# Mechanisms for Wear Tolerance among Bermudagrass (*Cynodon* spp.) Genotypes: Cell Wall Components and Leaf Anatomy

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**Abstract**

**Wear damage is one of the biggest problems associated with continuous use on sports and recreational turfgrass fields. Wear tolerance of 8 bermudagrass (*Cynodon* spp.) genotypes including 6 ecotypes collected from regional Australia and two commercial cultivars were evaluated in the field. Green cover after wear treatment of ecotypes 394 and MED3 was over 50% higher than the lowest genotypes. The wear tolerant genotypes also had significantly higher acid detergent fibre content, cellulose, lignin and total cell wall content than wear susceptible genotypes. Optical and scanning electron microscopies were used to study stolon and leaf transverse sections and leaf surface characteristics, respectively. Transverse sections of stolons and leaves, suggested that the fibre area surrounding vascular bundles of the wear tolerant genotypes was about 50% higher than the wear susceptible ones. Scanning electron micrographs of the leaf surface suggested that the arrangement of epidermal cells formed a ridged pattern. The gaps between the ridges of wear tolerant genotypes were shorter than those of the wear susceptible genotypes. Collectively, these results suggested that the mechanism of wear tolerance was associated with high fibre content in stolons and leaves. In addition, we suggest that the wear tolerant genotypes have a denser ridge pattern of epidermal cells probably giving the leaves greater tensile strength.**

## INTRODUCTION

The demand for wear tolerant turfgrasses has increased due to the more frequent use of sports fields, golf courses, and recreation areas. An understanding of the mechanisms that underlie wear tolerant turfgrasses will help in designing breeding programs to develop improved cultivars.

Variation for plant cell wall components (total cell wall-TCW, cellulose, lignin) and leaf anatomical characteristics are thought to be associated with wear tolerance. It has been reported that the higher content of cell wall components could increase cell wall strength and significantly enhance wear tolerance in Kentucky bluegrass (*Poa pratensis* L.) genotypes (Brosnan et al., 2005) and bermudagrass cultivars (Roche et al., 2009), while Trenholm et al. (2000) studied 3 bermudagrass cultivars and found that improved wear tolerance was associated with reduced TCW and increased lignin content. Anatomical characteristics of leaf transverse sections showed that the area of sclerenchyma fibres and lignified cells were positively associated with the content of cell wall components as well as the wear tolerance observed from two species, tall fescue (*Festuca arundinacea* Schreb.) and rough bluegrass (*Poa trivialis* L.) (Shearman and Beard, 1975).

To date no research has been conducted to study mechanisms of wear tolerance in bermudagrass with a focus on both cell wall components and anatomical characteristics, particularly using light and scanning electron microscopy.

In previous studies of wear tolerance in bermudagrass, commercial cultivars were used as study materials (Roche et al., 2009; Trenholm et al., 2000). However, there is enormous genetic variation for a range of morphological traits among bermudagrass

collections that have been assembled around the world, e.g., USA (Anderson et al., 2009; Wu et al., 2006) and Australia (Jewell et al., 2012). Therefore, wild ecotypes of bermudagrasses may be a valuable source of wear tolerance and no studies have compared wear tolerance of ecotypes to cultivars.

Therefore, the objectives of this paper were to describe the mechanisms for wear tolerance in bermudagrass and compare the wear tolerance of ecotypes with that of commercial cultivars.

## MATERIALS AND METHODS

The response of bermudagrass (*Cynodon* spp.) genotypes to wear tolerance was investigated at the Gatton Research Station (27.54°S; 152.34°E) of The University of Queensland. Eight genotypes including two commercial cultivars, 'Grand Prix' and 'Wintergreen' and 6 ecotypes collected from regional Australia (Western Australia, New South Wales and Queensland) were planted into 3×2 m plots arranged as a randomized complete block design (RCBD) with three replications on 11 December 2009. All the grasses were irrigated and mowed weekly. On 13 September 2010 about 9 months after planting when every plot had established a closed canopy, wear treatments were applied using a Traffic Simulator (Roche et al., 2009) which was a modified Brinkman design based on the self-propelled GA-SCW Traffic Simulator (Carrow et al., 2001). The Traffic Simulator used smooth rubber galvanised rollers (l m wide) to cause scuffing of the turf surface rather than studded rollers rotating at different speeds. The wear treatment consisted of 40 passes with the Traffic Simulator. About half of each plot was exposed to wear, and the other half was used as a control.

