Understanding the mechanisms involved in dollar spot reduction through rolling
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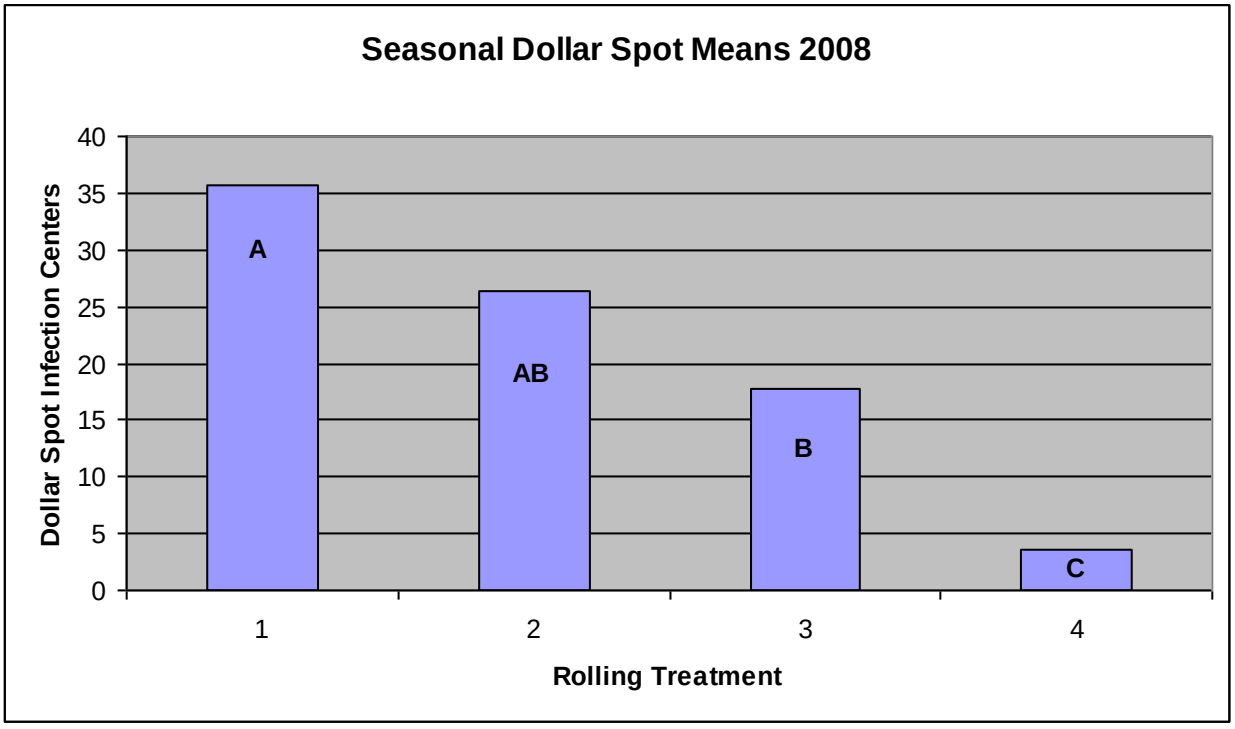
The rolling treatments administered to the 12' x 7' plots of creeping bentgrass growing on a USGA green were as follows:

