridization using RSP as a common parent. RSP Kentucky bluegrass was shown to have excellent summer stress tolerance (Bonos and Murphy, 1999) as well as heat tolerance (Wang and Huang, 2004) so it is not surprising that these entries maintained good rooting under heat stress with root weights of 0.20 g or more. Cultivars and selections with more roots in the upper 7-cm soil layer should be more able to capture water from irrigation or intermittent rain, allowing for more efficient water use during sod establishment.

7 – 14 cm Root Layer

This root layer is measured from 7 to 14 cm below the sod plug. There were significant differences in rooting ability in this layer in both years of the experiment

(Table 2). In 2007, Hampton produced the most roots by weight (0.18 g) in this layer. Champagne, Bedazzled, 'Diva', A94-703, Aura, and Langara also exhibited good root production under heat stress in the 7 – 14 cm layer (Figure 3) with root weights of 0.14 g or more. Princeton P-105, 'Fairfax', and Baron produced the least roots by weight (0.07 g) in the 7 – 14 cm layer (Figure 3). In 2008, A94-703 and Mercury produced the most roots by weight (0.10 g) followed by Mallard and A93-485 (0.09 g) in the 7 – 14 cm layer. Princeton P-105, Liberator, 'Chicago II', and Midnight all produced the least roots by weight (0.02 g) in the 7 – 14 cm layer (Figure 3). Princeton P-105 and Chicago II exhibit poor rooting with root weights of 0.02 g, interestingly, they are both Compact Type cultivars which are typically adapted to northern climates and higher latitudes (Huff, 2003), where summers are not as severe therefore summer stress tolerance may not be as important which could have resulted in less root growth under heat stress which was observed within these two entries.

14 – 21 cm Root Layer

This root layer was measured from 14 to 21 cm below the sod plugs. This layer only showed significant differences in root weight in 2007 (Table 2). Hampton showed the heaviest root weight (0.16g) in the 14 – 21 cm layer among all cultivars (Figure 4). Chicago II and Princeton P-105 showed the lightest root weight (0.06 and 0.05 g, respectively) in the 14 – 21 cm layer. Bedazzled, Champagne, Diva, and A93-485 also had intermediate root weights in the 14 – 21 cm layer (0.14, 0.13, 0.13, and 0.12 g, respectively) in 2007. These results are consistent with the other root layers.

21 – 28 cm Root Layer

This root layer was measured from 21 to 28 cm below the sod plugs. There were significant differences in root weights in 2007 but not in 2008 in this layer (Table 2). Hampton and Bedazzled produced the most roots by weight (0.14 and 0.11 g, respectively) in the 21 – 28 cm layer. Diva and A93-485 also exhibited high root weights (0.09 g) in 2007. Chicago II and Fairfax produced the least roots by weight (0.02 and 0.03 g, respectively) in the 21 – 28 cm layer (Figure 5). Increased root weight deeper in the soil profile under summer stress has been correlated to increased summer stress tolerance (Bonos and Murphy, 1999; Bonos et al., 2004). Therefore Hampton and Bedazzled should exhibit better summer stress tolerance and may have more successful sod establishment in the summer compared to Chicago II or Fairfax.

28 – 35 cm Root Layer

This root layer was measured from 28 to 35 cm below the sod plugs and is the deepest layer evaluated in this experiment. There were only differences in rooting in this layer in 2008. A94-703, Bedazzled, Mallard, and Hampton exhibited the highest root weights in this layer (0.006, 0.005, 0.005, and 0.005 g, respectively) (Figure 6). In the absence of rainfall or irrigation A94-703, Bedazzled, Mallard, and Hampton may be able to maintain transpirational cooling for a longer period of time, due to their access to water deeper in the soil profile. Mallard was also found to exhibit good drought tolerance without heat stress (Richardson et al., 2008). Our study suggests the drought tolerance observed in Mallard may be due to increased root weights in deeper profiles.

Total Root Weight

Significant differences in total root weight (0 – 35 cm below the sod plugs) were observed at week six in both years of this study (Table 2). In 2007, root weights ranged from 0.760 to 0.293 g. Hampton produced the most roots by weight in 2007 (0.760 g). Bedazzled, A93-485, Champagne, Diva, and Aura also exhibited high total root weights (0.636, 0.566, 0.544, 0.544, and 0.523 g, respectively) after six weeks under heat stress in 2007. Princeton P-105, Baron, Fairfax, and Chicago II produced the least roots by weight (0.303, 0.302, 0.297, and 0.293 g, respectively) in 2007 (Table 3). In 2008, root weights ranged from 0.500 to 0.123 g. A94-703, A93-485, Mercury, and Mallard produced the most roots by weight (0.500, 0.450, 0.430, and 0.425 g, respectively). Hampton, Bedazzled, and Champagne, also exhibited higher root weights (0.380, 0.364, and 0.360 g, respectively) than many other entries after six weeks under heat stress in 2008. Lakeshore, Liberator, and Midnight produced the least roots by weight (0.142, 0.131, and 0.123 g, respectively) (Table 3). Hampton, A93-485, Bedazzled, and Champagne consistently produced the heaviest root systems in both years of this study. Contrastingly, Princeton P-105 and Chicago II produced the least total roots in both years of this study. Both of these cultivars are compact types, which exhibit low above ground growth habit (Bonos et al., 2000; Murphy et al., 1997; Shortell et al., 2009). In general, above ground growth is correlated to below ground growth (Barbour and Murphy, 1984; Bonos and Murphy, 1999; Chloupek et al., 1999; Pederson et al., 1984) in plants, indicating that lower growing cultivars may have shallower/less root systems. However this is not the case for all compact cultivars. For example, Hampton, also a Compact Type cultivar, consistently exhibited high root weights in most layers and in total root weight under heat stress, indicating the need to thoroughly evaluate specific cultivars for rooting ability under summer stress conditions.

