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...Using Science to Benefit Golf



University of Kentucky scientists investigated whether morphological characteristics of creeping bentgrass leaves may be related its ability to resist infection by *Sclerotinia homoeocarpa*, the causal organism for dollar spot. Shown above are the “barbs” that occur on the leaf edges of creeping bentgrass.

PURPOSE

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Leaf Surface Morphology Among *Agrostis* Species and Cultivars and Correlation with Dollar Spot Severity

D.W. Williams and Michael S. Harrell

SUMMARY

University of Kentucky scientists investigated how morphological characteristics of creeping bentgrass leaves may be related its ability to resist infection by *Sclerotinia homoeocarpa*, the causal organism for dollar spot. The study found:

- All bentgrasses surveyed (*A. palustris* Huds. var. *stolonifera*, *A. canina* L., *A. tenuis* Sibth., and *A. idahoensis* Nash.) were glabrous. Trichomes were not present on either adaxial or abaxial surfaces.
- Stomatal surveys indicated that adaxial surfaces contained approximately twice as many stomata as abaxial surfaces in fairway-height turf.
- Significant correlations of stomatal densities with dollar spot severity were non-existent in both adaxial and abaxial surveys. There appears to be no relationship between stomatal density and dollar spot severity in fairway height bentgrass turf.
- There were no significant correlations between measured cuticle thicknesses and dollar spot severity.

Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) is one of the most expensive fungal diseases to control on bentgrass (*Agrostis* spp.) golf turf, both in environmental and financial terms. This is because the disease is endemic to all regions that propagate bentgrasses and damage can occur throughout the entire growing season. As such, golf course superintendents make regular preventative fungicide applications to reduce or eliminate turf damage from dollar spot often on a 14-21 day schedule from spring to autumn.

The dollar spot fungus infects leaves by hyphal penetration. The infection then spreads until entire leaves are affected. If left untreated, whole tillers including the crown may be damaged or killed. As the infection process often begins on

the surfaces of leaves, morphological traits may have an effect on the ability of the fungus to penetrate leaves and cause disease.

Previous research has shown highly significant differences in susceptibility of both bentgrass species and cultivars to dollar spot (2). Generally, creeping bentgrasses (*A. palustris* Huds.) are more prone to damage than colonial (*A. tenuis*) or velvet (*A. canina*) bentgrasses. Cultivars of creeping bentgrass also have significantly different levels of susceptibility. There have been no published studies on differences in leaf surface morphology among the currently available bentgrasses. This research investigates differences in leaf surface morphology among bentgrasses and attempts to correlate these morphological traits with the severity of damage from dollar spot. Specifically, surveys were conducted to quantify trichome size, density, and distribution, stomatal densities, and thickness of the waxy cuticle on leaf surfaces.



Figure 1. Dollar spot mycelium progressing in both directions on the surface of a 'Penncross' creeping bentgrass leaf

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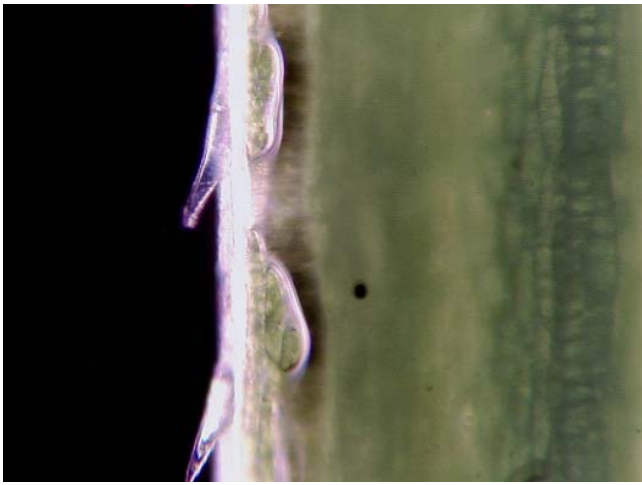


Figure 2. Barb-like extensions of the waxy layer along the leaf margin of creeping bentgrass

Materials and Methods

Leaf samples were harvested from the 1998 National Turfgrass Evaluation Program (NTEP) bentgrass trials at the University of Kentucky in Lexington, KY. Both putting green and fairways trials were evaluated. Species included were *A. palustris* Huds. var. *stolonifera*, *A. canina* L., *A. tenuis* Sibth., and *A. idahoensis* Nash.