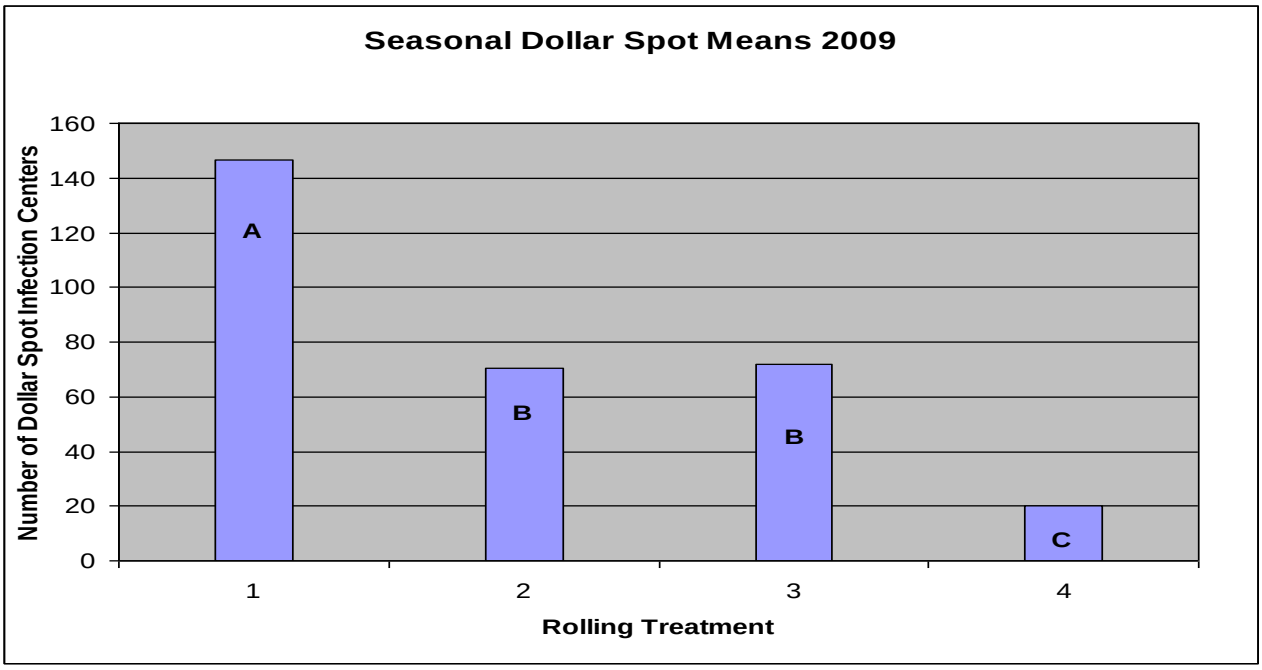
1. Control (no roll)
2. Rolled 1x in a.m. (immediately after mowing)
3. Rolled 1x in the p.m. (after dew has dissipated)
4. Rolled 2x in the a.m. (immediately after mowing)

The study involving rolling for the reduction of dollar spot has been underway for the past two summers (2008 and 2009), and has given some insight into dollar spot infection and proliferation on bentgrass putting greens.

Dollar spot counts and quality ratings were taken when disease pressure was evident, and clipping collections were made in 2008 and 2009 on numerous dates. A key finding in this study related to the removal of dew and guttation water is that in both years, rolling once in the a.m. and once in the p.m. were not significantly different from one another, but were significant from the control. The below graphs indicate the seasonal means for both 2008 and 2009 respectively.

