Relationship between Cell Wall Components and Nitrogen Use Efficiency in Dollar Spot Resistance in *Agrostis* Species and Cultivars

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SUMMARY

Dollar spot caused by *Sclerotinia homoeocarpa* is one of the most economically important diseases affecting golf greens. Dollar spot susceptibility varies among *Agrostis* species and cultivars, with velvet bentgrass (*Agrostis canina* L.) exhibiting superior resistance to dollar spot compared to creeping bentgrass (*Agrostis stolonifera* L.). Leaf surface properties including anatomical, physiological, and morphological traits may affect the ability of the fungus to penetrate leaves and cause infection. The relationship between leaf cell wall components and dollar spot severity has not been investigated in these species. Efficient use of nitrogen (N) and higher nitrogen use efficiency (NUE, greater productivity-to-plant N ratio) has been proposed in turfgrass to maintain turf function with less N, which is the current trend in the management of golf greens. NUE has been shown to be related to nitrate reductase activity (NRA) but its relationship to dollar spot has not been investigated. Our objectives were to evaluate dollar spot severity among cultivars of creeping and velvet bentgrass and determine relationships between cell wall components, NUE and NRA with dollar spot disease. Dollar spot, total cell wall (TCW), ligno- and hemi-cellulose contents, shoot NUE and NRA measurements were be made in the field on seven velvet bentgrass and seven creeping bentgrass entries as part of the 2003 NTEP Bentgrass Greens Test. A USGA green was used and maintained without fungicides during summer months, mowed at 5/32 inch height of cut, and fertilized with 3 pounds of total N per 1000ft^2 per year. Dollar spot severity counts were made in early September and October of 2007 while NRA and NUE were determined in July. Velvet bentgrass cultivars exhibited lower disease severity to dollar spot compared to creeping bentgrass except under severe disease pressure. Shoot NRA and NUE were positively correlated. Dollar spot tolerant velvet bentgrass cultivars exhibited significantly lower NRA and NUE compared

to dollar spot susceptible creeping bentgrass entries. NRA was positively correlated with dollar spot severity accounting for at least 50% of the total variation in disease severity. Dollar spot tolerant creeping bentgrass cultivars such as Declaration exhibited greater hemicellulose content compared to intolerant cultivars such as Independence. Leaf hemicellulose content accounted for at least 30% of the variation in *Agrostis* dollar spot severity. NUE was not correlated with dollar spot disease in *Agrostis* species and cultivars. These measurements will be repeated in 2008. However, first year results suggest that lower shoot NRA and greater leaf hemicellulose content, especially in dollar spot susceptible creeping bentgrass cultivars, may lower disease incidence. The data does not support the notion that NUE under the conditions of this test provides any influence on dollar spot severity. These results suggest that balanced N fertility in the management of creeping bentgrass is critical to balancing the competing effects of N fertility on dollar spot severity with leaf NRA and hemicellulose content in *Agrostis* species and cultivars.

INTRODUCTION

Creeping bentgrass (*Agrostis stolonifera* L.) and velvet bentgrass (*Agrostis canina* L.) are the preferred cool-season turfgrass of choice for use on putting greens in New England. Dollar spot caused by *Sclerotinia homoeocarpa* is one of the most economically important diseases that affect turfgrass, especially closely mown greens, tees and fairways (Goodman and Burpee, 1991). Significant difference exists in dollar spot susceptibility between species and cultivars of *Agrostis* (Chakraborty et al, 2005). Colonial bentgrass (*Agrostis tenuis* L.) and velvet bentgrass have good resistance to dollar spot when compared to creeping bentgrass (Chakraborty et al, 2005; Belanger et al., 2004; Williams and Harrell, 2005).

Dollar spot infection begins at the leaf surface and anatomical, physiological, and morphological traits may affect the ability of the fungus to penetrate leaves and cause infection. Some research has been conducted to investigate relationships between dollar spot resistance and leaf morphology (trichome, stomatal and cuticular wax distribution) in *Agrostis* (Williams and Harrell, 2005); however, no significant relationship between these traits and dollar spot was detected. Additional research is needed to investigate the relationship between cell wall components and dollar spot severity. Cell walls are a matrix consisting of cellulose, hemicellulose, and lignin (Taiz and Zeiger, 1972), which enable plants to withstand pressure from outside forces (weight, bending, crushing) such as wear from foot or vehicular traffic. Consequently, differences in cell wall composition may also affect the ability of plants to resist pathogen infection.

Grasses rich in lignin have demonstrated reduced fungal penetration and increased resistance to fungal pathogens (Sherwood and Vance, 1980).

Research has shown that lignin may be an important mechanism in leaf rust (*Puccinia coronata*) resistance in grasses (Delgado et al., 2002). Velvet bentgrass cultivars have been shown to have both superior wear tolerance and greater total cell wall content when compared to cultivars of creeping bentgrass (Dowgiewicz and Ebdon, unpublished data), suggesting leaf cell wall content may play a role in dollar spot resistance in these species. In addition, recent data indicated that one genomic region where a major dollar spot resistance gene was identified (Chakraborty et al., 2006) contained three key genes involved in the lignin biosynthetic pathway (Jung et al., unpublished data).

There has been a trend in recent years to achieve faster green speeds by reducing nitrogen (N) (Radko, 1985). However, lack of adequate N predisposes plants to greater damage from dollar spot (Endo, 1966). Nitrogen-supplemented growth promotes greater vigor and mowing frequency and in turn, removal of diseased tissue (Couch, 1995). Landschoot and McNitt (1997) demonstrated that dollar spot suppression was directly related to plant color and N availability. Greater vigor and dollar spot suppression may be achieved with less N by selecting for higher nitrogen use efficiency (NUE). Nitrogen use efficiency, defined as dry matter produced per unit N in grass clippings, has been shown to vary with the species and cultivar (Harris and Whittington, 1983; Kuo et al., 1995; Jiang and Hull, 1998). Turfgrass with higher NUE could be fertilized using less N while still maintaining high quality.