The results of total root weight are consistent with the results observed for each layer individually. In this study the entries that tended to have more roots in shallower profiles also had more roots in deeper profiles and higher total root weights, indicating that these cultivars and selections have the ability to maintain and grow roots throughout the soil profile under heat stress and therefore should be more stress tolerant (Bonos and Murphy, 1999; Bonos et al., 2004; Edbon and Kopp, 2004; Karcher et al., 2008). Additionally, the cultivars Chicago II, Liberator, and Eagleton exhibited thicker thatch layers than Royale and Monte Carlo (Table 4). There were no significant differences in percent organic matter between the cultivars and selections (data not shown). No correlations were found to exist between compressed thatch thickness and root weights at any depth in 2007 (data not shown). In 2008, compressed thatch thickness was weakly negatively-correlated to total root weight ($r^2 = -0.38$, $p = 0.05$) indicating that cultivars with increased thatch accumulation had less total root weight. However, because of the weak association to total root weights in 2008 and inconsistency between years the authors believe that thatch accumulation did not have a significant influence on the rooting ability between the cultivars and selections evaluated in this study.

The results reported here suggest that there is significant genetic variation in rooting ability among Kentucky bluegrass cultivars and selections under heat stress. This indicates that breeders should be able to select plants that are capable of growing more roots in the summer. These findings support other research by Bonos et al. (2004) and Lehman and Engelke (1991) in other perennial turfgrasses who found these traits to be variable, beneficial, and heritable. This characteristic allows for Kentucky bluegrass to potentially be used further south and extend the regions where this species can be grown.

Canopy Temperature Depression

Canopy temperature depressions are a measure of plant evaporative cooling through transpiration and indirectly quantify plant water status (Balota et al., 2007). Since all of the cultivars and selections evaluated in this experiment were kept well-watered the differences in canopy temperature depression represents the cultivars ability to cool through transpiration, and should be considered a heat stress avoidance method when the plants are supplied with adequate water unlike what has been studied by Throssell et al. (1997) who used this technique to predict drought stress and schedule irrigation events. There were significant differences between cultivars in mean canopy temperature depression throughout 2007. However there were no significant differences in 2008. According to our canopy temperature depressions and root weights the environment was more stressful in 2008 than 2007, leading to a lack of cultivar separation. Hampton and Champagne exhibited the coolest canopies or most negative canopy temperature depressions (Table 5). Hampton and Champagne were two of the cultivars that exhibited more root weights in most of the profiles evaluated when compared to the other entries. Princeton P-105 and Baron had positive canopy temperature depressions or canopies were hotter than ambient conditions (Table 5). These entries generally exhibited poor rooting ability in this experiment. Canopy temperature depressions were highly negatively correlated to root weight in the 0 – 7 cm profile (r^2 = -0.82, p = 0.001), 7 – 14 cm profile (r^2 = -0.79, p = 0.001), 14 – 21 cm profile (r^2 = -0.67, p = 0.001), and total root weight (r^2 = -0.83, p = 0.001) which is consistent with previous research on Kentucky bluegrass (Bonos and Murphy, 1999; Perdomo et al., 1996), indicating that cultivars with heavier root weights may be growing at a faster rate and transpiring faster than the intolerant cultivars. This measurement may be a good candidate for screening cultivars for the ability to produce roots in the summer, but this technique should be used with caution due to the inconsistent results we observed between years in this study.

Turfgrass Quality

Turfgrass quality is a visual measurement on a 1 – 9 scale, with 9 = best turf quality. Components of quality include color, brightness, leaf texture, density, uniformity, smoothness, and amount of overall damage. Turfgrass quality six weeks after planting ranged from 8 to 3 in 2007 and 2008. Bedazzled, A93-485, and ‘Cabernet’ generally exhibited high quality whereas Fairfax, Princeton P-105, Liberator, and Baron exhibited poor quality (Table 6). Turfgrass quality was weakly correlated to root weights in the 0 – 7 cm profile (r^2 = 0.47, p = 0.01), 7 – 14 cm profile (r^2 = 0.51, p = 0.01), 14 – 21 cm profile (r^2 = 0.51, p = 0.01), 21 – 28 cm profile (r^2 = 0.45, p = 0.02), and total root weight (r^2 = 0.53, p = 0.01), suggesting that increased root weights could have contributed to increased turf quality in the summer. However, it was not significantly correlated to root weights in 2008 and based on this variation should not be used as the only predictor of rooting ability.

Leaf Firing

Leaf firing is the chlorosis and senescence of the end of the leaf blades. Leaf firing is a sign of physiological damage caused by heat stress and has been used in the past to visually study plant heat tolerance (Nilson and Orcutt, 1996). Leaf firing was also rated on a 1 to 9 scale, with 9 = no leaf firing. There were significant differences in leaf firing in both years of this study (Table 2). Leaf firing six weeks after planting ranged from 9 to 4 in 2007 and 2008. Eagleton, Cabernet, Royale, Monte Carlo, and A94-703 (mostly typical Mid-Atlantic Types) generally showed little to no leaf firing, whereas Baron, Fairfax, Princeton P-105, and Aura showed significant leaf firing (Table 6). Leaf firing measurements were significantly correlated to root weights in the 0 – 7 cm profile ($r^2 = 0.42$, $p = 0.03$), 7 – 14 cm profile ($r^2 = 0.45$, $p = 0.02$), 14 – 21 cm profile ($r^2 = 0.40$, $p = 0.04$), and total root weight ($r^2 = 0.42$, $p = 0.03$) in 2007, however it was not correlated to root weight in 2008. Therefore, reduced leaf firing could have contributed to increased root weights but based on the results of this study was not the sole contributor and other variables were also at work.

Conclusion

In conclusion, there was a significant amount of genetic variability in the rooting ability of Kentucky bluegrass cultivars and selections under heat stress. This research has identified a number of cultivars or selections that show continued root growth six weeks after sodding during the summer months in New Jersey. Cultivars and selections with increased root weight in the summer, such as Hampton, A93-485, Bedazzled, and Champagne would have the ability to establish from sod significantly better during the hot summer period then other cultivars with poor root weight under heat stress. Additionally when developing new cultivars, selections that show increased root weights under heat stress can be preferentially used.