Green cover, the percentage of leaves that remain green, was determined using the method modified from Karcher and Richardson (2003). An image of the grass in each plot was taken by a digital camera (500D, Cannon, Japan). Subsequently, the images were analysed using SigmaScan Pro software (v.5.0.0, SPSS Inc., Chicago, USA). The percentage of green leaves was determined from the percentage of pixels with hue ranging from 40 to 120 and saturation ranging from 0 to 100. GC was collected at 0, 5, 14 and 28 days after wear treatment.

Cell wall components and leaf anatomy were studied for all the 8 genotypes. Aboveground material (leaf and thatch) was cut from one 30×30 cm quadrat in the control part of each plot to analyse cell wall components. Fibre measurements relate to the structural parts of the plant, which consist mainly of hemicellulose, cellulose and lignin. To determine this, cell contents were extracted by boiling in a neutral solution of detergent, leaving the Neutral Detergent Fibre (NDF) as a measurement of total fibre present. The insoluble part of NDF when boiled in a solution of detergent and sulphuric acid is referred to Acid Detergent Fibre (ADF). ADF includes cellulose and lignin. The Total Cell Wall (TCW) content is indicated by NDF (Brosnan et al., 2005). ADF, lignin and NDF were determined by the protocol from Goering and Van Soest (1970).

The 3$^{rd}$ fully expanded young leaf and the stolon at 3$^{th}$ and 4$^{th}$ node were collected to do anatomical sections. Transverse section of leaves and stolons were hand-cut with a fine microtome blade. A stain specific for fibre developed by Sass (1958) was used to identify fibre accumulation. Using light microscopy (BH-2 BHS Trinocular microscope, Olympus, Japan) photographs of each section were taken by a digital camera (PowerShot G5, Canon, Japan). The percentage of stained fibre of each leaf (Fig. 2) or stolon section and the number of vascular bundles per stolon cross section were calculated with the software SigmaScan Pro.

The most wear tolerant genotype and the most wear susceptible genotype were used to study the epidermal structure on the abaxial surface of leaf blades. The 3$^{rd}$ fully expanded young leaf was sampled from the control section of plots in the first wear treatment. Leaf samples were processed based on the protocol from Oross and Thomson (1982). The processed samples were observed with a JEOL 6610 SEM scanning electron microscope operated at 8 keV.

All the data were analysed as RCBD using a General Linear Model (GLM) option

in Minitab 15 (Minitab Inc., State College, Pennsylvania, USA).

## RESULTS

Generally, all the genotypes had over 95% green cover before wear treatment (data not shown). Genotypes MED3 and 394 had the highest green cover after wear treatment, about 50% higher than genotypes 19 and 718 which were the lowest (Fig. 1). The green cover of commercial cultivars, 'Grand Prix' and 'Wintergreen', were mid range, about 25% significantly lower than MED3 and 394.

The content of cell wall components such as TCW, Acid Detergent Fibre (ADF), cellulose and lignin of ecotypes MED3, 394 and 25a1 were significantly higher than 718, 19 and 212 (Table 1). For the content of TCW, ADF and cellulose, MED3, 394 and 25a1 were about 10-15% higher than 718, 19 and 212, while the genotypic variation of lignin was not large, but MED3 and 394 were still in the top level (Table 1). In addition, the green cover at 0 and 14 days after wear treatment were significantly and positively correlated to the content of TCW, ADF and cellulose, with $r=0.91$-$0.95$, $P<0.01$ and $n=8$.

Genotypes were significantly different from each other for fibre area both in leaf and stolon cross sections (Table 2). Ecotype 394 and MED3 had the highest leaf and stolon fibre area about 47 and 43% higher, respectively, than 718, 19 and 212 which had the lowest fibre area in leaf and stolon (Table 2). Number of vascular bundles/cross sectional area of MED3 was the highest, about 42% higher than the lowest ones such as 394 and 25a1 (Table 2). The leaf and stolon fibre area were significantly and positively correlated to the content of TCW, ADF and cellulose, with $r=0.82$-$0.92$, $P<0.01$ and $n=8$.

From SEM micrographs of the epidermis on the abaxial surface of leaf blades sampled from MED3 and 'Wintergreen', it could be found that the arrangement of epidermal cells formed a ridged pattern, and there was a gap between successive ridges (Fig. 3). This gap between ridges was narrower in leaves of MED3 (Fig. 3a) compared to 'Wintergreen' (Fig. 3b).

## DISCUSSION

The principal mechanism of wear tolerance observed in this study was probably associated with a higher percentage of cell wall components in leaves and stolons. This result was consistent with the findings of Brosnan et al. (2005) and Roche et al. (2009) rather than Trenholm et al. (2000). Cellulose could provide plant tissues with a high tensile strength through a tightly packed group of polysaccharide chains. This suggests that plants with higher cellulose content may be more wear tolerant. Lignin is a highly branched polymer of phenylpropanoid groups that is able to increase mechanical rigidity and strengthens stems and vascular tissues (Taiz and Zeiger, 2006). At the anatomical level, wear tolerant genotypes had a larger fibre area in the leaf and stolon cross sections, which may explain the higher percent of cell wall components.

