* At the 2nd flowering 8 mature plants were infected – 6 from T and 2 from MT

Smut fungus infection and detection in the perennial grass *Andropogon virginicus*

Jacquelyn Harth and Janet Morrison

Department of Biology, The College of New Jersey, Ewing NJ 08628

Abstract

Andropogon virginicus, a dominant grass in mid-Atlantic old fields, can be attacked by the smut fungus *Sporisorium ellisii*. This pathogen causes systemic or partial infections, reduces/eliminates seed production, and increases mortality. Our ability to establish infections experimentally is crucial for further research on host resistance and the modes of transmission and infection. We collected 25 partially infected plants from a New Jersey population. These had some tillers infected, indicating susceptibility and providing a source of fungal inoculum (teliospores), and some uninfected tillers, providing a source of seeds matched to inoculum. We prepared four inoculum types: teliospores matched from the same plant as the target seed, mixed teliospores, mycelial cultures grown from single, matched teliospores, and mixed mycelial cultures. We applied each inoculum to four seeds, four seedlings, and four seed-grown adults per maternal plant, with controls. The inoculated adult plants flowered, at which point infection can be detected (sporulation in the grass spikelets), but we saw no infections for any inoculation type. We are monitoring plants inoculated at the seed and seedling stage until flowering. Since visual detection of infection must wait until flowering, we also are developing a molecular method of detection, which tests for the presence of *S. ellisii* DNA within the plant. We have developed primers that amplify *S. ellisii* DNA but not *A. virginicus* DNA, and have tested different plant tissues from field-collected infected plants. So far, in plants collected in both winter and summer, we have detected smut DNA only in root crown tissue.

Introduction

Plant diseases are important factors in host plant ecological success. They can influence competition, reproductive status, and the genetic structure of host plant populations. Host plants and pathogens can act as agents of natural selection upon each other, thereby influencing evolution within both plant and pathogen populations.

Andropogon virginicus (broomsedge), an ecologically important, warm season perennial grass, is commonly infected with the smut fungus *Sporisorium ellisii*. This pathogen destroys the host's reproductive capability and causes increased mortality rates. Some populations are completely free of infection, while others have fairly high infection prevalence.

The natural pathway of infection is currently unknown, and experimental infection has, so far, not been achieved. Infection with *S. ellisii* can only be determined visually upon flowering, when sori filled with fungal teliospores have replaced the reproductive structures of its host plant. However, preliminary lab results using a new molecular technique that detects fungal DNA in host plant tissue showed that *S. ellisii* overwinters in the root crown of *A. virginicus*.

We seek to 1) develop a reliable method of experimental infection for use in future studies, and 2) further develop the molecular detection method in order to track the pathogen in the plant body, as it travels from root crown to shoot tip.

Results

Inoculation Experiment

Infection rates were generally low, with the highest rate (as of July 2010) for seedlings inoculated with teliospores taken from the seedlings' partially infected maternal parent. In seeds and seedlings, 69% of infections were systemic across the whole plant (all shoots infected) and 31% were partial (some shoots uninfected). For the inoculated mature plants, however, only 37% of infections were systemic. All infected shoots, however, were themselves systemically infected (all flowers infected).

Plant stage at inoculation	Smut fungus inoculum type	Percent infected at first flowering
Seeds	T: teliospores from maternal parent only	7.5 %
	MT: mixed teliospores	4.7 %
	M: mycelial culture from maternal parent only	5.6 %
	MM: mixed mycelial culture	0.0 %
Seedlings	T	18.2 %
	MT	2.9 %
	M	0.0 %
	MM	0.0 %
Mature *	T	0.0 %
	MT	0.0 %
	M	0.0 %
	MM	0.0 %

* At the 2nd flowering 8 mature plants were infected – 6 from T and 2 from MT.

Fungal Detection PCR

We have been unable to repeat the summer results (below) with the same DNA sample or samples from the fall collections. Therefore we explored the issue of fungal:plant DNA concentrations (right).