Trichomes were evaluated using scanning electron microscopy (SEM). Stomata were quantified by taking leaf surface imprints from applications of a clear polyacrylate from both adaxial and abaxial surfaces and viewing by compound microscope with an ocular micrometer. Cuticle surveys were accomplished by compound microscope with an ocular micrometer. These traits were statistically correlated with dollar spot severity data collected from the respective NTEP trials. In all surveys, 10 sub-samples were evaluated from each entry for each trait.

Results

By SEM analyses, all bentgrasses surveyed were glabrous in that trichomes were not present on either adaxial or abaxial surfaces. This agrees with previous reports on wild-type bentgrasses (1). There were, however, many cultivars that exhibited what appeared to be barb-like extensions of waxy layers along leaf margins (Figure 2). These extensions did not occur on the leaf surfaces proper, only along leaf margins.

The typical processes by which leaf tissue is prepared for SEM can be destructive. It is

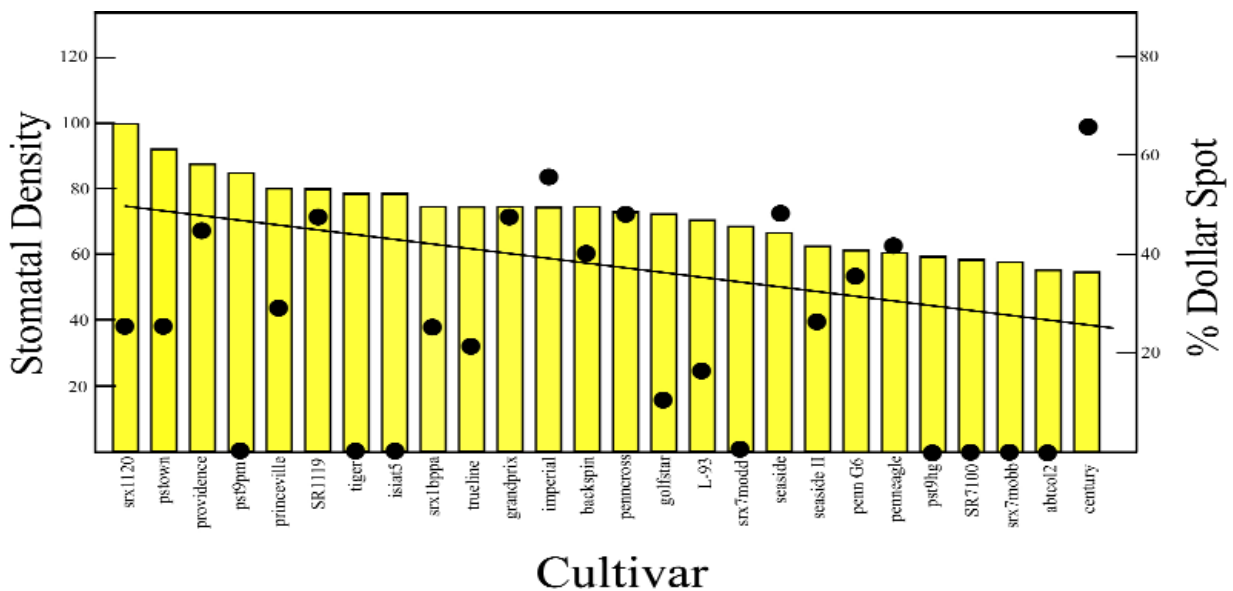


Figure 3. Adaxial stomatal density and dollar spot severity of bentgrasses managed at fairway height. Bars indicate stomatal density (stomata per mm²) plotted on the left Y axis. Line regresses data points of dollar spot severity for each cultivar (% plot affected) plotted on the right Y axis.

