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Project Title: **Characterizing growth and life history of silvery-thread moss in cool-season putting greens: assessing vulnerability to stress in the life cycle**

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Summary
During 2017, we successfully cloned (pure plant lines) 17 genotypes of silvery-thread moss (STM, *Bryum argenteum* Hedw.) from golf course putting greens and 17 genotypes from native habitats. As of our last report (Oct 2016), we had initiated an experiment comparing the life history and growth dynamics of 7 golf course + 7 native habitat lines of STM. During 2017 we completed this experiment using all 34 clonal lines (17 golf course + 17 native habitat genotypes). Last year we reported preliminary results, and at present we have the final data from this comprehensive experiment. The primary differences between the golf course vs. the native genotypes of STM revolve around an ability to germinate rapidly, grow vigorously, and to produce a mat of brown rhizoids that serve to anchor the shoots to the substrate. Interestingly, the golf course putting green environment appears to select for this group of traits from plants dispersing into the putting green from native habitats. Evidence for this selection pressure is the presence of several clonal lines collected from native habitats exhibiting similar growth characteristics to STM lines derived from putting greens. This finding suggests that traits exhibited in putting green mosses are present in native habitats yet seldom expressed in native habitats.

Overview
The purpose of this project is to address a biological concern in golf course greens, the silvery-thread moss (STM), known scientifically as *Bryum argenteum*. This moss has infested golf course putting greens across the USA, and golf course superintendents have expressed concerns regarding effective eradication approaches. We initiated this project with these goals:

1. Accumulate representative collections of STM from a variety of golf courses and representative collections of STM from non-golf course habitats, place these genotypes into pure culture, and compare their life history and stress responses. In essence, we wish to determine how different the golf course strains of this species are compared to populations <u>not</u> in golfing greens. Understanding these differences will help us formulate better treatment plans for eradication.

2. Evaluate the effectiveness of administering specific stresses, including the application of carfentrazone, at various points in the life cycle of STM. These life cycle stages include (in sequence from juvenile to adult) protonema, rhizoid, juvenile shoots, adult shoots, and asexual reproductive structures.

Progress to Date for Year 2 (2017)

1. Completed the establishment of 34 genotypes of STM at the University of Nevada, Las Vegas. As of October of the previous year (2016), we had successfully cloned to pure lines 12 genotypes of STM from golf course greens and 10 genotypes of STM from "off golf course" habitats. We refer to these locations as "*Green*" and "*Native*" habitats, respectively. We now have 34 pure cultures of STM: 17 from golf courses across N. America, and 17 from *Native* habitats from across N. America. These *Green* golf course samples include STM from courses in (numbers of genotypes in parentheses) Alberta (1), California (6), Colorado (1), Illinois (1), Minnesota (1), Nevada (1), Ohio (3), Oregon (1), and South Dakota (2). The *Native* samples include mosses (STM) from localities in the following states: Arizona (2), California (2), Georgia (1), Kentucky (1), Massachusetts (1), Nevada (4), New Mexico (1), Oregon (3), Pennsylvania (1), and Washington (1). Once this process was completed, we made a set of "backup" cultures of each of the 34 genotypes and placed these under low light conditions in sealed Petri dishes for long term storage.

2. Completed Experiment 1: Comparing the Growth Dynamics of Golf Course STM to "Off Course" STM. The major conclusions of this experiment indicate that *Green* STM mosses incorporate a markedly different set of life history (growth) traits compared to *Native* STM mosses. We show these differences by including several figures taken from our draft MS (**Figures 1–5**). **Figure 1** shows the differences visually (the habitat and taller shoots). **Figure 2** shows how shoot induction (the time to the first shoot production in culture) is significantly faster in *Green* mosses. **Figure 3** shows how the *Green* mosses produce many more shoots over the first 21 days in culture compared to *Native* mosses. **Figure 4** shows the dramatic difference in rhizoid production in *Green* mosses, which indicates to us that rhizoid production may be critical in the moss holding its position in the putting green. **Figure 5** shows the tendency of *Green* mosses to devote less energy to asexual reproduction through bulbils (detachable shoot fragments) than *Native* mosses. This indicates a tradeoff is present wherein *Green* mosses put their energies into shoot production instead of specialized asexual reproduction.

