Effects of smut fungus infection on the early successional grass *Andropogon virginicus*.

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Abstract

Andropogon virginicus is an important early successional species. This perennial C_4 bunchgrass often is found dominating old fields along the East coast of United States, and is introduced in California and Hawaii. In the east, populations of *A. virginicus* often harbor a pathogenic smut fungus, *Sporisorium ellisii*. Infected plants are smaller and exhibit lower photosynthesis rates than healthy plants, and mortality is greater in infected plants (70% compared to 43% in healthy plants in one population from 2005 through 2006). This fungus remains largely asymptomatic until the grass flowers, when infected individuals display fungal sori instead of normal flowers and fruits. As a result, we do not yet know the pattern of infection throughout the host tissue; while most infected plants exhibit systemic infection (all flowers on all shoots are replaced by sori), we can not tell if fungal hyphae are growing throughout the plant. Additionally, infections appear to be perennial; most plants infected one year remain infected the next year, but it is possible that this may actually be re-infection. Therefore, to facilitate our further study of this plant-pathogen interaction we are developing a molecular marker method to diagnose *S. ellisii* infection within host plant tissue, by utilizing *S. ellisii* specific primers developed from within the ribosomal ITS sequence.

Introduction

Andropogon virginicus is a common early successional C4 perennial grass that is native to eastern North America. In the recent past it has spread from its native range into California, where it is naturalized, and into Hawaii, where it is deemed invasive. In the native range this grass often harbors a pathogenic smut fungus, *Sporisorium ellisii*, which infects floral structures, replacing them with fungal sori. Preliminary findings indicate that the disease is restricted to the native range.

Natural plant populations continuously fluctuate as they are influenced by environmental factors. We suspect that change in populations of *A. virginicus* throughout time is not only influenced by intrinsic mortality rates, but also by the costs associated with the presence of a pathogenic fungus as well. These costs may include decreased physiological vigor, growth rate and reproduction, and even increased mortality. We are working to understand population-level consequences of infection by studying such costs within individuals. We sampled *A. virginicus* individuals in a natural population over three years, with regard to size, the presence and severity of infection (total infection, partial infection, and healthy), and mortality. In addition, a set of infected and uninfected plants were compared for photosynthesis rates.

It is impossible to easily detect *S. ellisii* infection within *A. virginicus* until flowering in late summer, when visible sori develop. To continue the study of this plant-pathogen interaction under both field and greenhouse conditions in a timely and efficient manner, it would be very useful to be able to quickly and accurately identify infected individuals at a much earlier stage. Therefore, we are developing a PCR-based detection method to assess infection status of the plant. We approach this task by screening *A. virginicus*, *S. ellisii*, and two other common smut fungi for amplification of a DNA sequence obtained from basidiomycete-specific ITS primers (ITS 1-F and ITS 4-B). We require that the marker amplifies in the fungal DNA but not in the plant DNA. Also, to make the marker specific for our particular smut fungus we are designing unique primers from within its amplified ITS sequence.

Methods

Molecular methods

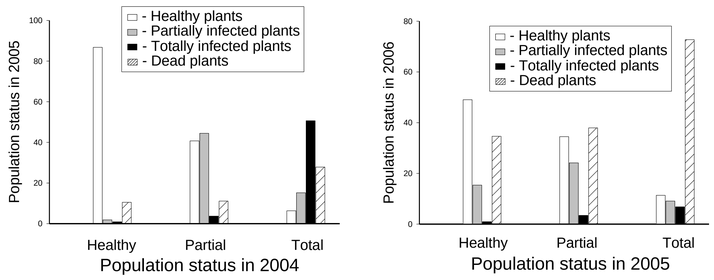
- All DNA was extracted using QIAGEN DNeasy kits. *Sporisorium ellisii* DNA was extracted from field-collected teliospores and *A. virginicus* DNA was extracted from leaf tissue obtained from uninfected greenhouse grown plants.
- PCR was performed using 95C° melting temperature, 50-58C° annealing temperature, and 72C° extension temperature, for 35 cycles with a 10 minute final extension.
- PCR products were visualized on 1.5% agarose gel stained with ethidium bromide.

Field methods

- 480 1m2 plots were established in an old field plant community. In each plot with *A. virginicus* present a focal plant was tagged and, once a year for three years, each was assessed for mortality, numbers of healthy, partially infected, and fully infected shoots, and height of tallest shoot.
- Healthy and fully infected plants located along a transect in the same field were measured for photosynthesis *in situ*, using a Li-Cor 6400 portable photosynthesis system.

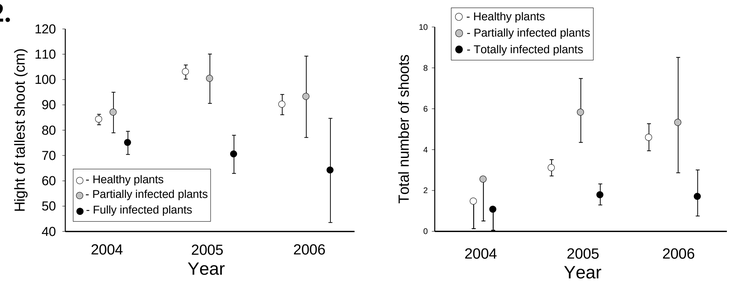
Results

Figure 1.