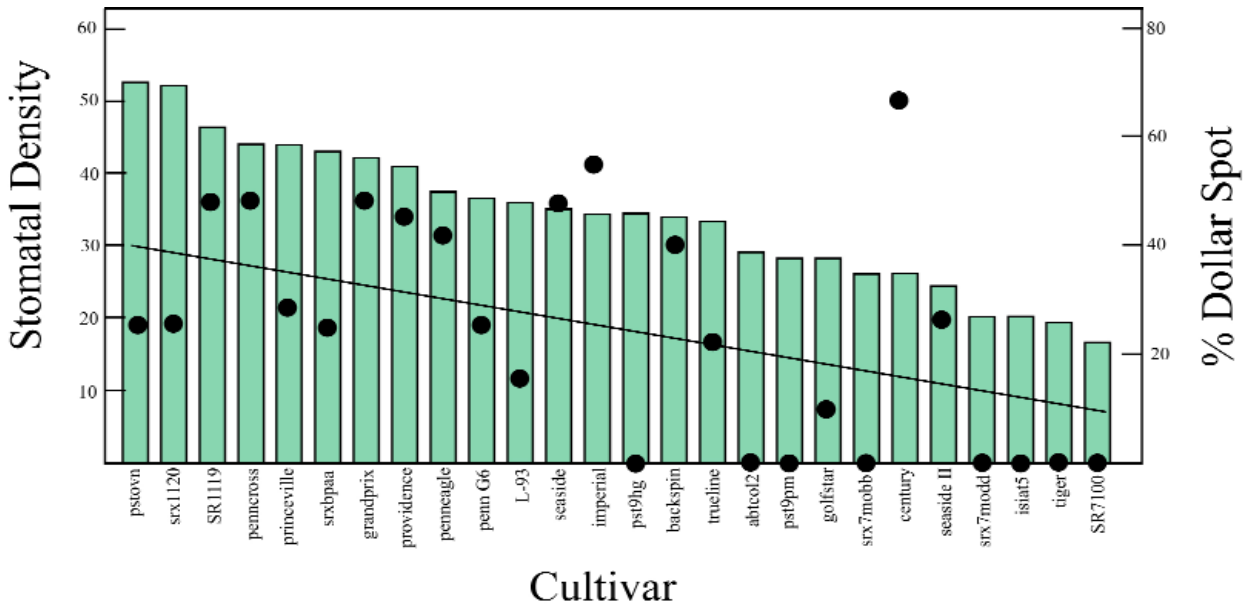


Figure 4. Abaxial stomatal density and dollar spot severity of bentgrasses managed at fairway height. Bars indicate stomatal density (stomata per mm²) plotted on the left Y axis. Line regresses data points of dollar spot severity for each cultivar (% plot affected) plotted on the right Y axis. Cultivars are on the X axis.

therefore possible that trichomes on leaf surfaces were damaged during tissue preparation. Further research is ongoing to determine if this did in fact occur in these studies, including surveys of fresh tissue by confocal microscopy.

Stomatal surveys indicated that adaxial

surfaces contained approximately twice as many stomata as abaxial surfaces in fairway-height turf (Figures 3, 4). This agrees well with previously published work (3). Significant correlations of stomatal densities with dollar spot severity were non-existent in both adaxial and abaxial surveys. There appears to be no relationship between stomatal density and dollar spot severity in fairway height bentgrass turf.

Interestingly, when comparing the same bentgrass cultivars at both fairway (15mm) and putting green (5 mm) heights of cut, putting green turf had nearly twice the stomatal density of fairway-height turf (Figure 5). There did appear to be a stronger trend when correlating stomatal density with dollar spot severity in putting green turf (Figures 6, 7). It is interesting to note that as stomatal density decreased, dollar spot severity tended to increase. However, this relationship was very weak and not statistically significant.

There were differences in the thicknesses and forms of waxy layers/cuticles among the bentgrasses (Figures 8, 9). However, there were no significant correlations between measured thicknesses and dollar spot severity (Figure 9).