3. Developed a draft manuscript titled, "*Divergence in Life History and Developmental Traits in Bryum argenteum Accessions from Golf Course Putting Greens*". The authors of the manuscript (MS) are, Zane Raudenbush, Joshua Greenwood, Nicholas McLetchie, Sarah Eppley, Steven Keeley, Richard Castetter, and Lloyd Stark. We anticipate completing the statistics for the MS and submitting the MS early in next year's cycle, ideally before January of 2018. The abstract of this draft MS follows.

Abstract

●*Premise of the study:* Silvery thread moss, *Bryum argenteum*, is an undesirable invader of golf course putting greens across North America, establishing colonies and proliferating despite practices to minimize its existence. In order to understand the growth dynamics of this species, our goal was to grow genotypes of "*green*" (growing in putting greens) and "*native*" (growing in habitats outside of putting greens) of *B. argenteum* in a common garden experiment and assess life history traits.

●*Methods:* Seventeen collections of *green* and 17 collections of *native B. argenteum* were cloned to single genotypes, decontaminated through subculturing, and raised through a minimum of two asexual generations in the lab. A culture of each genotype was initiated using a single

detached shoot apex and allowed to grow for 6 months under conditions of inorganic nutrients present and absent. Observations included protonemal germination, extension rate and cover, first shoot induction and number of shoots, shoot height, gemma and bulbil production, aerial rhizoid cover, sex expression and number of inflorescences, and chlorophyll fluorescence and content parameters.

●*Key results:* Genotypes of *B. argenteum* from putting greens exhibited earlier shoot regeneration and shoot induction, faster protonemal extension, longer (higher) shoots, produced fewer gemmae and bulbils and greater aerial rhizoid cover, and showed similar tendencies of chlorophyll fluorescence properties and chlorophyll content. Cultures receiving no inorganic nutrients exhibited lower chlorophyll content, much reduced growth, and bleaching of shoots.

●*Conclusions:* Mosses from putting greens establish and grow faster and yet do not produce as many specialized asexual propagules. Rhizoid production is much greater in putting green mosses. The highly managed putting green environment has either selected for a suite of traits allowing the moss to effectively compete with grasses, or genotypic diversity is very high in this species, allowing a set of specialized genotypes to repeatedly colonize the putting green from native habitats.

4. Conducted a pilot experiment on the desiccation tolerance of the rhizoid mat of *Bryum argenteum* clonal lines (genotypes) from Golf Course Putting Greens. Preliminary results indicate that this portion of the life cycle is critical to the establishment of STM in greens. Rhizoids are nonphotosynthetic extensions from the shoot that anchor the shoot and maintain the integrity of the colony of shoots against incursion from grasses. Our pilot data indicate that rhizoids are capable of producing new rhizoids and are indeed tolerant to drying out entirely, and therefore deserve our attention as a focus of research.

Plans for Year 3 (2018)

1. Submit our first Manuscript developed with USGA funding. We are currently completing the statistical section and then will complete the Introduction and portions of the Discussion.

2. Initiate our Second Major Experiment on STM incorporating 3 Factors: (1) Desiccation Tolerance, (2) Carfentrazone Treatment, and (3) Nutrient effects. Using a subset of the 17 *Green* + 17 *Native* STM genotypes, we will initiate a series of 2-factor experimental treatments to determine the effects of desiccation, carfentrazone, and nutrient additions, on the health of STM plants.

