Effectiveness of a Chemical Hybridizing Agent on *Cynodon Dactylon* in Inhibiting Anther Dehiscence

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Elevated temperatures and heightened emissions have led to a surge in airborne pollen dispersed by wind-pollinated plants, resulting in pronounced fluctuations during pollen seasons, particularly in the allergy-susceptible region of Southern Nevada. Despite escalating severity of allergies, no comprehensive solution has been discussed. This research aims to investigate the efficacy of synthetic detergent in curtailing pollen release from *Cynodon Dactylon* (Bermuda grass), grass preferred by Southern Nevada residents for its resilience to drought but notorious for releasing copious amounts of pollen. Both lab-cultivated grass and grass purchased from a nursery were employed in this study. Two trays, one featuring newly grown grass and another with purchased grass, constituted the control groups. Concurrently, two additional sets of trays - one with newly grown grass and the other with purchased grass - were sprayed with a 1:9 ratio solution of synthetic detergent and water. Pollen counts were taken utilizing petroleum jelly as an adhesive applied on microscope slides, with acetocarmine dye imparted coloration in the otherwise transparent pollen grains. Initial pollen counts taken of the control groups showed an average of 226 grains. Results indicated a significant reduction in pollen grains by about 37.8% in lab cultivated grass and 47% in purchased grass. Assessing the efficacy of synthetic detergent in inhibiting pollen release not only addresses the immediate concerns of mitigating pollen-related allergies in Southern Nevada but also holds broader implications for developing strategies to alleviate the intensified impact of climate-induced changes on allergen exposure.

Keywords: Southern Nevada, Bermuda grass, pollen allergies, synthetic detergent, chemical hybridizing agents

Introduction

The issue of pollen sensitization has been steadily rising, affecting many local Southern Nevada residents in the form of seasonal allergic rhinitis. Pollen, released by many invasive plants that are wind pollinators, has always persisted as an obstacle to residents' wellbeing for years. However, due to increases in climate change, plants have been releasing more pollen; the regular allergy seasons of spring and fall have also been fluctuating in recent years as a result of rising greenhouse gasses [1–3]. Systems of recording pollen counts in place, such as the University of Nevada, Las Vegas's pollen monitoring program. Despite these systems, a comprehensive solution addressing the root of the problem has not been researched thus far. This research project intends to find a way to inhibit anther dehiscence, the release of pollen from anther, from plants in order to resolve the persistent matter of pollen sensitivity common throughout Southern Nevada. *Cynodon Dactylon*, an invasive species known as bermuda grass, is one of the main culprits of increasing pollen allergies [4]. Due to its drought survival capabilities, it is preferred by Southern Nevada residents for yards. Its pervasive and pollen releasing qualities make it favorable for this study. Synthetic detergent, a common chemical hybridizing agent (CHA) used to sterilize anthers in plant breeding techniques, was used to reduce anther dehiscence in the Bermuda grass. Synthetic detergent works to induce male sterility by disrupting the plant cell membranes, therefore interfering with another development [5–7]. Since detergent is cost-effective and not as well researched, it was preferred for this study not only to find a solution to allergies but also to add to this growing field. The hypothesis was: If synthetic detergent is regularly sprayed on *Cynodon Dactylon*, then the amount of pollen released by the plant will decrease, and as a result, help reduce pollen sensitization in the context of climate change. This was proven correct as the detergent successfully reduced the amount of pollen being released by the grass by about 38% and 47% in two trays of grass.

Methods and Materials

To address increasing sensitivity of humans to airborne pollen due to climate change, solving the root of the issue holds significant promise. In order to inhibit pollen development or anther dehiscence, CHAs, substances that can kill or temporarily sterilize anthers in plants, were considered. Some researchers detail using genetic modification of plants to prevent production of functional pollen or physical removal of anthers by hand, but

CHAs are the most feasible to use according to the majority of plant breeding studies and based on what was accessible in a school lab. While there are many plants that contribute airborne pollen, *Cynodon Dactylon* (Bermuda grass) was best to use for experimentation due to its classification within the poaceae family, recognized to release a significant amount of pollen compared to other plants. It is commonly found in the yards of Southern Nevada residents as well. Grass was best suited for use in a classroom laboratory setting, as it does not have any long reaching branches. The CHA that was tested was the synthetic detergent produced by the brand Tide. While spraying the grass with a chemical that is known to kill gametes may seem detrimental to the growth of the plant, the chemical being used in this experiment has not been shown to have any significant negative effects on the female gametes[8,9]. The chemical also causes temporal male sterility, so the development of the plants would not be permanently damaged and the surrounding environment is not majorly impacted. To begin experimentation, Bermuda grass seeds were planted in two trays with proper lighting from a plant UV light, water, and soil requirements necessary for growth. After anthers had developed, microscope slides with one side covered in petroleum jelly were placed on the top of a covering on the grass. The side with the petroleum jelly faced the grass so any airborne pollen was captured. The temperature of the room and the lamp that provided light for the grass to grow were regulated to stay under 100° F so the petroleum jelly did not melt or catch on fire. One of the two trays was regularly sprayed with a spray bottle 1-2 times a week with a solution of synthetic detergent diluted with water-10 mL detergent and 90 mL of water, the concentration recommended by most researchers using detergent as a hybridizing agent. The second tray acted as the control, representing current pollen conditions. The purchased grass from the nursery underwent the same process, with one tray being the control and the other being the test. All other conditions, like regular watering and lighting, were adhered to throughout the experiment. The maintenance for Bermuda grass is 1 inch of water biweekly and ambient temperature of 75° F, and the conditions for germination and growth of both growths were the same based on information provided by the grass supplier.