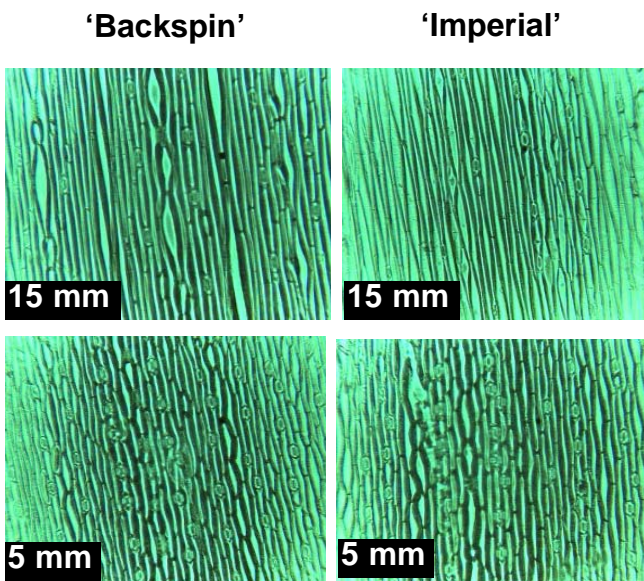


Figure 5. Leaf surface imprints of two cultivars managed as fairway (15mm) and putting green (5mm) turf. Note the increased stomatal densities in putting green samples.

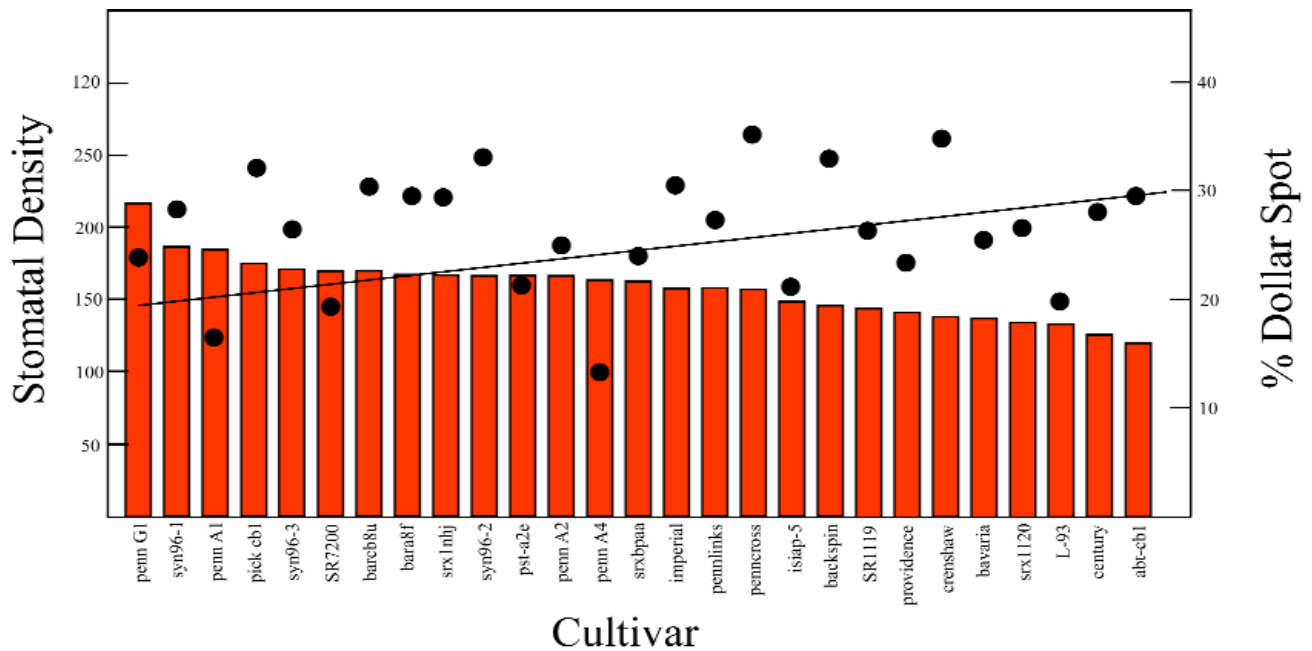


Figure 6. Adaxial stomatal density and dollar spot severity of bentgrasses managed at putting green height. Bars indicate stomatal density (stomata per mm²) plotted on the left Y axis. Line regresses data points of dollar spot severity for each cultivar (% plot affected) plotted on the right Y axis. Cultivars are on the X axis.