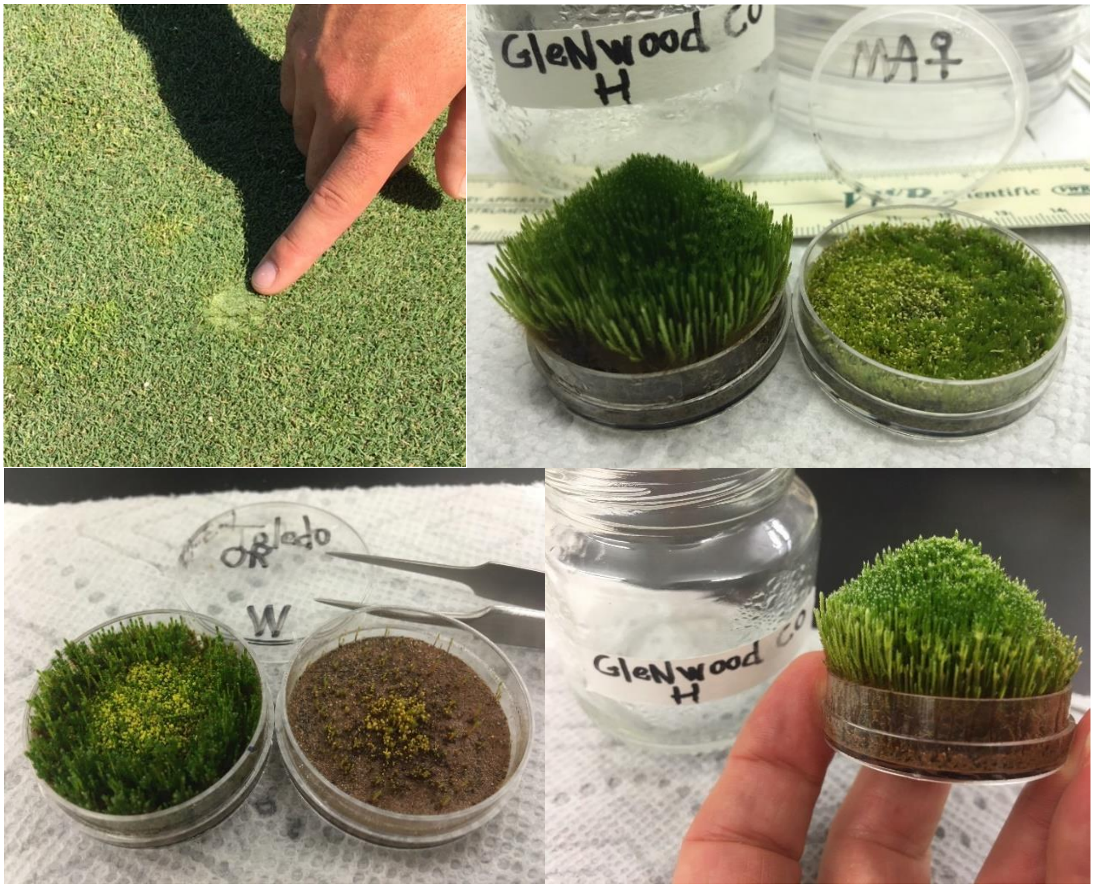

Figure 1. The visual differences in life history traits (habitat and taller shoots) between *Green* and *Native* mosses.

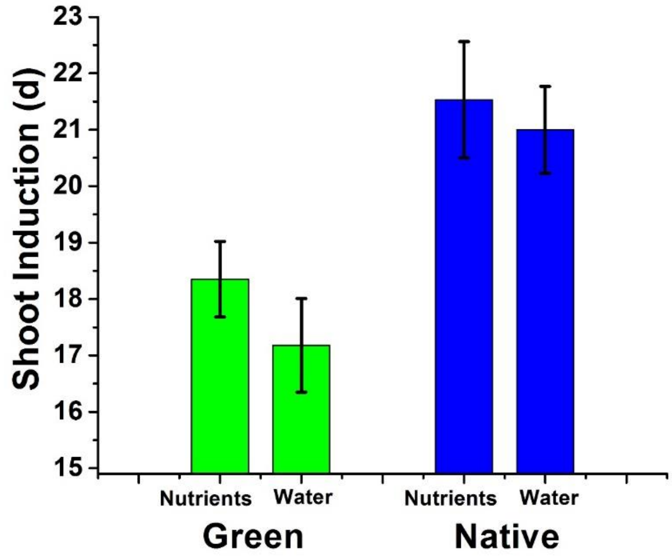

Figure 2. Shoot induction (the time to the first shoot production in culture) is significantly faster in *Green* mosses.