In order to analyze data quantitatively, a pollen count was conducted for each tray. After about 24 hours, the slides were removed from the tops of the trays. 1-2 drops of acetocarmine dye and a coverslip were placed on each slide so that they could be observed under a microscope. Number of pollen grains on each slide were counted and recorded. Data collected was recorded in the form of a table detailing the days that the chemical was used and number of pollen grains per slide for each tray. In terms of practicalities, 5-6 weeks was required for the bermuda grass to grow enough to develop pollen grains. However, the grass planted from seeds proved to take more time than expected to grow, which is why the purchased grass was brought into the experiment in the first place. Detergent testing took place over a two-week period. Synthetic detergent is a harmless soap used for washing clothes, but gloves were used regardless.

Results

Spraying concentrations of synthetic detergent and water reduced pollen counts taken in both lab-cultivated grass (Fig 2) (labeled "seeds") and purchased grass (Fig 1) (labeled "purchased") from a nursery over a period of two weeks. Three pollen counts (Fig 3) were taken after grass was sprayed three times during this period. Microscope slides were inspected at 400 magnitude and the number of grains were counted. Averages were taken to manage inter-slide variability. Lab-cultivated grass showed an average of 220 grains in the control, 137 grains in the tray sprayed with detergent, and an average reduction of 83 grains. Overall, the lab-cultivated grass showed a 37.8% reduction in pollen grains. Purchased grass displayed an average of 171 grains in the control, 90 grains in the tray sprayed with detergent, and an average reduction of 81 grains.

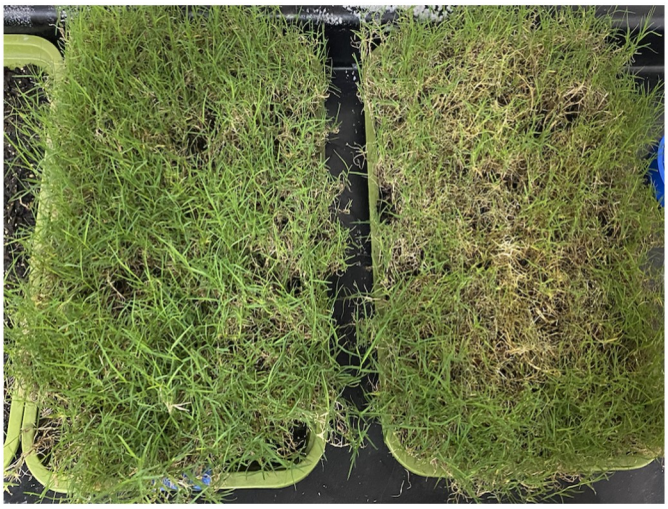

Fig. 1 Picture of purchased grass at the end of the two-week testing period. On the left is control, on the right is detergent sprayed.

There was a 47% reduction in grains for purchased grass. The numbers that were averaged are shown in the graph (Fig 4). Browning took place in the purchased grass but lab-cultivated had minor differences between the control and detergent sprayed trays. In sum, the results indicate that spraying synthetic detergent on Bermuda grass effectively curtailed the production and release of pollen grains, with the purchased grass demonstrating a slightly higher percentage of reduction.

Fig. 2 Picture of lab cultivated grass at the end of the two-week testing period. On the left is control, on the right is detergent sprayed.

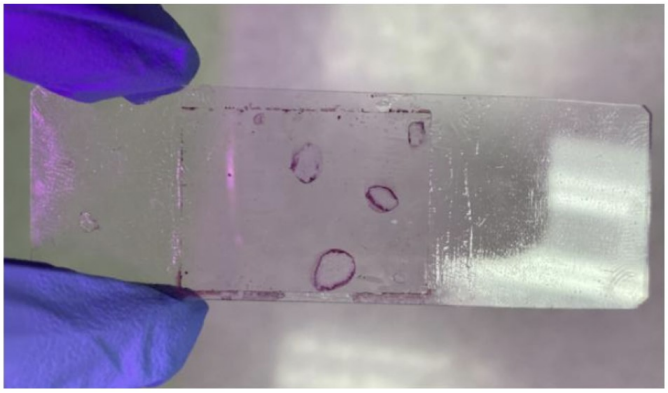

Fig. 3 Picture of sample pollen count

Statistical Analysis

In order to determine significance of results, ANOVA without replication was performed through XLMiner in Google Sheets. Averages 220 for lab grown, control; 137 for lab grown, detergent sprayed; 171 for purchased, control; 90 for purchased, detergent sprayed were placed in a table in Google Sheets for ANOVA test.