Turfgrass quality and leaf firing although weakly-correlated to root weights in some of the profiles and timings, did not consistently correlate over the course of the experiment. These results could indicate that turfgrass quality and leaf firing observations may provide a glimpse into turfgrass root growth, but based on the observed variation and inconsistent correlations to root weights these measurements should not be used as the only predictor of Kentucky bluegrass root growth under heat stress. Canopy temperature depressions were strongly correlated in 2007 but there were no significant differences between entries in 2008. Further research should be conducted to confirm the utility of canopy temperature depressions as an indicator for rooting under heat stress.

This could mean that larger root systems by weight are not always responsible for Kentucky bluegrass summer stress tolerance under well-watered conditions and that other parameters must be conferring the observed results. Root viability has been shown to be important in stress tolerance in other grasses (Huang et al., 1997) and perhaps differences in root viability are responsible for the inconsistent correlation between increased root weights, canopy temperature depressions, and decreased leaf firing over the different years that was determined here. This indicates that progress in improving the rooting ability of Kentucky bluegrass under heat stress can only be expected if selection is based on root characteristics such as increased root weight under heat stress and possibly even high root viability. This is problematic because both measurements are time consuming and laborious which limits the number of cultivars and selections that can be effectively screened. However, even though this slows progress, the identification of cultivars and selections with increased root weight under heat stress can help to increase the longevity of the stand in the northeastern United States, extend the season for sod production and planting, and possibly even identify more appealing characteristics that are efficiently measured and better related to the root weight and root viability of the turfgrass plant.

The utilization of Kentucky bluegrass cultivars that are capable of generating large root weights under heat stress can lead to more efficient water use during sod establishment, which will conserve our natural resources and protect the environment. Additionally, the development of Kentucky bluegrasses with improved rooting ability under heat stress could allow Kentucky bluegrass to be used further south and extend the utilization of the species.

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Figure 1. Minimum and maximum air temperatures during the six week rooting experiment of 25 Kentucky bluegrass cultivars and selections evaluated at the Rutgers University Horticultural Research Farm II in North Brunswick, NJ, in 2007 and 2008.

Figure 2. Root weights in the 0 - 7 cm profile of 25 Kentucky bluegrass cultivars and selections in response to heat stress six weeks after planting in 2007 and 2008 at the Rutgers University Horticultural Research Farm II in North Brunswick NJ.

Figure 3. Root weights in the 7 – 14 cm profile of 25 Kentucky bluegrass cultivars and selections in response to heat stress six weeks after planting in 2007 and 2008 at the Rutgers University Horticultural Research Farm II in North Brunswick NJ.

Figure 4. Root weights in the 14 – 21 cm profile of 25 Kentucky bluegrass cultivars and selections in response to heat stress six weeks after planting in 2007 and 2008 at the Rutgers University Horticultural Research Farm II in North Brunswick NJ.

Figure 5. Root weights in the 21 – 28 cm profile of 25 Kentucky bluegrass cultivars and selections in response to heat stress six weeks after planting in 2007 and 2008 at the Rutgers University Horticultural Research Farm II in North Brunswick NJ.

Figure 6. Root weights in the 28 – 35 cm profile of 25 Kentucky bluegrass cultivars and selections in response to heat stress six weeks after planting in 2007 and 2008 at the Rutgers University Horticultural Research Farm II in North Brunswick NJ.

Table 1. Kentucky bluegrass entries evaluated for root growth under heat stress at the Rutgers University Horticultural Research Farm II, in North Brunswick, NJ, in 2007 and 2008.

	Cultivar or Selection	Classification Type
1	Baron	BVMG
2	Chicago II	Compact
3	Diva	Compact
4	Princeton P-105	Compact
5	Bewitched	Compact
6	A00-1400	Compact
7	Mercury	Compact
8	Hampton	Compact
9	Bedazzled	Compact-America
10	Langara	Compact-America
11	Mallard	Compact-America
12	Royale	Compact-America
13	A96-1338	Compact-America
14	Liberator	Compact-Midnight
15	Midnight	Compact-Midnight
16	Cabernet	Mid-Atlantic
17	Eagleton	Mid-Atlantic
18	A93-485	Mid-Atlantic
19	A94-703	Mid-Atlantic
20	Aura	Mid-Atlantic
21	A03-56	Other
22	Fairfax	Other
23	Monte Carlo	Other
24	Lakeshore	Shamrock
25	Champagne	Shamrock

Table 2. Mean squares of analysis of variance of rooting characteristics of 25 Kentucky bluegrass cultivars and selections in response to heat stress six weeks after planting at the Rutgers University Horticultural Research Farm II in North Brunswick, NJ, in 2007 and 2008.

	Root Weights						Canopy Temperature Depression	Turfgrass Quality	Leaf Firing
	0 - 7cm	7 - 14cm	14 - 21cm	21 - 28cm	28 - 35cm	Total			
2007									
Rep	0.007*	0.003	0.002	0.002	$2E^{-4}$	0.036	2.16*	3.37**	1.44*
Cultivar	0.006***	0.003**	0.002*	0.002***	$1E^{-4}$	0.045***	2.89***	2.16***	1.21***
2008									
Rep	0.001	$1E^{-5}$	$4E^{-5}$	$1E^{-4}$	$2E^{-6}$	0.001	2.55	2.27	0.35
Cultivar	0.009**	0.002**	0.001	$3E^{-4}$	$1E^{-5}$*	0.029**	1.41	3.25**	3.35*

*, **, *** Denotes significance to the 0.05, 0.01, and 0.001, respectively.

Table 3. Total root weights (0 – 35 cm profile) of Kentucky bluegrass cultivars and selections evaluated under heat stress at the Rutgers University Horticultural Research Farm II in North Brunswick, NJ in 2007 and 2008.