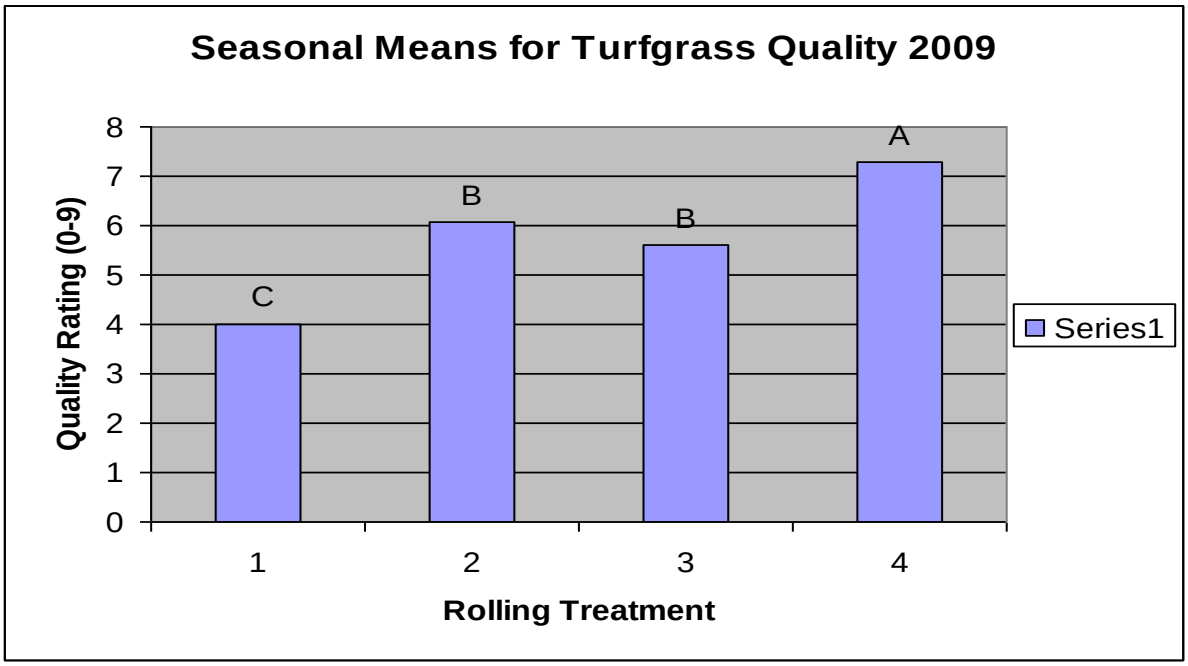


Means followed by different letter are significantly different according to LSD=12.38 α =0.05



LSD=34.96 α=0.05

This finding indicates that rolling twice in the a.m. is the best management practice with regard to controlling dollar spot, and as seen below has no adverse effect on turfgrass quality (0-9 rating, 0 being completely dead 9 being excellent turf quality). It also nullifies the current theory that the second removal of dew or gutation after mowing is the major factor contributing to dollar spot reduction. **The fact that rolling in the P.M. reduced dollar spot significantly from the control in both years shows that other factors besides dew removal are contributing to the reduced disease incidence**.



Microbial Populations

Microbial populations in the turfgrass rootzone are of interest with regard to rolling as well. Possible increases in microbial competition may be due to the consistent rolling of the turfgrass plots. We have collected, prepared and sent samples to be analyzed via Phospholipid Fatty acid (PLFA) analysis. This procedure provides direct information on the entire microbial community in three key areas: Viable (living) biomass, community composition or population "Fingerprint", and microbial activity. We are confident that if differences are to be found, this procedure will indicate them. 20 soil cores from the upper root zone of each plot were taken and homogenized to get a representative soil sample. The analysis is being performed by Dr. Teri Balser's soil ecology lab at the University of Wisconsin, and results should be here within the next 4-5 weeks.

We are also looking at other plant produced enzymes that may play a role in defense and are known to be induced by mechanical stimulation (i.e. rolling, rubbing, wounding). The extraction and quantification of these enzymes and proteins require much time and experimentation, but may be keys to answering the rolling question.

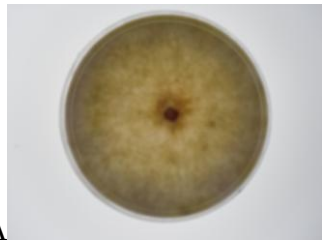
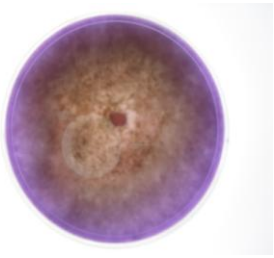
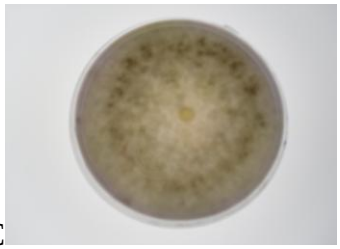
Pathogen Work