Higher NUE by turfgrass is associated with efficient uptake by roots and efficient utilization and transport from root to shoot (Jiang et al., 2001). The initial step in incorporation of N into plant tissues is nitrate reduction, which is catalyzed by the enzyme nitrate reductase. Ambient nitrate levels increase with N supply, and in turn, stimulate nitrate reductase activity (NRA), which is most evident in leaf tissue. Leaf tissue N and shoot growth increases with N supply while NUE has been shown to decrease with N in *Agrostis* species and cultivars (Kuo et al., 1995) and Kentucky bluegrass (*Poa pratensis* L.) cultivars (Jiang and Hull, 1998). As such, an inverse relationship is often observed between NRA and NUE, although a positive correlation between NRA and NUE of a tissue has also been reported (Bushoven and Hull, 2001).

Superior shoot productivity is often correlated with high shoot NRA in crop plants (Campbell et al., 1993). High productivity does not necessarily equate to higher NUE especially under conditions where N is not growth limiting, which is typical of golf greens. Furthermore, unlike crop yields, superior yield in turfgrass does not necessarily equate to superior turf performance. In *Agrostis* species no clear relationship has been observed between NRA and productivity (Harris and Whittington, 1983). Colonial bentgrass, growing under infertile conditions, exhibited higher NRA compared to creeping bentgrass growing under more fertile conditions (Harris and Whittington, 1983). Alternatively, under high levels of nitrate-N, NRA activity was greater in creeping bentgrass than colonial bentgrass. Bushoven and Hull (2001) reported no significant relationship (correlation) between shoot NRA and NUE in creeping bentgrass cultivars. The aforementioned research with *Agrostis* were not field evaluations but rather laboratory conditions using soilless media. Additional research is needed at the species and cultivar level in *Agrostis* to understand the metabolic basis for NUE in order to develop grasses that would require less N input under conditions typical of golf greens.

Agrostis species have been shown to vary in NUE (Harris and Whittington, 1983; Kuo et al., 1995; Bushoven and Hull, 2001). Colonial and velvet bentgrass are recognized as having better tolerance to infertile conditions compared to creeping bentgrass, a species better adapted to high N fertility (Beard, 1973; Harris and Whittington, 1983). Kuo et al. (1995) observed higher NUE with strongly creeping type *Agrostis* species such as creeping bentgrass when compared to colonial bentgrass. Nitrogen use efficiency can be an important attribute for sustaining adequate productivity for managing dollar spot under low N environments. Furthermore, maintaining adequate productivity with less N can promote greater total cell wall content because excessive nitrogen-induced shoot growth can reduce various cell wall components and wear tolerance (Beard, 1973; Shearman and Beard, 1975). On the basis of the information above, the following objectives are proposed (i) evaluate dollar spot severity among cultivars of creeping and velvet bentgrass and (ii) determine the relationship between dollar spot resistance and cell wall components, NUE, and NRA.

MATERIALS AND METHODS

Creeping and velvet bentgrass cultivars as part of the 2003 National Turfgrass Evaluation Program (NTEP) Greens Test were assessed for dollar spot severity, NUE, NRA and cell wall content. Plots were 3 by 6 ft and were established in October 2003 at the Joseph Troll Turfgrass Research Facility, South Deerfield, MA. The *Agrostis* species and cultivars represent diverse plant morphology, pedigree, place of origin, and disease resistance. The genetic material offers potential opportunity to select for diverse tolerance to dollar spot as well as anatomical and physiological characteristics. Plots were arranged as a randomized complete block design with 3 replicates and were maintained under well-irrigated conditions to prevent stress and mowed daily at 5/32 inch. Plots were maintained at 3.5 pounds of nitrogen per 1000 ft^2 per year and fungicides were withheld during summer months to promote dollar spot activity.

Measurements (indicated below) were made in the field on seven velvet bentgrass entries (Experimental, Greenwich, Legendary, SR-7200, Venus, Vesper, and Villa) and seven creeping bentgrass entries (Authority, Bengal, CY-2, Declaration, Independence, Penn A-1, and Penncross).

Cell Wall Components

Leaf fiber analysis was assessed to determine the amount of total cell wall content (entire fibrous portion), lignocellulose, and hemicellulose according to the methods of Goering and Van Soest (1970). Polyester bag technology (PBT) (Contreras Lara, 1999; Komarek et. al, 1994) was used for this analysis as well. This procedure required acid and neutral detergent testing with different reagents to measure quantities of cell wall constituents. The neutral detergent fiber (NDF) procedure was used to determine the percent total cell wall content (TCW) on a dry weight basis. Lignocellulose content was determined on a dry weight basis using the acid detergent fiber method (ADF). The difference between the quantity of NDF and ADF served to estimate the percent hemicellulose (NDF-ADF).

Filter bags (ANKOM Technology, Macedon, NY) were used in both fiber procedures. These polyester bags had a uniform pore size of 30 μm. Bags were weighed and filled with approximately 0.1 g of dried sample. Bags were then placed in an 11 ball flask, and depending on the analysis, moistened with 70 ml of the appropriate detergent solution (neutral detergent solution or acid detergent solution). All solutions were prepared according to the methods of Goering and Van Soest (1970). The flask was heated to maintain temperature between 95 and 100ºC, and continuously agitated. After 60 minutes for neutral detergent fiber analysis and 70 minutes for acid detergent fiber analysis, the bags were removed from the flask and washed with boiling water to remove any detergent solution. They were then soaked in acetone for 3 minutes and oven dried for 60 h at 70ºC. Oven dry weights were then recorded and converted to percentages [{(initial weight-final weight)/initial weight)} X 100] with the percentage of neutral detergent fiber representing the total cell wall content, the percentage of acid detergent fiber representing the lignocellulose content, and the difference between the two (NDF-ADF) representing the hemicellulose content.