	Cultivar or Selection	Total Roots (g) 2007	2008
1	A00-1400	0.327	0.311
2	Aura	0.523	0.277
3	A03-56	0.428	0.217
4	A93-485	0.566	0.450
5	A94-703	0.508	0.500
6	A96-1338	0.437	0.263
7	Baron	0.302	0.293
8	Bedazzled	0.636	0.364
9	Bewitched	0.473	0.255
10	Cabernet	0.426	0.257
11	Champagne	0.554	0.360
12	Chicago II	0.293	0.186
13	Diva	0.544	0.299
14	Eagleton	0.463	0.262
15	Fairfax	0.297	0.252
16	Hampton	0.760	0.380
17	Lakeshore	0.432	0.142
18	Langara	0.494	0.265
19	Liberator	0.429	0.131
20	Mallard	0.487	0.425
21	Mercury	0.421	0.430
22	Midnight	0.434	0.123
23	Monte Carlo	0.498	0.319
24	Princeton P-105	0.303	0.192
25	Royale	0.378	0.300
	LSD at 5% =	0.208	0.174

Table 4. Compressed thatch thickness (mm), using a 1kg weight, of Kentucky bluegrass cultivars and selections evaluated for root growth under heat stress at the Rutgers University Horticultural Research Farm II in North Brunswick, NJ, in the summer of 2008.

	Cultivar or Selection	Compressed Thatch Thickness (mm)
1	A00-1400	15.3
2	Aura	16.9
3	A03-56	15.7
4	A93-485	13.5
5	A94-703	12.6
6	A96-1338	15.4
7	Baron	14.7
8	Bedazzled	16.5
9	Bewitched	15.2
10	Cabernet	16.6
11	Champagne	16.8
12	Chicago II	18.0
13	Diva	14.9
14	Eagleton	17.2
15	Fairfax	15.3
16	Hampton	15.2
17	Lakeshore	16.8
18	Langara	16.8
19	Liberator	17.2
20	Mallard	12.3
21	Mercury	15.8
22	Midnight	16.9
23	Monte Carlo	11.6
24	Princeton P-105	15.5
25	Royale	11.5
	LSD at 5% =	2.7

Table 5. Canopy temperature depressions of Kentucky bluegrass cultivars and selections evaluated under heat stress six weeks after planting at the Rutgers University Horticultural Research Farm II in North Brunswick, NJ, in the summers of 2007 and 2008.

	Cultivar or Selection	2007	2008
1	A00-1400	0.3	0.8
2	Aura	-1.7	1.8
3	A03-56	-0.2	1.2
4	A93-485	-1.3	1.6
5	A94-703	-0.3	0.5
6	A96-1338	-0.3	0.4
7	Baron	1.0	1.3
8	Bedazzled	-2.0	0.2
9	Bewitched	-0.7	0.1
10	Cabernet	-0.7	0.6
11	Champagne	-2.0	0.4
12	Chicago II	1.0	0.7
13	Diva	0.7	0.5
14	Eagleton	-1.0	0.2
15	Fairfax	0.7	-0.2
16	Hampton	-2.3	-0.8
17	Lakeshore	-0.2	-0.3
18	Langara	0.5	0.7
19	Liberator	-0.3	-0.3
20	Mallard	-0.7	0.8
21	Mercury	-0.7	1.1
22	Midnight	-0.5	0.0
23	Monte Carlo	-0.7	0.6
24	Princeton P-105	0.7	-0.1
25	Royale	0.3	-0.1
	LSD at 5% =	1.1	NS

Table 6. Turfgrass quality (TQ) and leaf firing (LF) ratings of Kentucky bluegrass cultivars and selections evaluated for root growth under heat stress for six weeks at the Rutgers University Horticultural Research Farm II in North Brunswick, NJ, in the summers of 2007 and 2008.

	Cultivar or Selection	2007 TQ[1]	2007 LF[2]	2008 TQ	2008 LF
1	A00-1400	8	6	6	6
2	Aura	7	6	4	5
3	A03-56	6	7	6	7
4	A93-485	7	6	8	9
5	A94-703	7	7	6	7
6	A96-1338	8	7	6	7
7	Baron	6	6	5	5
8	Bedazzled	8	7	6	6
9	Bewitched	7	7	8	7
10	Cabernet	8	8	7	7
11	Champagne	7	7	3	4
12	Chicago II	6	7	5	5
13	Diva	7	6	5	6
14	Eagleton	8	8	6	6
15	Fairfax	6	6	5	5
16	Hampton	8	7	6	5
17	Lakeshore	7	8	5	6
18	Langara	6	7	5	5
19	Liberator	6	7	5	5
20	Mallard	7	7	6	6
21	Mercury	7	6	5	5
22	Midnight	7	7	6	6
23	Monte Carlo	8	8	5	7
24	Princeton P-105	6	6	5	5
25	Royale	6	7	6	7
	LSD at 5% =	1	1	2	2

[1]Turfgrass quality, where 9 = best turf quality
[2]Leaf firing, where 9 = least leaf firing

Figure 1. Minimum and maximum air temperatures during the course of the experiment at the Rutgers University Horticultural Research Farm II in North Brunswick, NJ, in 2007 and 2008.