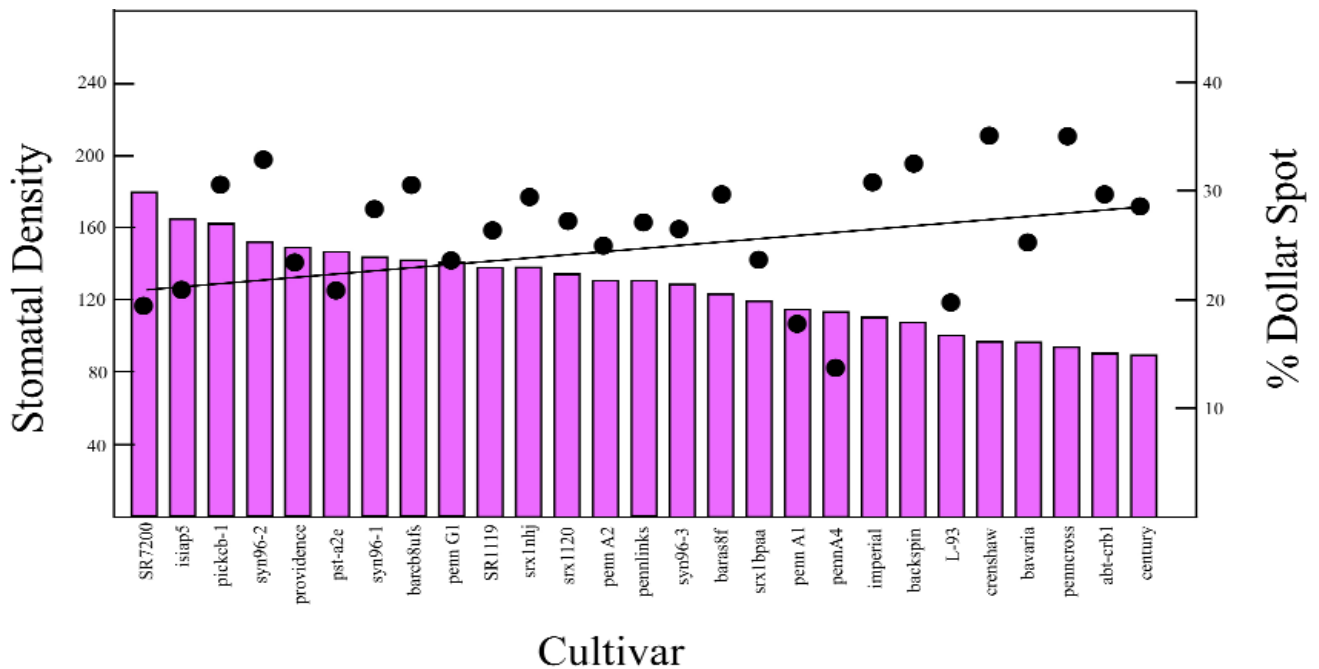


Figure 7. Abaxial stomatal density and dollar spot severity of bentgrasses managed at putting green height. Bars indicate stomatal density (stomata per mm²) plotted on the left Y axis. Line regresses data points of dollar spot severity for each cultivar (% plot affected) plotted on the right Y axis. Cultivars are on the X axis.