Mean for lab grown counts was 178.5, and mean for purchased was 130.5. Mean for controls was 195.5, and mean for detergent sprayed was 113.5. Grand mean was 154.5. Error sum of squares was 1. Total degrees of freedom was 3.

Mean squares and F-values between lab grown grass and purchased were 2304 and 6724 between controls and detergent sprayed.

P-values between lab grown and purchased was 0.0132609935997985, and between control and detergent sprayed was 0.00776327092203688. Both P-values are less than 0.05, so both are statistically significant. Therefore, the source of the grass and treatment with detergent were factors that affected pollen counts.

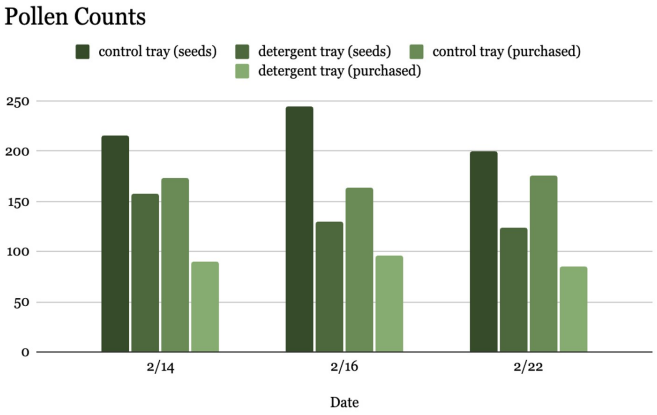

Fig. 4 Graph of pollen counts over two week testing period. Dates represent the days that counts were conducted.

Confidence intervals were calculated using the formula $CI = \bar{x} \pm z\left(\frac{s}{\sqrt{n}}\right)$:

- Lab grown, control: $[170.3, 269.7]$
- Lab grown, detergent sprayed: $[74.9, 199.1]$
- Purchased, control: $[133.8, 208.2]$
- Purchased, detergent sprayed: $[65.2, 114.8]$

There is no overlap between purchased control and detergent sprayed, and between lab grown control and purchased detergent sprayed. So, there is statistical significance between these groups.

Discussion

Regularly applying synthetic detergent on Bermuda grass leads to a reduction in anther dehiscence, thereby mitigating pollen sensitization considering climate change, especially in Southern Nevada. The chemical caused damage to the cell membranes of the anthers in the grass, chemically inhibiting the development of pollen grains and their release. Climate change caused fluctuations in the typical allergy seasons and a general increase in pollen release. While there are pollen count systems, a solution to the increase is still yet to be considered. After a two-week trial with four trays - two lab-cultivated and two purchased - there was a reduction in pollen grains of 37.8% and 47%, respectively. As determined by ANOVA and confidence intervals,

there is statistical significance between the purchased control group and purchased detergent sprayed group. As seen in the graph, the control of the seeds tray varied slightly, but otherwise every other tray stayed consistent. The differences in the control groups are attributed to the height variation between lab-cultivated and purchased grass. Though a more extended trial period would enhance accuracy, the steady control data and decreased grains after detergent testing suggest promising results. Still, the lack of enough testing time proves to be the most significant limitation of this study because long term effects on the grass by the detergent could not be examined, making it difficult to see how the detergent would be performed in the real world. Human error could have also occurred in the counting of the pollen grains in the microscope. Humidity was not monitored, which could have affected the release of pollen, but all plants were kept in the same environment. Further research can be done by testing different types of grass or CHAs, or continuing use of synthetic detergent to observe its long-term effects on the grass, especially since the testing period was only two weeks due to lab time constraints. In terms of possible application in the future, more research would need to be done to examine if the strategy of spraying detergent could be viable for plants other than Bermuda grass, such as mulberry or European olive trees that also release copious amounts of pollen. Finding success in this method of using synthetic detergent to reduce dehiscence has significant implications for those suffering in regions like Southern Nevada where pollen allergy is quite common. Not only is Bermuda grass advantageous for testing due to its substantial amount of pollen release, but it is also prevalent in Southern Nevada and is the cause for a large part of the airborne pollen in this area. The results of this project could provide greater understanding in reducing pollen sensitization to greatly benefit Southern Nevada residents.

Conclusion

Synthetic detergent proved to be successful in reducing anther dehiscence in Bermuda grass in both labs cultivated and purchased over a period of two weeks. The lab cultivated grass experienced a 37.8% reduction in pollen grains, while the purchased grass experienced about a 47% reduction in pollen grains after being sprayed with concentrations of detergent over two weeks. Pollen allergies have grown to be detrimental to Southern Nevada residents with the increased incidence of climate change, with Bermuda grass being a lead pollen producer in the region. The implications of this research are quite large, specifically for allergy sufferers. Special allergy treatments may prove to be inconvenient, so substantial results that validate that synthetic detergent causes temporal male sterility could be applicable in the context of reducing the amount of airborne pollen in the months that allergy is most severe. As a result, the development of the plants the rest of the year would not be affected. While much research has been conducted on how grass pollen is harmful to humans, not many solutions to the issue have been presented; this experiment on Bermuda grass could be the basis for further research in the field of aerobiology and reduction of pollen release for decreased allergy.

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