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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 2016, we accumulated material of the invasive putting green moss, *Bryum argenteum*, from 15 golf courses in the USA, and material of the same species from natural habitats from 17 localities throughout the USA. This material was collected and shipped to us (at UNLV) in dried condition, placed into culture on sieved sand media, and subcultured until the culture of moss was free from algal contaminants. Our culture technique produces single clonal lines (genotypes) of each moss collection. When we had produced 7 such genotypes from putting greens and 7 such genotypes from non-putting green habitats, we initiated an experiment comparing putting green genotypes with non-putting green genotypes in terms of the growth dynamics of each group. Our preliminary results indicate that the Silvery-Thread Moss (STM, also *Bryum argenteum*) has evolved a suite of traits that allow it to successfully compete with bent grass in putting greens. These traits include the ability to germinate rapidly, accelerated growth rates, the production of denser and taller shoots, the tendency to produce large mats of rhizoids (the "rooting" function of mosses), and to avoid sexual reproduction in favor of vegetative growth. Such accelerated growth rates could leave this species vulnerable to stress at a particular phase in the life cycle, and this will be the subject of research in Year 2 (2017)

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 not 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 1 (2016)

Establishing genotypes of STM at the University of Nevada, Las Vegas. Dr. Zane Raudenbush (now at The College of Wooster, Ohio) distributed information slips with instructions on how to collect this moss from greens at two USGA national conferences. The response was good, with superintendents sending core samples of the moss to Dr. Llo Stark at UNLV. In addition, Dr. Raudenbush made collections from golf courses in Ohio, and Raudenbush and Stark (along with potential postdoctoral student Joshua Greenwood) visited and collected this moss from a rural Nevada golf course. We have received at least one sample of STM from a total of 15 golf courses. These golf course samples include courses from Alberta (Canada), California, Colorado, Illinois, Minnesota, Nevada, Ohio, Oregon, and South Dakota. Of these 15 genotypes (a specific genetic strain from the golf course in question), 12 have been purified in culture and the remaining 3 are in the process of purification. The purification is accomplished by placing shoots of STM into culture, allowing the moss to proliferate (with contaminants), and then after a few weeks of growth subculturing the shoot apices of the moss. Such an approach is successful in freeing the collection from algal and bacterial contaminants because the aerial portions of the moss are normally free of contaminants. This subculturing was repeated (usually two or three times) until a pure culture was obtained. Contaminants usually included assorted green algae and cyanobacteria. From receipt of sample to a pure culture of a specific genotype takes about 3 months in the lab.

In order to compare the responses of STM from golf course greens with STM genotypes collected from non-golf course habitats, we received and collected specimens from a total of 17 localities from a variety of urban and natural "off course" habitats. These "off course" collections are from Arizona, California, Georgia, Kentucky, Massachusetts, New Mexico, Nevada, Oregon, Pennsylvania, and Washington. The habitats range from sidewalk cracks, along streets, in parks, or in native habitats on soil. Of these 17 genotypes, we have purfied 10 in culture, and expect to have all 17 genotypes in pure culture in a few months. We are shooting for 15 genotypes of STM from golf course greens, and 15 genotypes of STM from "off course" habitats, and should reach that number in a few months.

2. Experiment 1: Comparing the Growth Dynamics of Golf Course STM to "Off Course" STM. We initiated an experiment using 7 golf course genotyes and 7 "off-course" genotypes of STM, following the progression of life stages as follows: protonema, shoots, bulbils (asexual reproductive structures), and gametangia (sperm or egg clusters). We are interested in detecting differences in growth dynamics and the expression of life history stages between mosses from golfing greens vs. mosses of the same species not inhabiting golfing greens. The experiment is in its 12th week as of this writing (October 2016), and some interesting trends merit mention:

A. Moss from the putting greens germinate faster.

B. Moss from the putting greens grow faster laterally as measured by protonemal proliferation.

C. Moss from the putting greens produce shoots sooner, as assessed by the day of shoot induction.

D. Moss from the putting greens grow faster in height as measured by shoot height.

E. Moss from the putting greens produce a higher number of shoots per unit area as measured by shoot counts.

F. Moss from the putting greens produce greater cover of rhizoids (the rootlike filaments extending from shoots) as measured by rhizoid cover.

G. Moss from the putting greens produce fewer specialized asexual reproductive structures as measured by bulbil counts and protonemal gemma counts.

H. Moss from the putting greens express sex later, as assessed by the number of perigonia (sperm containing structures) and perichaetia (egg containing structures), and when they express sex, it is typically higher on the shoot.

I. Moss from the putting greens, to date, are exclusively female.

J. Moss from the putting greens appears to have a higher nutrient need.

These life history and growth dynamic differences between strains from golf course greens and strains from habitats excluding golf courses are exciting because they indicate recent evolution pressure on golf course putting green habitats. Our "common garden" approach to comparing the growth dynamics of specific genotypes represents one of the strongest approaches to discern between differences related to genetic vs. environmental causes. Because of common culture conditions, any differences we see between green and off-green genotype cultures can be attributed to genetic differences (as opposed to a plastic response). The above differences are many, and indicate strong selective pressures acting on golf course greens over the last few hundred years, a very short evolutionary timeframe. These are likely to be among the life history features that give this moss a competitive advantage in putting greens, and relate to the ability to colonize rapidly, spread laterally rapidly, produce shoots quickly that grow tall and dense, extend rhizoids into the sand and laterally, require lots of nutrients, and not be reliant on sexual reproduction.

Plans for Year 2 (2017)

1. Complete Experiment 1. We currently are comparing 7 genotypes of golf course STM with 7 genotypes of "off course" STM. We need to increase the number of genotypes (strains) tested to about 15 in order to be statistically sound in experimental design, and we should be able to complete this experiment early in Year 2.

2. Initiate Desiccation Tests on STM. We will begin stressing the STM mosses with drying (desiccation) stresses at key phases of their life history, in an attempt to pinpoint weak links in the life history of this moss weed.

Note

Because the project was funded at 50% of budget, we have delayed hiring a graduate student or laboratory tech person until the second year. In 2017 we anticipate hiring a half-time graduate student, lab tech, or postdoctoral student (probably Mr. Joshua Greenwood of UNLV) who will assist Dr. Stark in conducting sets of experiments on environmental stress effects on STM strains from golf course greens.