Nitrogen Use Efficiency and Nitrate Reductase Activity

Nitrogen use efficiency and NRA were performed on clippings collected from field plots. Nitrogen use efficiency measurements were based on clippings collected from all field plots on 9 July, 2007. Leaf tissues were oven dried at 70ºC for 48 h and samples were analyzed for total N content using micro-Kjeldahl procedures (Eastin, 1978). Nitrogen use efficiency (g dry weight/mg tissue N) was derived from total N content using the formula: NUE = 1000/mg N/g dry matter.

Nitrate reductase activity was assayed using the *in vivo* method for cool-season turfgrass described by Jiang and Hull (1998). Shoots (leaf clippings) were collected by harvesting tissues during the morning hours on 9 and 12 July 2007. Leaf segments were placed into 25-ml flasks containing 5ml of incubation medium consisting of 0.1 M potassium phosphate buffer (pH 7.5), 50 mM KNO_3, and 0.39 M 2-propanol. Incubation flasks were placed in a vacuum desiccator and evacuated to 6 mm Hg for 2 minutes. After releasing the vacuum, flasks were stirred to ensure tissues were submerged in incubation medium and again evacuated, followed by purging with N_2 gas. This evacuation/purging procedure was repeated once, and the flasks were immediately stoppered and transported to a 30ºC water bath shaker, and covered with black plastic to exclude light. After 15 minutes, 0.5-ml aliquots were transferred from each flask to 10-ml test tubes to determine the initial NO_2- concentration. Leaf NRA was based upon NO_2- increase in the incubation medium between 15 and 45 minutes. Nitrite concentration was determined by adding to the test tubes, 1 ml 29 mM sulfanilamide solution in 2.4 M HCl followed by 1 ml 11.6 mM N-(1-napthyl)-ethylenediamide dihydrochloride in 0.12 M HCl (Snell and Snell, 1949). Absorbance was measured at 540 nm using a spectrophotometer (Spectronic Genesys Series, Spectronic Instruments Inc., Rochester, NY). The NRA was expressed as μmol NO_2^- produced per gram fresh tissue per hour.

Dollar Spot Assessment

The University of Massachusetts Turfgrass Disease Diagnosis Laboratory confirmed the identity of dollar spot disease. Dollar spot severity was evaluated by counting the total number of disease centers (spots) per plot. Counts were made initially on 2 September followed by 1 October 2007. Severity of dollar spot disease was moderate at the 2 September rating while the 1 October rating represented heavy dollar spot disease pressure. No significant dollar spot disease was observed before the September rating date.

Statistical Analysis

Field and lab measurements that were conducted during the 2007 growing season will be repeated in 2008. Dollar spot severity, NUE, NRA, TCW, hemi- and ligno-cellulose were analyzed using analysis of variance (ANOVA) procedures. Cultivar sum of squares were partitioned into single degree of freedom (df), orthogonal contrasts, to test for the difference between the combined means of velvet and creeping bentgrass cultivars. Contrasts were also performed to test for differences within velvet and creeping bentgrass species. Least significant difference (LSD) values are reported for comparisons at the 0.05 level. Correlation coefficients between cultivar means (n=14) were calculated to investigate the relationship between dollar spot severity and various cell wall components, NUE and NRA measurements from the field.

RESULTS AND DISCUSSION

Dollar Spot

Significant differences were observed between cultivars within *Agrostis* during the 2 September and 1 October evaluation dates (Table 1). At the 2 September rating creeping bentgrass entries exhibited approximately twice the number of disease spots compared to velvet bentgrass entries, averaging 11.1 and 5.8 spots per plot, respectively. Differences were detected within velvet bentgrass and creeping bentgrass entries during the September rating period. Creeping bentgrass cultivars ranged from 3.3 spots (Declaration) to 34.7 spots per plot (Independence) while velvet entries ranged from 1.3 spots (Experimental) to 16.7 spots per plot (Vesper). By the 1 October rating, no statistical difference was detected between velvet and creeping bentgrass cultivars in dollar spot severity. Disease pressure was significantly greater at the October rating date, increasing by as much as 10-fold for creeping bentgrass and 14-fold for velvet bentgrass when compared to the September rating date. Accordingly, any advantages afforded by planting velvet bentgrass cultivars were lost under heavy disease pressure. Numerous entries exhibited acceptable dollar spot tolerance (≤ 5 spots per plot) in September, including Declaration and Penn A-1 creeping bentgrass as well as the following velvet bentgrass entries: Experimental, Greenwich, Legendary, Venus and Villa (Table 1). Independence creeping bentgrass and Vesper velvet bentgrass were the most susceptible entries to dollar spot for their corresponding species regardless of the rating date.

Dollar spot counts at the 2 September rating were highly correlated ($r=0.84$, $P \leq 0.001$) with disease evaluations determined on the 1 October date suggesting the relative ranking of cultivar disease severity was similar between the two rating dates. However, none of the *Agrostis* entries afforded acceptable tolerance to dollar spot during the 1 October rating, counts ranged from 60 spots (Villa velvet bentgrass) to 197 spots per plot (Independence creeping bentgrass) (Table 1). No statistical difference was detected between velvet entries on the 1 October dollar spot rating date. Conversely, differences in dollar spot severity were observed between creeping bentgrass entries during the heavy disease pressure of early October. Specifically, CY-2, Declaration and Penn A-1 exhibited significantly fewer disease infection centers compared to Authority and Independence creeping bentgrass. These creeping bentgrass cultivars represent potentially tolerant (i.e., CY-2, Declaration and Penn A-1) and susceptible (i. e., Authority and Independence) cultivars.