This is the first report showing the relationship between SEM micrographs of leaf epidermis and wear tolerance. We found the arrangement of epidermal cells formed a ridged pattern, and the wear tolerant genotype had a denser ridge pattern with narrower gaps between ridges than the wear susceptible genotype. This type of ridge pattern could possibly give leaves greater tensile strength.

Two ecotypes had considerably superior wear tolerance compared to the commercial cultivar, suggesting that these ecotypes could potentially be used as genetic resources for further breeding and that screening additional ecotypes be may reveal even higher wearing genotypes.

## CONCLUSIONS

The mechanisms of wear tolerance identified for bermudagrass in this study were possibly associated with a higher percentage of cell wall components in leaves and stolons as well as a denser ridge pattern of epidermal cells giving leaves greater tensile strength. More studies using a larger number of genotypes will be conducted in the future.

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## Tables

Table 1. Chemical analysis of cell wall components in samples of leaf and thatch taken from 8 bermudagrass genotypes in a wear trial conducted at the Gatton, Australia campus of The University of Queensland. The cell wall components included Total Cell Wall (TCW) and Acid Detergent Fibre (ADF) which is cellulose plus lignin. All components are expressed as percentages (w/w) on a dry matter basis. Within each column, means followed by the same letter are not significantly different based on least significant difference (l.s.d.) (P=0.05).

| Genotype | TCW (%) | ADF (%) | Cellulose (%) | Lignin (%) |
|---|---|---|---|---|
| MED3 | 73.6a | 35.7ab | 22.4a | 13.3ab |
| 394 | 74.1a | 36.6a | 22.9a | 13.7a |
| 25a1 | 72.8a | 34.2abc | 21.9a | 12.3bc |
| Grand Prix | 72.8a | 33.5bc | 19.8bc | 13.7a |
| Wintergreen | 71.5ab | 32.6cd | 20.0b | 12.6bc |
| 718 | 69.6bc | 30.4de | 18.2c | 12.2bc |
| 19 | 67.3cd | 29.1e | 18.0c | 11.1c |
| 212 | 66.5d | 30.4de | 18.2c | 12.2bc |

Table 2. Fibre area in leaf and stolon cross sections and the number of vascular bundles in stolon cross sections of 8 bermudagrass genotypes in a wear trial conducted at the Gatton, campus of The University of Queensland, Australia. Data were calculated from the photos of leaf and stolon anatomical sections taken by optical microscopy (×40). Within each column, means followed by the same letter are not significantly different based on least significant difference (l.s.d.) (P=0.05).

| Genotype | Leaf anatomical sections | Stolon anatomical sections | |
|---|---|---|---|
| | Fibre area ($\mu m^2$ per leaf section) | Fibre area ($mm^2$ per stolon section) | Number of vascular bundles (count $mm^{-2}$) |
| MED3 | 1352.8b | 2.76a | 54.6a |
| 394 | 1682.4a | 3.05a | 31.9de |
| 25a1 | 1298.4b | 2.82a | 31.7e |
| Grand Prix | 1339.1b | 2.75a | 39.8c |
| Wintergreen | 1312.2b | 2.73a | 32.7de |
| 718 | 794.5d | 1.58b | 46.9b |
| 19 | 827.9d | 1.73b | 47.4b |
| 212 | 1078.6c | 1.68b | 36.1cd |