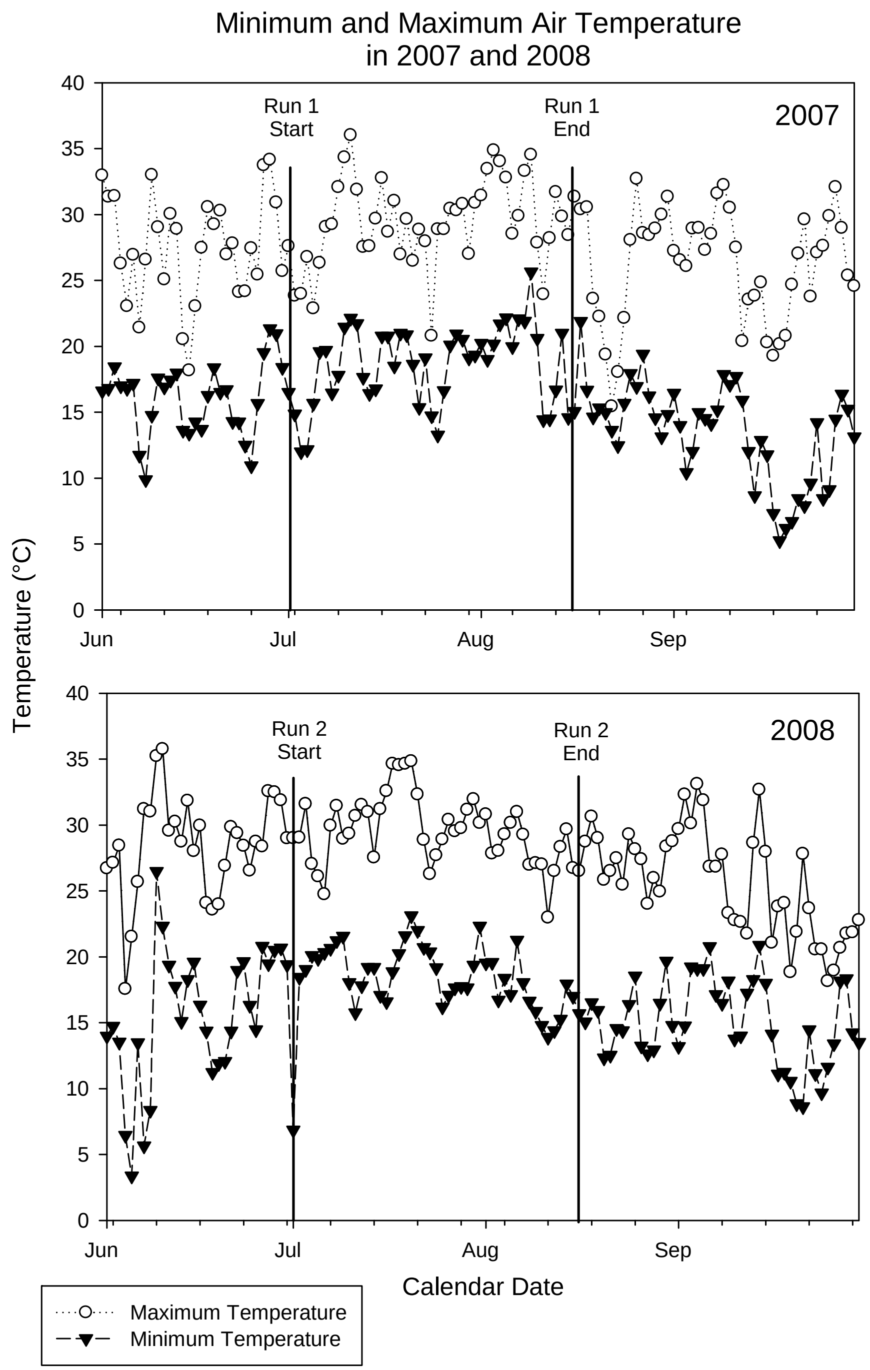
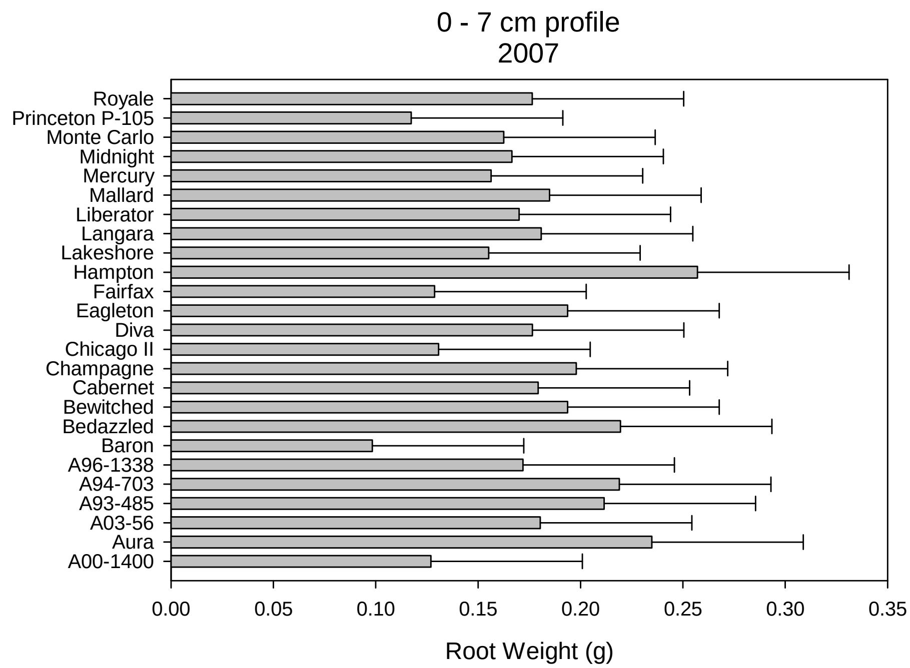


Figure 2. Root weights in the 0 - 7 cm profile of 25 Kentucky bluegrass cultivars and selections in response to heat stress six weeks after planting in 2007 and 2008 at the Rutgers University Horticultural Research Farm II in North Brunswick NJ.