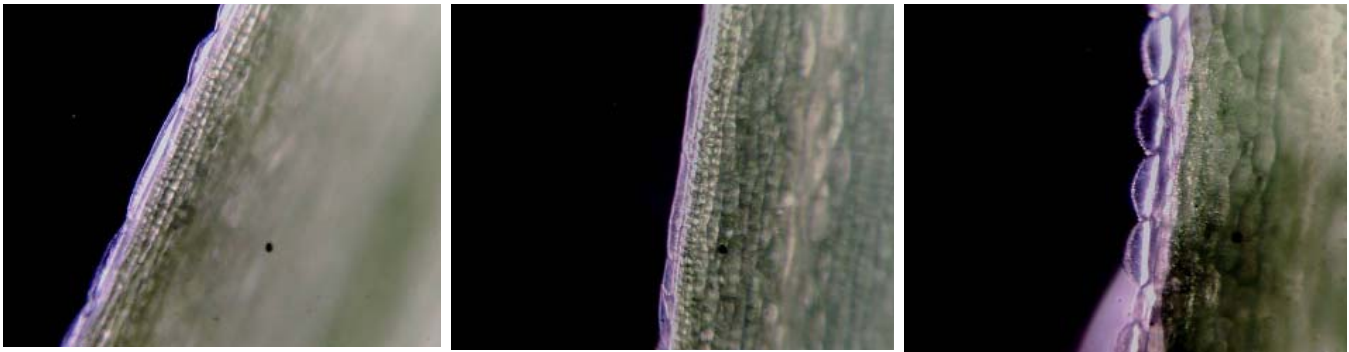


Figure 8. Differences in the thickness and forms of the waxy-layer/cuticle among bentgrass cultivars

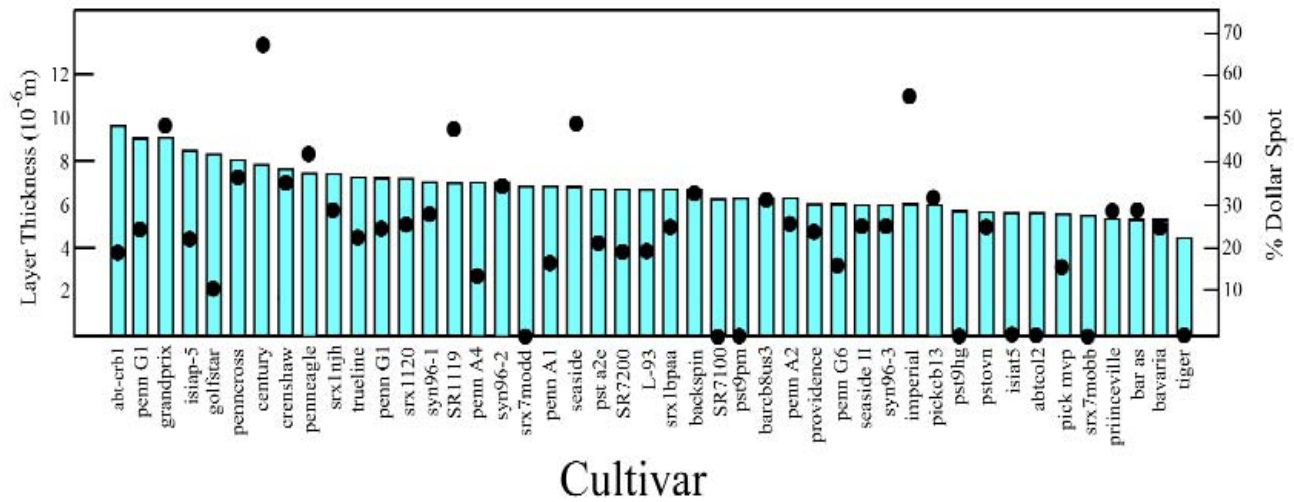


Figure 9. Thickness of the waxy-layer/cuticle and dollar spot severity of bentgrasses managed at fairway and putting green height. Bars indicate thickness in micrometers plotted on the left Y axis. Data points indicate dollar spot severity (% plot affected) plotted on the right Y axis for each cultivar.

Discussion

There were no consistent or significant correlations between the leaf surface characteristics measured in this study and the severity of dollar spot. Although regular turf management practices would not be expected to have large impacts on leaf surface characteristics, knowledge of any relationships between leaf surface morphology and disease severity would be a very useful tool for plant breeders and molecular biologists work-

ing towards lower levels of susceptibility or total disease resistance.

Ongoing research at the University of Kentucky is investigating better methods of surveying leaf surface characteristics of the bentgrasses. These efforts are concentrated on improved methods for quantifying both trichomes and cuticle thickness. Additionally, we are currently conducting a study in cooperation with Rutgers University investigating potential biochemical mechanisms of reduced susceptibility to

dollar spot among the bentgrasses. Our goal is to contribute to improved disease resistance through either classical plant breeding efforts and/or molecular manipulation of existing biochemical defenses in hopes of reducing both the environmental and financial impacts of managing bentgrass as golf turf.

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