Cell Wall Components

Differences in cell wall components were identified due to the effects of species and cultivar. Greater hemicellulose content was observed with velvet bentgrass entries when compared to creeping bentgrass (Table 1). Although differences were detected between *Agrostis* cultivars in TCW, ligno- and hemi-cellulose, only hemicellulose components were statistically different when comparing between velvet and creeping bentgrass entries. As such, greater cell wall content and thickening due to hemicellulose may contribute to velvet bentgrass superior tolerance to dollar spot. No significant relationship between cell wall constituents and dollar spot severity was detected with TCW and lignocellulose. Generally, as hemicellulose content increased in leaf tissue, a corresponding decrease in the number of dollar spot infection centers was observed. A stronger relationship between hemicellulose content and dollar spot severity was observed under greater disease pressure that was exhibited during the 1 October rating. Specifically, the correlation (*r*) between dollar spot disease and hemicellulose in leaf tissue corresponded to –0.47 ($P \leq 0.10$) and –0.56 ($P \leq 0.05$) for the 2 September and 1 October rating periods, respectively. Hemicellulose content in leaf tissues accounted for approximately 32% of the total variation in dollar spot disease that was observed during the 1 October rating date (Figure 1).

A strong relationship between dollar spot and leaf hemicellulose content was also observed between creeping bentgrass cultivars (Figure 2). Unlike velvet bentgrass cultivars, significant differences between creeping bentgrass entries in dollar spot severity (1 October rating) and hemicellulose content was detected (Table 1). Creeping bentgrass entries such as Declaration, which exhibited greater resistance to dollar spot (fewer infection centers), was associated with greater leaf hemicellulose content when compared to susceptible cultivars such as Independence. Hemicellulose content in leaf tissues accounted for as much as 47% ($r = -0.69$, $P \leq 0.05$) of the variation in dollar spot disease for cultivars of creeping bentgrass. These results suggest that breeding for greater hemicellulose content in shoots of creeping bentgrass may potentially increase resistance to dollar spot for this economically important disease and species.

NUE and NRA

Nitrogen use efficiency was significantly greater in creeping bentgrass cultivars compared to velvet bentgrass (Table 1). Similarly, Kuo et al. (1995) observed significantly higher NUE values for creeping bentgrass cultivars compared to less aggressive spreading types such as colonial bentgrass. We also observed a significant relationship in *Agrostis* between NUE and stoloniferous growth habit expressed as the percentage of total stems that are stolons. Creeping bentgrass cultivars exhibited a significantly greater capacity and more vigorous spreading type growth habit than velvet bentgrass cultivars. Creeping bentgrass cultivars produced significantly greater percentages of stems as stolons when compared to velvet bentgrass cultivars, 65% and 51%, respectively. Strongly stoloniferous creeping bentgrass entries such as CY-2, Independence and Penncross produced at least 70% of the total number of stems as stolons while velvet entries that were weekly stoloniferous such as Greenwich, SR-7200 and Villa exhibited fewer than 45% of the total number of stems as stolons. Generally, as the percentage of total stems that develop into stolons increased in *Agrostis*, a corresponding increase in NUE was observed ($r=0.57$, $P \leq 0.05$) (Figure 3). The capacity for stoloniferous growth accounted for as much as 32% of the total variation in NUE in *Agrostis*. No significant relationship was observed between the capacity for stolon growth and dollar spot severity.

No difference within velvet bentgrass and creeping bentgrass cultivars was observed in NUE (Table 1). Therefore, much of the variation in NUE was due to the effect of species and not cultivar. Creeping bentgrass entries ranged from 20.9 (Declaration) to 22.6 (Penncross) g dry weight per mg tissue N while velvet entries ranged from 20.0 (Legendary) to 21.4 (Experimental) g dry weight per mg tissue N (Table 1). These ranges reported here are consistent with those observed by Kuo et al. (1995) for creeping bentgrass maintained under high N (50 ppm N). In their study, NUE values averaged approximately 20 g dry weight per mg tissue N compared to the overall average of 21.3 g dry weight per mg tissue N observed in our study. Alternatively, under low N levels of 3 ppm, Kuo et al. (1995) observed significantly higher NUE for creeping bentgrass with values ranging from 36 to 43 g dry weight per mg tissue N. Similarly, Bushoven and Hull (2001) observed NUE values ranging from 42 to 60 g dry weight per mg tissue N. Tissue N content for creeping bentgrass reported by Bushoven and Hull (2001) ranged from 1.8% (Penncross) to 2.6% (Pennlinks) while our total N content in leaf tissues were approximately twice the reported values observed by Bushoven and Hull (2001) ranging from 4.4% (Penncross) to 5.0% (Legendary).

As stated earlier, increases in NO_3 supply will increase leaf tissue N and shoot growth and decrease NUE (Kuo et al., 1995; Jiang and Hull, 1998). Our N fertilization program in the field applied approximately 3 pounds of total N per 1000 ft^2 per year. This N rate is most likely considerably higher in available NO_3 than the aforementioned low N study of Bushoven and Hull (2001) given the 2-fold higher leaf N content of tissues observed in our study. A recent survey of golf courses in New England reported an average annual N rate of 3.2 pounds of N per 1000 ft^2 (Owen and Ebdon, 2006), which is close to the N rate used in our field study. Therefore, our results may be more relevant to actual fertility programs used by golf course superintendents of New England.