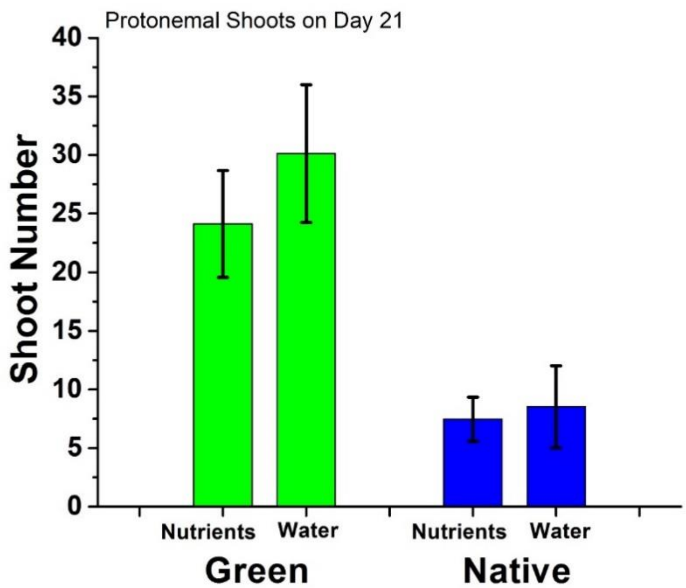

Figure 3. *Green* mosses produce many more shoots over the first 21 days in culture compared to *Native* mosses.

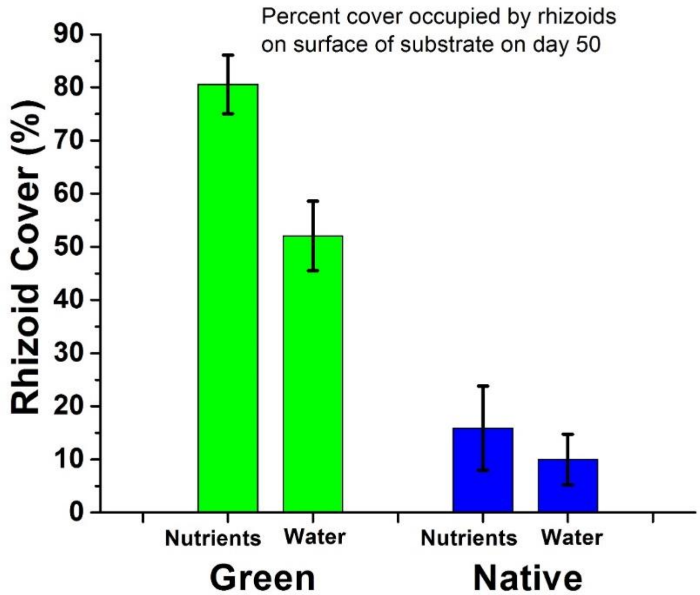

Figure 4. The difference in rhizoid production in *Green* mosses, which indicates to us that rhizoid production may be critical in the moss holding its position in the putting green.

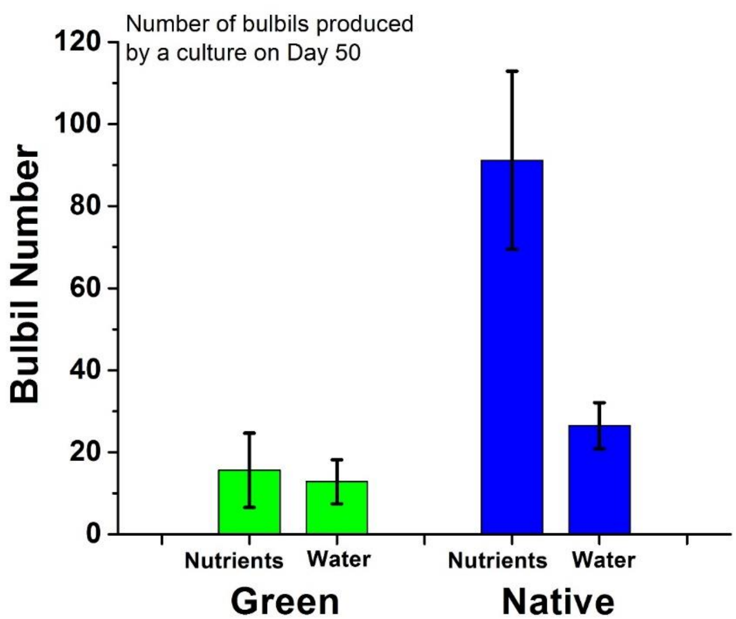

Figure 5. The tendency of *Green* mosses to devote less energy to asexual reproduction through bulbils (detachable shoot fragments) than *Native* mosses.