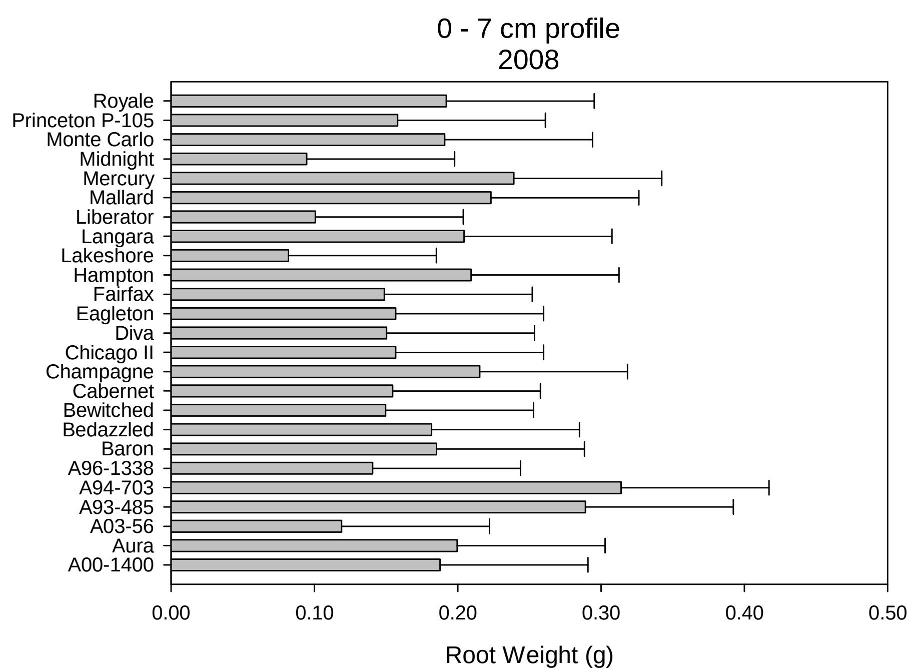
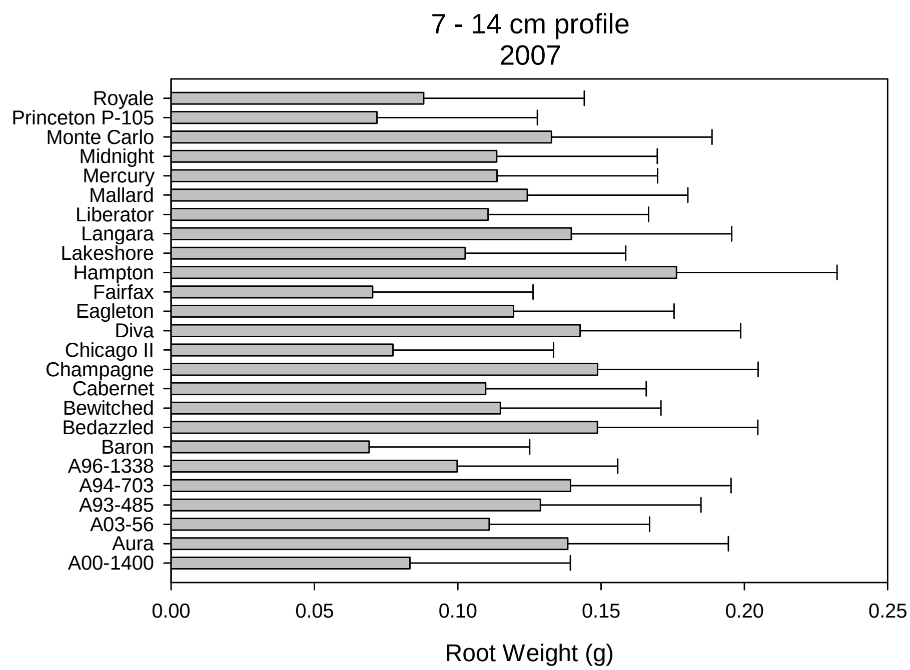


Figure 3. Root weights in the 7 – 14 cm profile of 25 Kentucky bluegrass cultivars and selections in response to heat stress six weeks after planting in 2007 and 2008 at the Rutgers University Horticultural Research Farm II in North Brunswick NJ.



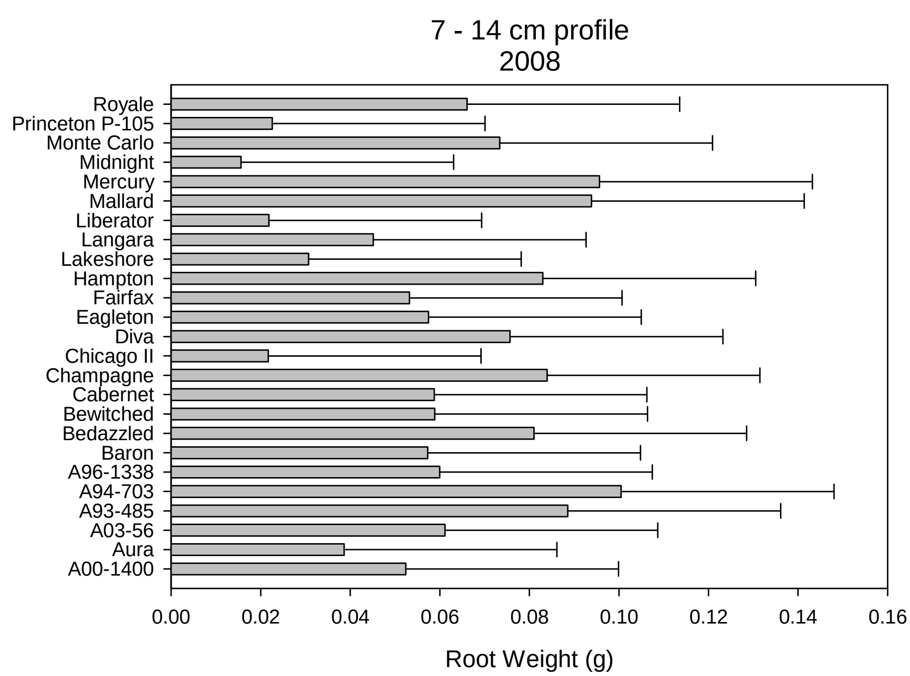
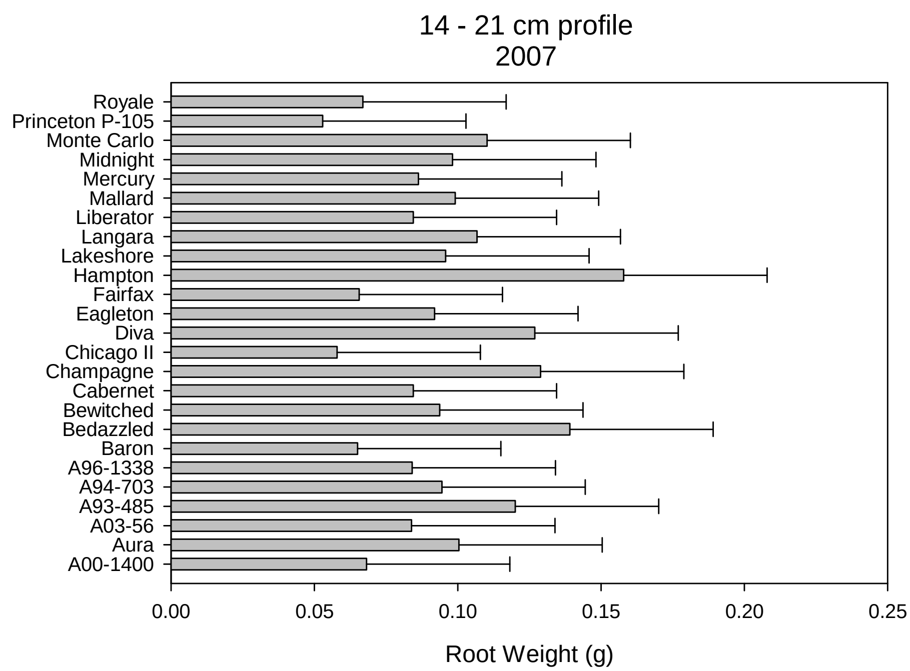