Nitrate reductase activity measurements conducted on the two dates in July were highly correlated, r=0.92 ($P \leq 0.001$). Nitrate reductase activity measured on 9 and 12 July were positively correlated with NUE, r=0.51 ($P \leq 0.10$) and 0.59 ($P \leq 0.05$), respectively. Accordingly, *Agrostis* shoot NUE increased with increases in shoot NRA. Approximately 35% of the total variation in NUE was accounted for by NRA during the 12 July NRA measurement period (Figure 4). Like NUE, NRA was positively correlated with the capacity for stolon growth in *Agrostis*, r=0.58 ($P \leq 0.05$) and 33% of the total variation in NRA was explained by the percentages of total stems as stolons (Figure 3).

A general inverse relationship is typically observed between NRA and NUE. However, Bushoven and Hull (2001) observed no significant relationship between shoot NUE and NRA in creeping bentgrass, while a positive correlation was observed in perennial ryegrass between root NUE and NRA. Jiang and Hull (1998) also observed a positive relationship between NRA and NUE with some Kentucky bluegrass cultivars such as Baron. Furthermore, Bushoven and Hull (2001) reported Penncross to have among the highest shoot NUE and NRA of the nine creeping bentgrass cultivars that were evaluated, which is consistent with Penncross NUE and NRA (on 12 July) that was observed in our study (Table

1). Penncross creeping bentgrass was the only entry evaluated that was common to our study and the study conducted by Bushoven and Hull (2001).

Velvet bentgrass entries exhibited significantly lower shoot NRA compared to creeping bentgrass cultivars on both measurement dates and in turn, less dollar spot disease (Table 1). A significant, positive correlation was detected between NRA recorded on 9 and 12 July and dollar spot disease recorded in September (r=0.49, $P \leq 0.10$ and 0.71, $P \leq 0.01$, respectively) and with dollar spot recorded in October (r=0.50, $P \leq 0.10$ and 0.73, $P \leq 0.01$, respectively). At least 50% of the total variation in dollar spot severity during the late summer-to-early fall period was accounted for by nitrate reductase activity recorded on the 12 July measurement date (Figures 5 and 6). Dollar spot susceptible creeping bentgrass cultivars such as Independence exhibited significantly higher NRA when compared to more tolerant cultivars such as Declaration or Penn A-1 (Figure 5). Additionally, dollar spot susceptible velvet bentgrass entries such as Vesper exhibited significantly higher NRA when compared to dollar spot tolerant cultivars such as Legendary (Figure 5). No significant relationship was observed between NUE and dollar spot severity. Therefore, lower shoot NRA in *Agrostis* was associated with fewer dollar spot infection centers while NUE was statistically unimportant in determining the severity of dollar spot disease.

Harris and Whittington (1983) noted that NRA varied between colonial bentgrass and creeping bentgrass depending on the availability of N. Under low N, colonial bentgrass, which is adapted to less fertile regions, exhibited higher NRA compared to creeping bentgrass. Conversely, under high N, creeping bentgrass exhibited higher NRA than colonial bentgrass. Velvet bentgrass as a species is also better adapted to infertile soils when compared to creeping bentgrass (Beard, 1973). Like colonial bentgrass, velvet bentgrass may exhibit higher NRA under low N and in turn, lower NRA compared to creeping bentgrass under higher N. The 3.5 pound annual N rate used in our test may be more favorable to mature creeping bentgrass than velvet bentgrass cultivars. Accordingly, this may explain the higher NRA with creeping bentgrass compared to velvet bentgrass under the conditions of our test. Furthermore, under lower N approaching 2 pounds N per 1000 ft^2 per year, higher NRA with velvet bentgrass may be observed compared to creeping bentgrass. Further research is needed to test this hypothesis. Lastly, research has shown that NRA may show a negative, positive or no relationship with growth rate (Harris and Whittington, 1983) and with NUE (Jiang and Hull, 1998; Bushoven and Hull, 2001) because growth rate and NUE are dependent on the growing environment, species and NO_3 availability. Therefore, a simple relationship between NRA and NUE may not necessarily exist requiring additional research. However, according to these first year results, if the goal is to keep dollar spot incidence as low as possible then priority should be given to selecting *Agrostis* cultivars with low shoot NRA and high hemicellulose content.

CONCLUSIONS

This study was an attempt to establish relationships between dollar spot severity and nitrate reduction, utilization and cell wall components in cultivars of creeping and velvet bentgrass grown in the field under typical golf green maintenance conditions. Significant variation in dollar spot disease, NUE, NRA and cell wall constituents were observed. Lower disease severity was associated with velvet bentgrass cultivars when disease pressures from dollar spot were moderate, but any advantage with velvet bentgrass cultivars was lost under heavy disease pressure. Shoot NRA and NUE were positively correlated; the more dollar spot tolerant velvet bentgrass cultivars exhibited significantly lower NRA and NUE compared to the more dollar spot susceptible creeping bentgrass entries. The capacity for stoloniferous growth accounted for approximately 30% of the variation in NUE and NRA in *Agrostis*. Nitrate reductase activity was positively correlated with dollar spot severity accounting for at least 50% of the total variation in disease severity. Leaf hemicellulose content accounted for at least 30% of the variation in *Agrostis* dollar spot severity. The more dollar spot tolerant velvet bentgrass entries exhibited greater hemicellulose content in leaf tissues than creeping bentgrass. Creeping bentgrass cultivars showed significant variation in dollar spot disease under moderate and high disease pressures. Dollar spot tolerant creeping bentgrass cultivars such as Declaration exhibited greater hemicellulose content (and lower NRA) compared to intolerant cultivars such as Independence. These results suggest that lower dollar spot disease in *Agrostis*, especially creeping bentgrass, was associated with lower leaf NRA and greater leaf hemicellulose content. Annual N application rates applied to golf greens in the New England region range from 1.9 to 4.5 pounds per 1000ft^2 (Owen and Ebdon, 2006). Lower leaf NRA and greater leaf hemicellulose content can be achieved by keeping N as low as possible, which in turn has the competing effect of promoting greater dollar spot incidence. Therefore, superintendents are advised to balance the competing effects of N fertility on dollar spot severity with leaf NRA and hemicellulose content by using balance N fertility in the management of creeping bentgrass greens.