Throughout both years fully infected plants showed higher mortality, with a marked increase during the last year when overall mortality rates increased across the board. Plants tended to remain in their respected groups throughout time, with limited transition from partial to total status with some recovery.

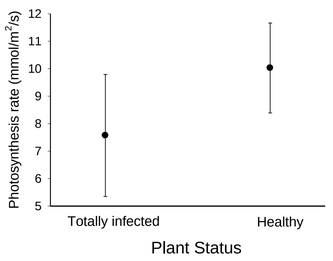
Figure 2.



Both healthy and partially infected plants were taller during first 2 years. (ANOVA: 2004 df=2,329; F=10.98; P=0.0001. 2005 df=2,273; F=41.72; P=0.0001. 2006 df=2,166; F=4.25; P=0.0159.)

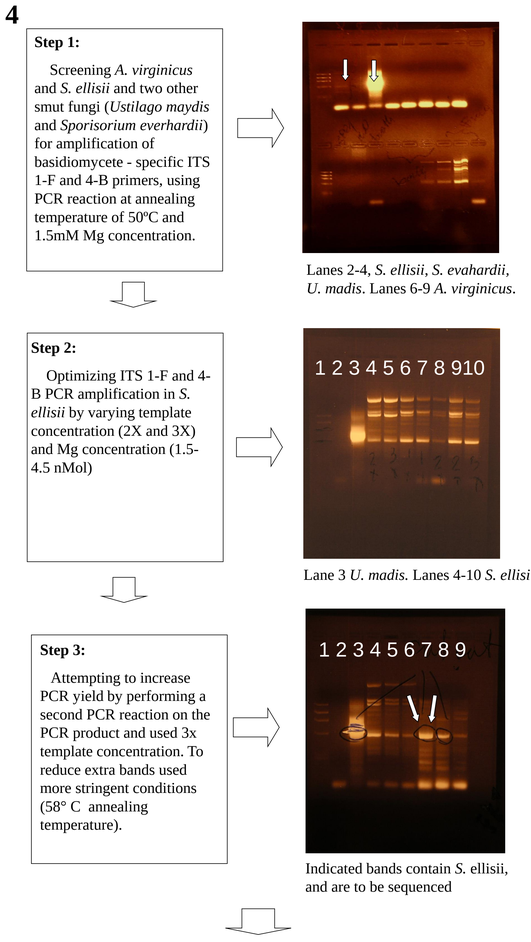
During first two years, partially infected plants were noticeably bigger. During last year, both healthy and partially infected plants were bigger.
(ANOVA: 2004 df=2,329; F=26.67; P=0.0001. 2005 df=2,272; F=19.84; P=0.0001. 2006 df=2,166; F=2.69; P=0.071.)

Figure 3.



Healthy plants displayed higher photosynthetic rates. (One tailed t test, t=1.825, df=50 P=0.037)

Figure 4



Discussion

Invasiveness can be facilitated by escape from natural enemies, and subsequent alleviation of resistance or tolerance costs. We suspect the parasitic smut fungus to exert physiological costs upon *A. virginicus* individuals. In order to determine if such costs exists, we followed a grass population throughout time, while monitoring individuals in various stages of infection. Fully infected plants experienced dramatic negative effects from the pathogen. Reproduction was eliminated completely, and they had a much higher mortality rate, especially in the second year when mortality was high across the board. They also had lower photosynthesis rate, and were smaller as measured by both number of shoots and height. In contrast, while partially infected plants had reduced seed production in infected shoots, overall they were larger than healthy plants. This suggests that during the initial stages the presence of smut fungus may induce an onset of a vigorous growth period, possibly in an attempt to overcome the pathogen, or that the larger plants make better targets. Some plants were able to overcome the disease; about half of partially infected plants transitioned to healthy status in the following year, with a much smaller proportion of fully infected plants recovered. Some turned from totally infected into partially infected, however it is not clear if that is due to recovery or emergence of new shoots. A greater proportion of partially infected plants exhibited total infection the following year than did healthy plants, suggesting that the partial status is likely to be an intermediate step on the path to total infection.

These results indicate that there is a cost to infection by this parasitic smut fungus, which reaches its peak upon full infection of the individual. It is plausible, then, that escape from this pathogen could facilitate invasiveness of this species in California or Hawaii, where the pathogen has, so far, not been observed.

Preliminary molecular data based on five trials indicated that basidiomycete specialized ITS primers do not amplify within *A. virginicus*, but do amplify within the genomes of the tested smut fungi. These early results indicate great potential for using ITS 1-F and 4-B as efficient markers to detect the presence of *S. ellisii* in host plants in the greenhouse during controlled inoculation experiments, where the only basidiomycete present will be *S. ellisii*. Development of unique primers will allow detection of *S. ellisii* in plants under field conditions where other fungi may be present. This PCR-based fungal detection technique will be a very useful research tool, allowing for rapid screening for susceptibility pre-flowering and mapping of fungal growth throughout the plant body during growth and winter dormancy in this perennial grass.

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