## Figures

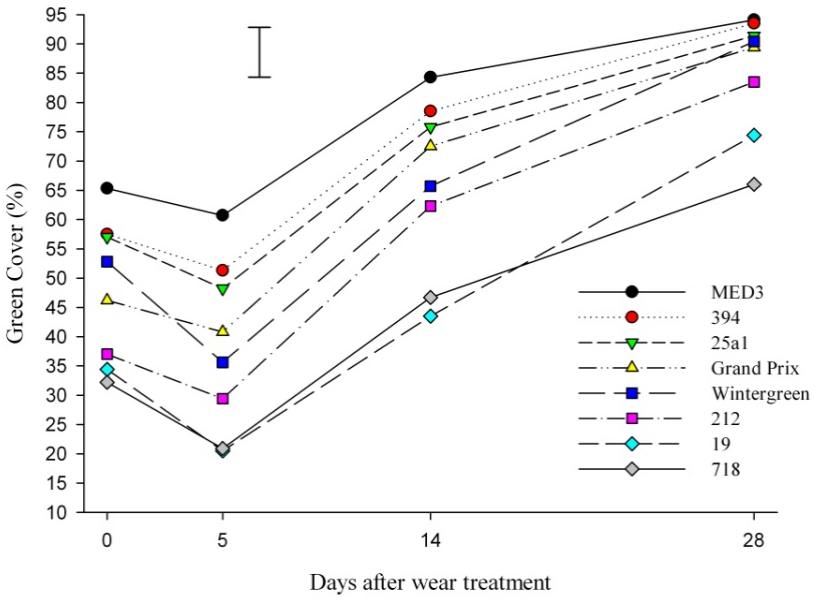


Fig. 1. Green cover of 8 bermudagrasses genotypes in a wear trial conducted at the Gatton campus of The University of Queensland. The error bar indicates least significant difference (l.s.d.) at P=0.05. All genotypes were >95% green cover prior to wear treatment.

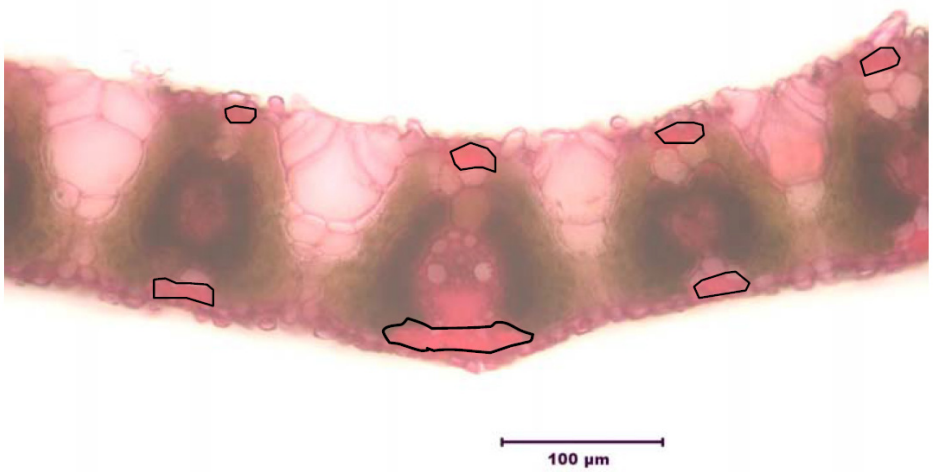


Fig. 2. A photomicrograph of a leaf cross section of genotype MED3. The black frames show leaf fiber areas at each vascular bundle of leaf cross sections.

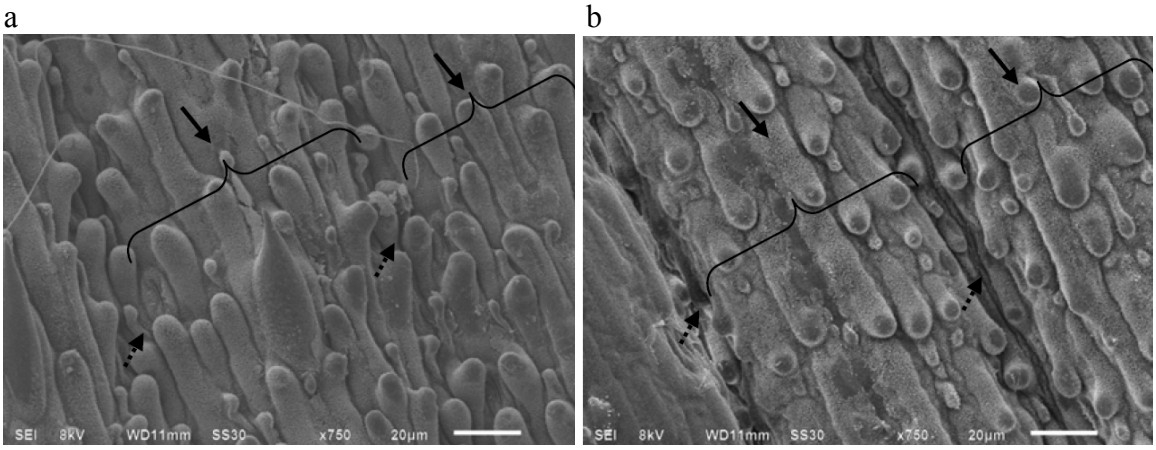


Fig. 3. Scanning Electron Micrographs (SEM) of the epidermal abaxial surface on leaf blades of bermudagrass genotypes, MED3 (a) and 'Wintergreen' (b). The arrow with dark line indicates the ridge, and the arrow with the dashed line indicates the gap between ridges. The SEM's showed that MED3 had wider and deeper gaps than 'Wintergreen'. Scale bars are 20 µm long and ×85 magnification.