REFERENCES

Beard, J. B. 1973. Turfgrass science and culture. Prentice-Hall. Englewood Cliffs, NJ.

Belanger, F. C., S. Bonos, and W. A. Meyer. 2004. Dollar spot resistant hybrids between creeping bentgrass and colonial bentgrass. Crop Sci. 44:581-586.

Brosnan, J. T., J. S. Ebdon, and W. M. Dest. 2005. Characteristics in diverse wear tolerant genotypes of Kentucky bluegrass. Crop Sci. 45:1917-1926.

Bushoven, J. T., and R. J. Hull. 2001.Nitrogen use efficiency is linked to nitrate reductase activity and biomass partitioning between roots and shoots of perennial ryegrass and creeping bentgrass. Intl. Turf. Soc. Res. J. 9:245-252.

Campbell, C. A., R. P. Zentner, F. Selles, B. G. McConkey, and F. B. Dyck. 1993. Nitrogen management for spring wheat grown annually on zero-tillage: Yields and nitrogen use efficiency. Agron. J. 85:107-114.

Couch, H. B. 1995. Disease of turfgrasses. 3rd edition. Krieger Publ., Malabar, Fla.

Chakraborty, N., T. Chang, M. D. Casler, and G. Jung. 2005. Response of bentgrass cultivars to *Sclerotinia homoeocarpa* isolates representing ten vegetative compatibility groups. Crop Sci. 46: 1237-1244.

Chakraborty, N., J. Curly, S. Warnke, M. D. Casler, and G. Jung. 2006. Mapping QTL for dollar spot resistance in creeping bentgass (*Agrostis stolonifera* L.). Theoretical and Applied Genetics. 113:1421-1435.

Contreras Lara, D. 1999. Two techniques for measuring neutral detergent (NDF) and acid detergent fibers (ADF) in forages and by products. Arch. Zootec. 48: 351-354.

Delgado, N. J., M. D. Casler, C. R. Grau, and H. G. Jung. 2002. Reactions of smooth bromegrass clones with divergent lignin of etherified ferulic acid concentration to three fungal pathogens. Crop Sci. 42:1824-1831.

Easin, E. F. 1978. Total nitrogen determination for plant material containing nitrate. Anal. Biochem. 85:591-594.

Endo, R. M. 1966. Control of dollar spot of turfgrass by nitrogen and its probable basis. Phytopathology 56:877 (Abstr).

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). USDA Agric. Handb. 379. U. S. Gov. Print. Office, Washington, DC.

Goodman, D. M., and L. L. Burpee. 1991. Biological control of dollar spot disease of creeping bentgrass. Phytopathology 81: 1438-1446.

Harris, P., and W. J. Whittington. 1983. The relationship between nitrate reductase and plant productivity in *Agrostis* species. New Phytol. 95:3-8.

Jiang, Z., and R. J. Hull. 1998. Interrelationships of nitrate uptake, nitrate reductase, and nitrogen use efficiency in selected Kentucky bluegrass cultivars. Crop Sci. 38:1623-1632.

Jiang, Z., W. M. Sullivan, and R. J. Hull. 2001. Nitrate uptake and metabolism in Kentucky bluegrass as affected by nitrate levels. Intl. Turf. Soc. Res. J. 9:303-310.

Komarek, A. R., Robertson, J. B., and P. J. Van Soest. 1994. A comparison of methods for determining ADF using the filter bag technique versus conventional filtration. ASAS/ADSA Meetings. USA.

Kuo, Y., Wehner, D. J., and T. W. Fermanian, 1995. Nitrogen utilization in creeping bentgrass. Agronomy Abstracts. p 154.

Landschoot, P. J., and A. S. McNitt. 1997. Effect of nitrogen fertilizers on suppression of dollar spot disease of *Agrostis stoloniferia* L. Intl. Turf. Soc. Res. J. 8:905-911.

Owen, M., and J. S. Ebdon. 2006. Winter survival of annual bluegrass and creeping bentgrass greens in the northeast. University of Massachusetts Extension Turf program.

Radko, A. M. 1985. Have we gone too far with low nitrogen on greens? USGA Green Section Record. March/April. 23(2):26-28.

Sherwood, R. T. ,and C. P. Vance. 1980. Resistance to fungal penetration in graminea. Phytopathology 70:273-279.

Shearman, R. C., and J. B. Beard. 1975. Turfgrass wear mechanisms: II. Effects of cell constituents on turfgrass wear tolerance. Agron. J. 67:211-215.

Snell, F. D., and C. T. Snell. 1949. p. 84. Colorimetric methods of analysis. Van Nostrand. Princeton NJ.

Taiz, L. and Zeiger, E. 1972. Plant Physiology, Second Edition. Sinauer Associates. Sunderland, MA.

Williams, D. W., and M. S. Harrell. 2005. Leaf surface morphology among Agrostis species and cultivars and correlation with dollar spot. USGA Turfgrass and Environmental Research. 4(10):1-6.

Table 1. Mean squares and means from ANOVA of nitrate reductase activity (NRA), nitrogen use efficiency (NUE), dollar spot severity and cell wall components from field plots of *Agrostis* species and genotypes maintained as a golf green in 2007.