Figure 4. Root weights in the 14 – 21 cm profile of 25 Kentucky bluegrass cultivars and selections in response to heat stress six weeks after planting in 2007 and 2008 at the Rutgers University Horticultural Research Farm II in North Brunswick NJ.



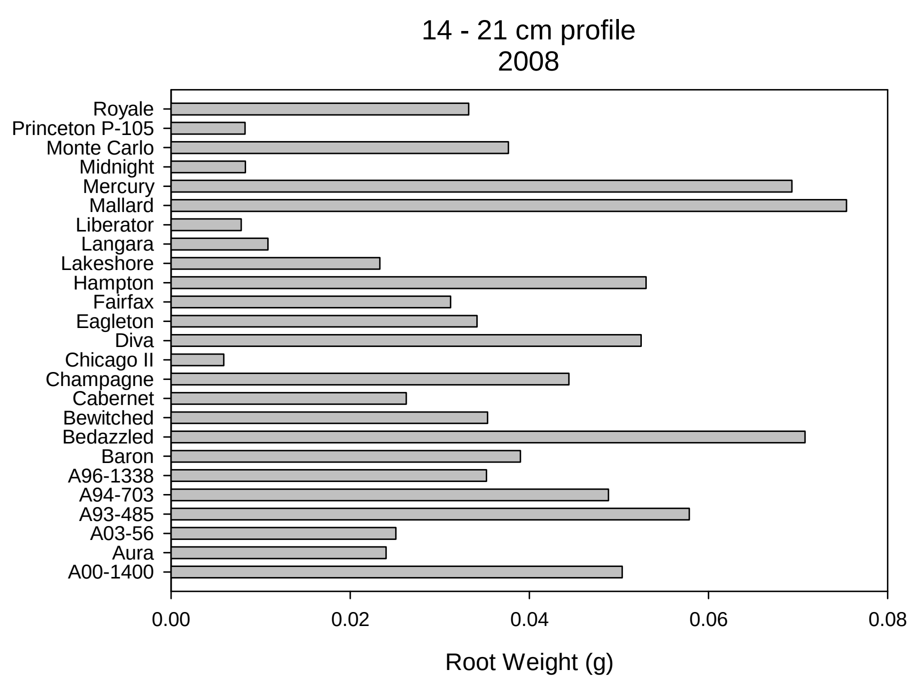
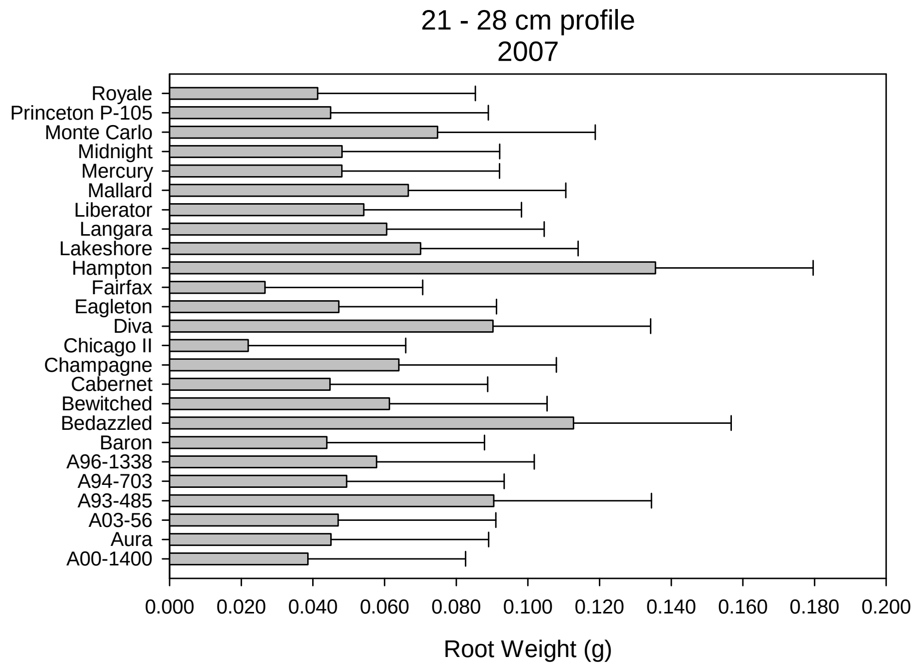


Figure 5. Root weights in the 21 – 28 cm profile of 25 Kentucky bluegrass cultivars and selections in response to heat stress six weeks after planting in 2007 and 2008 at the Rutgers University Horticultural Research Farm II in North Brunswick NJ.