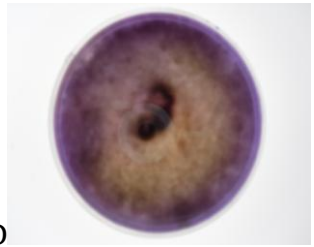
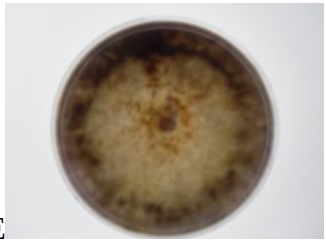
Initially, we began extracting proteins from collected clippings looking for a specific enzyme called oxalate oxidase (OxO), which is commonly found in grasses, and has been shown to be induced, and when expressed in some other plants, to have defense related activities, especially against pathogens that produce oxalic acid such as *Sclerotinia sclerotiorum.* Our initial findings with regard to OxO were insignificant, meaning activity was not correlated with rolling treatments or turfgrass quality.

We decided to look into the pathogen's production of oxalic acid as well as extracellular enzymes it may secrete in order to infect turfgrass, since this work has not yet been done with *Rutstoemia flocossum,* and determining if oxalic acid plays a major role in the infection process would aide in determining if OxO an enzyme that degrades oxalic acid, is important in the plant's defense. Using plating techniques, conducive to showing oxalic acid production, we tested multiple VCG's of the dollar spot fungus for the production of oxalic acid, pectin degrading enzymes, cellulase, and xylanase. Along with this we also tested the virulence of these strains to see if virulence was correlated with the production of oxalic acid and extracellular enzymes. Oxalic acid was shown to be produced by every VCG tested, but at different amounts and different times. This difference in acid production however, seemed to not have a significant role in the virulence of the strain, a possible reason as to why OxO activity may not be an important factor in dollar spot resistance. Pectinase, cellulase and xylanase are all enzymes that play major roles in the maceration and degradation of grass cell wall polysaccharides. These enzymes and their secretion were found to be induced depending on the substrate *R. flocossum* was growing on; for example: Pectin degrading enzymes were found to be produced by *R. flocossum* when grown on a pectin rich medium, however when grown on isolated creeping bentgrass cell walls, little to no pectin degrading enzymes were found to be produced. On the other hand, cellulose

degrading enzymes were produced in abundance when the pathogen was grown on creeping bentgrass cell walls, but not when grown on pectin medium. This finding shows that the dollar spot pathogen is very diverse, and like other higher fungi is able to degrade different substrates to obtain nutrients in different environments. (See supplemental graphs and photos).

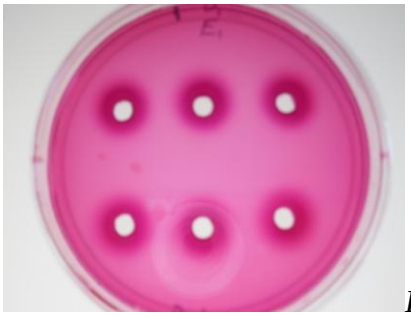
Oxalic acid production on bromophenol blue amended PDA plates. Plates change from purple to yellow when acid is produced. Each VCG is indicated by letter.