		NRA		NUE	Dollar spot		Cell wall components, 9 July		
F-test source	df	9 July	12 July	9 July	2 Sept.	1 Oct.	Total cell wall	Ligno-cellulose	Hemi-cellulose
		MS							
Block	2	0.18**	0.04	0.14	0.5	9232*	214.7***	22.3	374.9***
Cultivar	13	0.18***	0.49***	1.43*	232.8***	5346*	7.5**	3.2†	3.5**
Creeping vs. velvet	1	1.02***	2.95***	8.90***	288.1**	6000	5.7	0.1	7.1*
Among velvet	6	0.07†	0.15*	0.75	98.8*	1980	9.1**	4.7*	1.3
Among creeping	6	0.19***	0.43***	0.86	357.1***	8603**	6.3*	2.1	5.0**
Cultivar		Cultivar means							
Creeping		μmol g^{-1} hr^{-1}		g dwt mg N^{-1}	No. of spots per plot		%		
Authority		1.08	1.85	22.1	11.3	167	66.2	22.1	44.1
Bengal		0.46	0.98	21.6	11.3	102	67.1	22.9	44.2
CY-2		0.76	1.32	21.7	6.7	64	66.3	20.7	45.6
Declaration		0.80	1.33	20.9	3.3	64	69.8	22.1	47.7
Independence		0.85	1.92	21.4	34.7	197	65.6	21.4	44.2
Penn A-1		0.46	0.97	21.7	3.3	72	65.9	20.6	45.3
Penncross		0.57	1.31	22.6	6.7	85	66.1	21.0	45.1
Creeping mean		0.71	1.38	21.7	11.1	107	66.7	21.6	45.1
Velvet									
Experimental		0.34	0.86	21.4	1.3	101	67.2	20.9	46.3
Greenwich		0.33	0.82	20.9	3.7	75	69.5	22.7	46.8
Legendary		0.27	0.68	20.0	1.7	77	64.3	19.6	44.7
SR-7200		0.72	1.20	21.0	10.7	73	67.7	21.8	45.9
Venus		0.40	0.75	21.1	4.3	65	66.6	20.6	46.0
Vesper		0.42	1.09	20.9	16.7	134	67.7	21.5	46.2
Villa		0.33	0.57	20.2	2.3	60	69.2	23.3	45.9
Velvet mean		0.40	0.85	20.8	5.8	84	67.5	21.5	46.0
LSD (0.05)		0.30	0.40	1.3	10.9	81	2.6	2.2	1.6
CV (%)		32.0	21.2	3.7	76.8	50.5	2.3	6.1	2.1

***, **, *, † Significant at $P \leq 0.001$, 0.01, 0.05, and 0.10 levels, respectively.

Figure 1. Relationship between hemicellulose content and dollar spot severity (recorded on 1 October, 2007) in *Agrostis* species and cultivars.

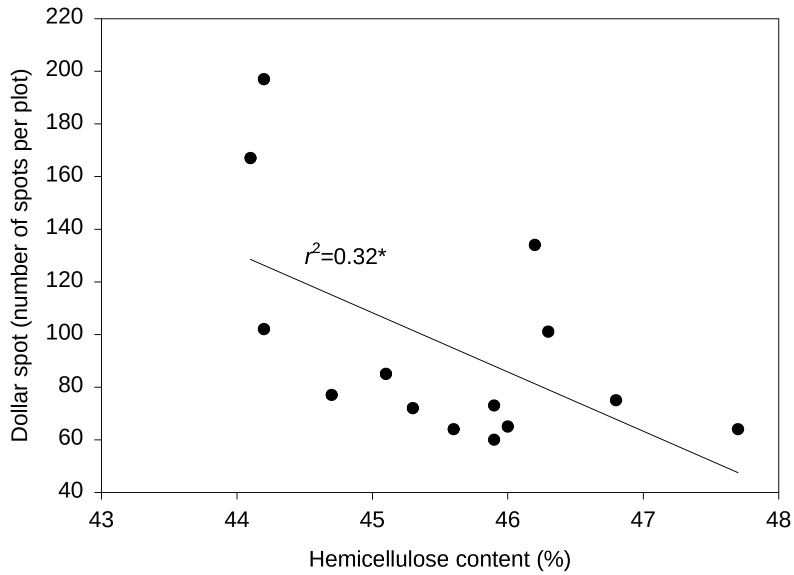

Figure 2. Relationship between hemicellulose content and dollar spot severity (recorded on 1 October, 2007) in cultivars of creeping bentgrass.

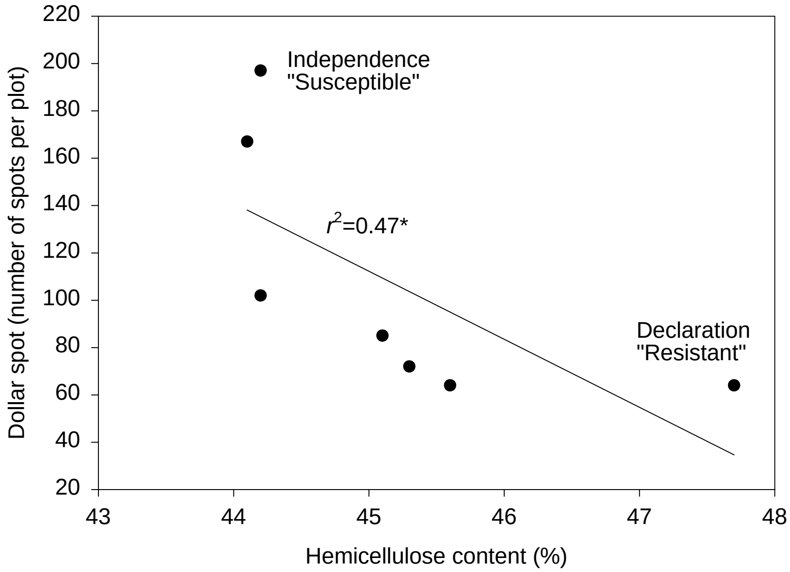

Figure 3. Relationship between the capacity for stoloniferous growth and nitrogen use efficiency (NUE) and nitrate reductase activity (NRA) in *Agrostis* species and cultivars.