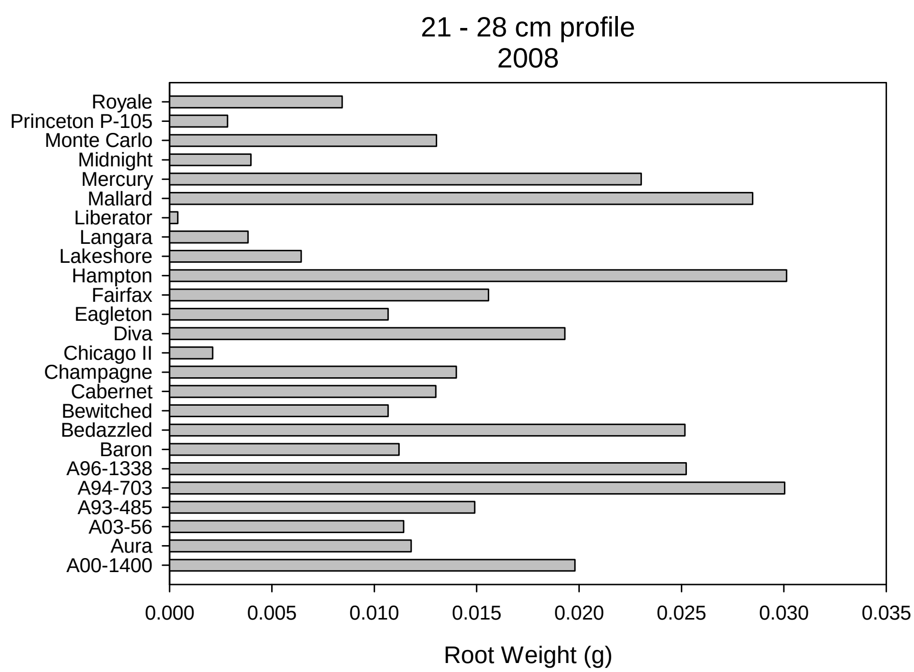
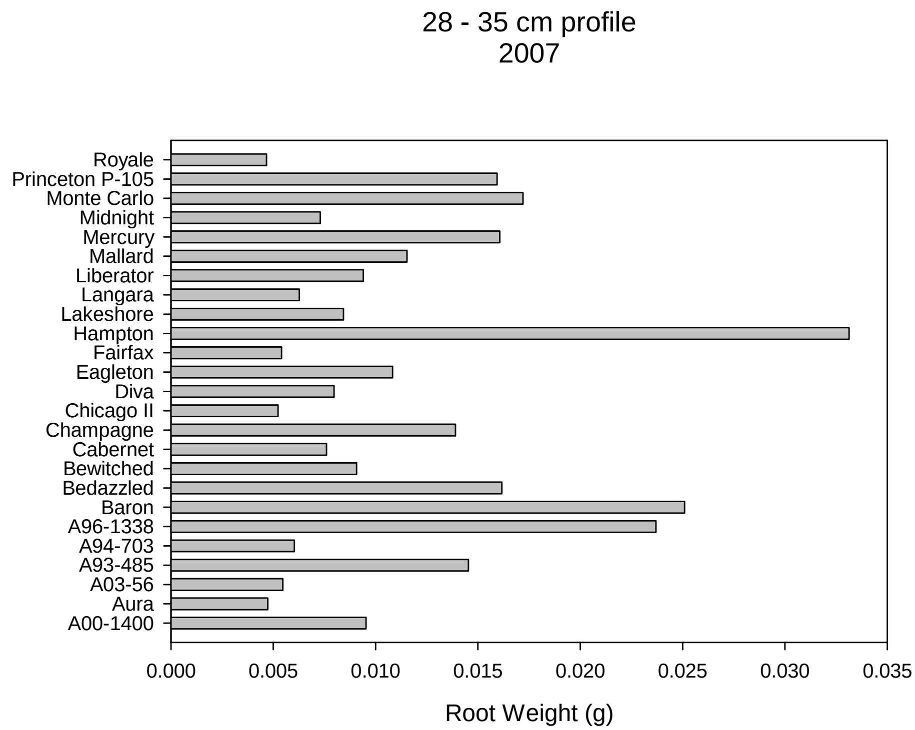
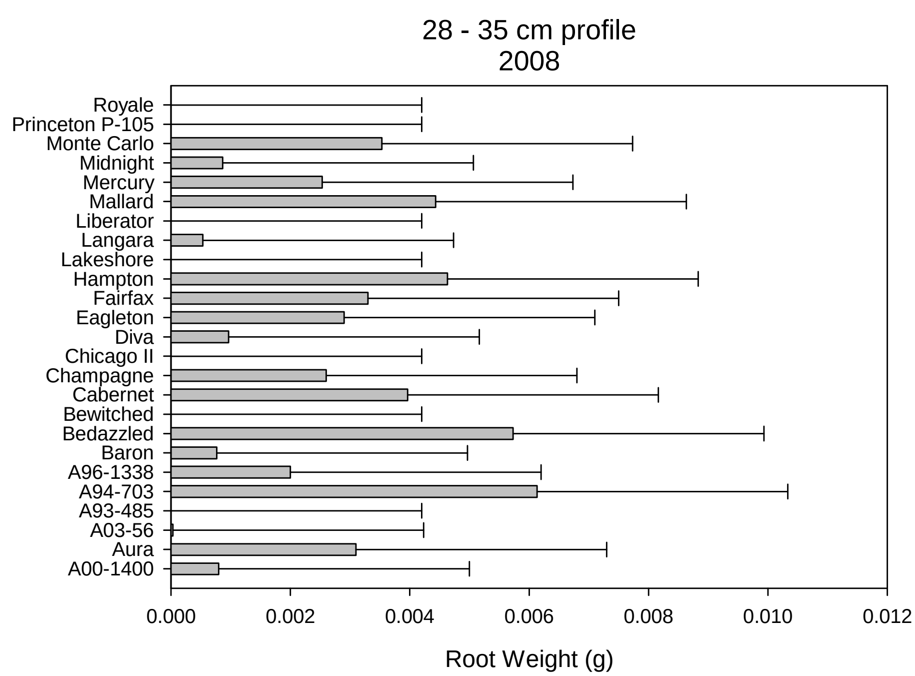


Figure 6. Root weights in the 28 – 35 cm profile of 25 Kentucky bluegrass cultivars and selections in response to heat stress six weeks after planting in 2007 and 2008 at the Rutgers University Horticultural Research Farm II in North Brunswick NJ.





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Publications

Shortell, R., W.A. Meyer, and S.A. Bonos. 2008. "Classification and Inheritance of Morphological and Agronomic Characteristics in Kentucky Bluegrass (*Poa pratensis* L.)" HortScience 44(2):274-279.

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