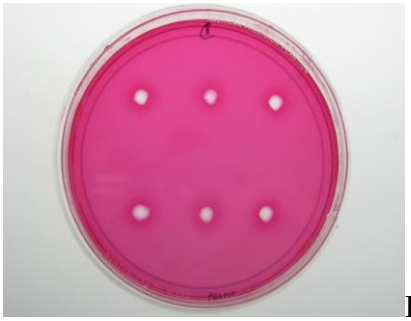
A  B  C 

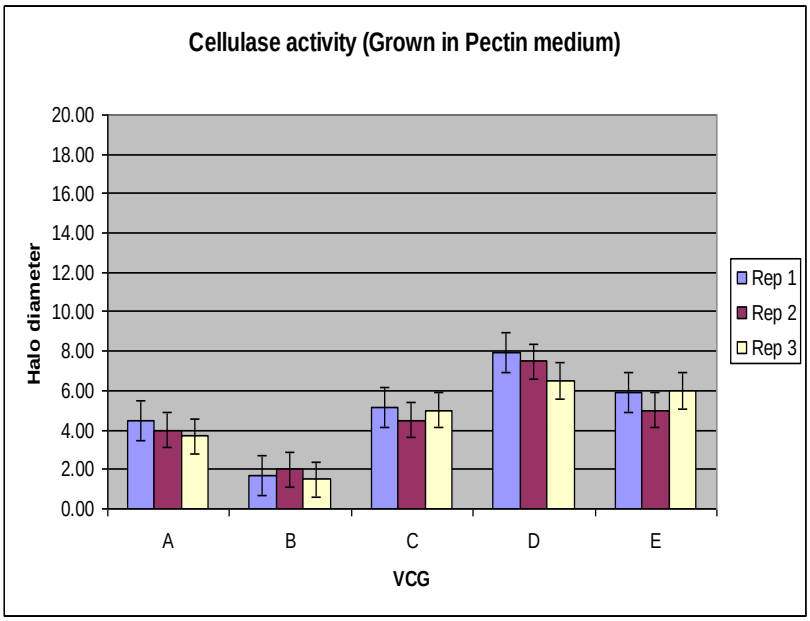
D  E 

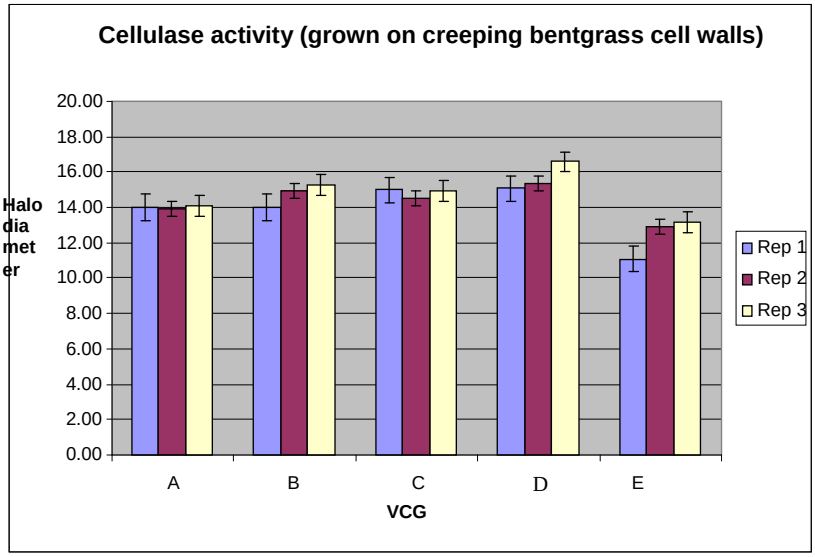
Note the differences in color change and consistency between VCG's

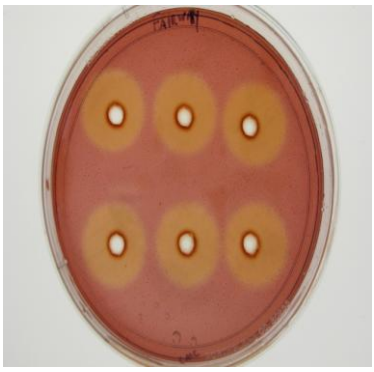
Pectin Degrading Enzyme Production

 *R. flocossum* Grown on Pectin amended medium. Note the dark halo formation around the wells, indicating enzyme production.

 R. Flocossum grown on creeping bentgrass cell wall amended medium. Note minimal halos around wells, indicating little to no enzyme production.







***Note the much higher cellulase activity on isolates that were grown on bentgrass cell walls as opposed to the pectin medium.