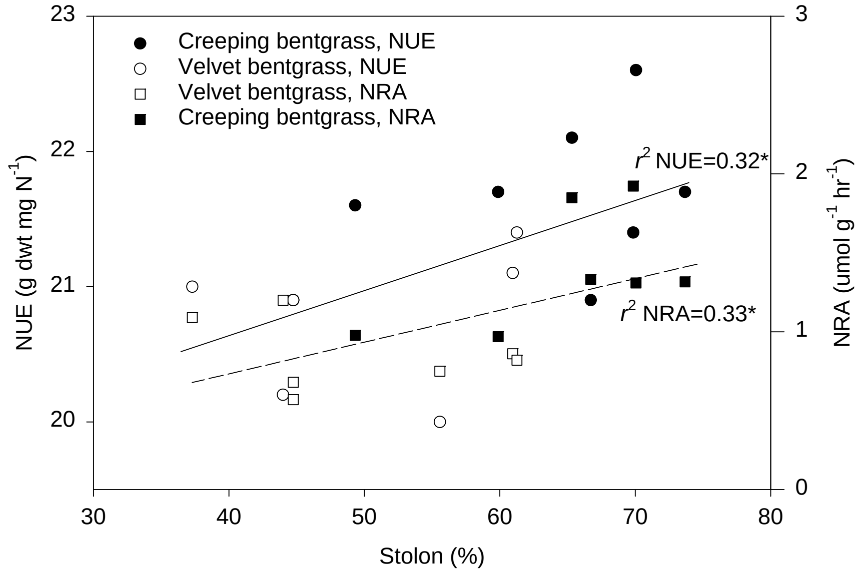

Figure 4. Relationship between nitrate reductase activity (NRA) recorded on 12 July and nitrogen use efficiency (NUE) in *Agrostis* species and cultivars.

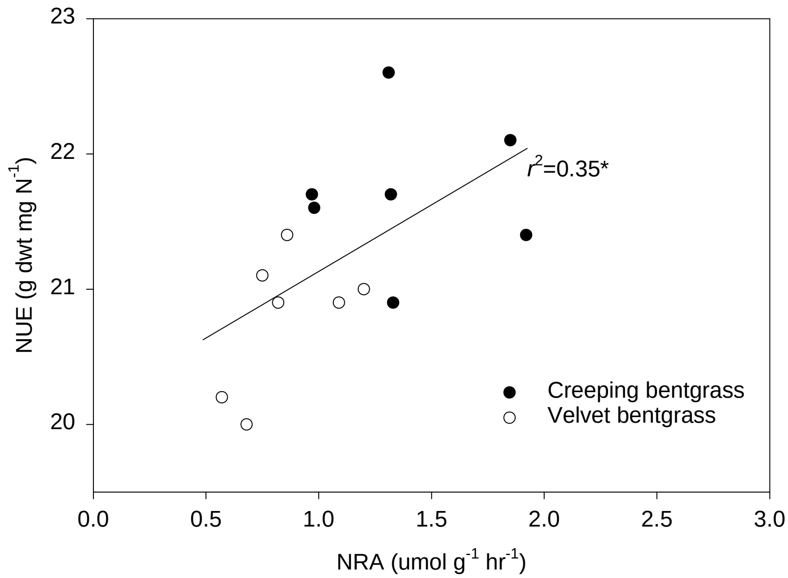

Figure 5. Relationship between nitrate reductase activity (NRA) and dollar spot severity (2 September) in *Agrostis* species and cultivars.

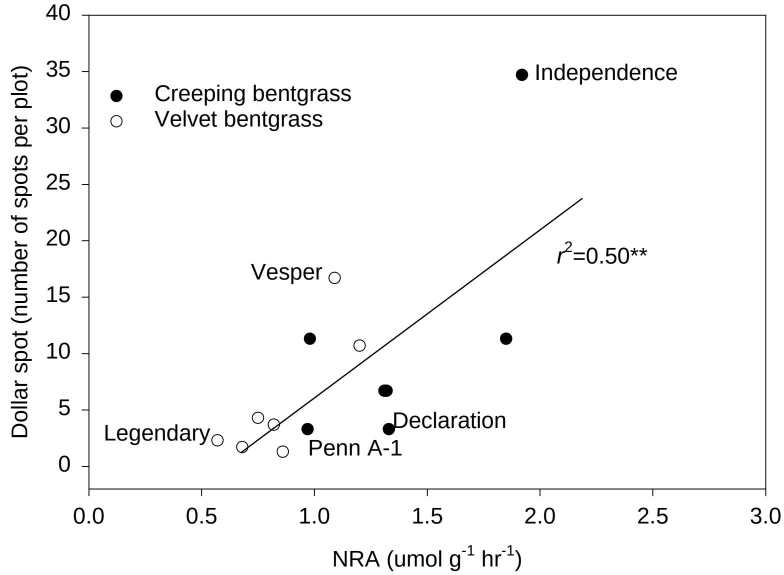

Figure 6. Relationship between nitrate reductase activity (NRA) and dollar spot severity (1 October) in *Agrostis* species and cultivars.