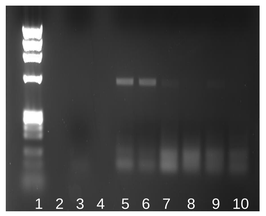

Figure A. Gel showing DNA extracted from the root crown (lanes 5 and 8), shoot base (lanes 6 and 9), and shoot tip (lanes 7 and 10) of a totally infected plant (lanes 5,6,7) and a healthy plant (lanes 8,9,10). Bands are visible at 600bp for all tissues extracted from the infected plant with the strongest in lanes 5 and 6 (root crown and shoot base). Lane 9 also shows a 600bp band suggesting new infection may be present in a previously healthy plant.

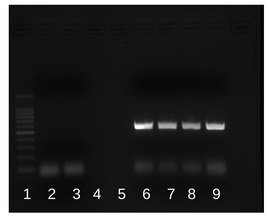

Figure B. Gel showing pure *S. ellisii* DNA at decreasing concentrations: 0.1, 0.08, 0.06, and 0.04 (lanes 6,7,8, and 9 respectively). The strong bands in lanes 6, 7, 8, and 9 suggest that the limit of detection of *S. ellisii* DNA. is below 0.04 ng/ul. Additionally lane 3 (pure *A. virginicus* DNA) did not show a 600bp band.

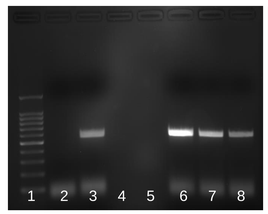

Figure C. Gel showing decreasing ratios of *S. ellisii* DNA to *A. virginicus* DNA : 0.08:100, 0.08:500, and 0.08:1000 (lanes 6,7, and 8 respectively). The strong bands found in lanes 6-8 suggest that low *S. ellisii* to *A. virginicus* concentrations do not affect the limit of detection for S. *ellisii* DNA.

Methods

Inoculation Experiment

Four types of inoculum :

1. Teliospores from the same partially infected plant as the target seed.
2. Mixed teliospores from the same and nine different plants.
3. Mycelial cultures grown from single teliospores from the same plant as the seed.
4. Mixed mycelial cultures grown from spores from the same and nine different plants.

Suspended teliospores and mycelial fragments in plain agar paste to aid adhesion to target plant tissue.

Experimental design :

- Treated four seeds each from field-collected plants #1-25 with each inoculum type, and assigned to random positions in one of four experimental blocks in the greenhouse : (seeds from 25 parents) x (4 inoc. types/parent) x (4 blocks) = 400 seeds (replication of 100 inoculations per inoculum type).
- Repeated procedure for mature plants and seedlings grown from seed from plants #1-25.

Inoculation :

- Seeds : Treated with a 500 ppm gibberellic acid solution, allowed to germinate for 3 days, and then dipped in inoculum and planted (July '09).
- Mature plants and seedlings : Cut most central tiller and applied 1 ml of inoculum over the wound, using a sterile pipette (mature: July '09; seedlings: Oct. '09)

Cut tiller with teliospore inoculum applied.

Fungal Detection PCR

Tested specificity and sensitivity of the polymerase chain reaction (PCR) using DNA primers designed to target *S. ellisii* DNA, but not *A. virginicus* DNA, using a range of concentrations of genomic DNA from various samples :

Sample	DNA Concentration (ng/µl)				
Pure S. ellisii DNA	2.5	0.1	0.08	0.06	0.04
Mixture of S. ellisii and A. virginicus DNA	2.5:50	0.1:50	0.08:50	0.06:50	0.04:50

- A specially developed ITS primer was tested against pure fungal DNA extract from *S. ellisii* teliospores, and *A. virginicus* DNA extracted from uninfected greenhouse plants.
- Totally infected plants from the field were tagged and collected in the summer, fall, and spring. DNA was extracted from three locations along the plant body: root crown, shoot base, and shoot tip.
- The primer was tested against the extracted DNA to test for the presence of *S. ellisii* in the plant body, and to verify that this primer can amplify fungal DNA within *A. virginicus*.

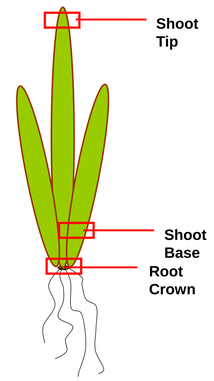

Figure 1. Diagram of *A. virginicus* showing the three locations DNA was extracted from the plant body.

Discussion and Future Directions

Inoculation – The most effective inoculation method we tested was to inoculate seedlings with teliospores collected from the seedling's partially infected maternal parent. The mixed teliospore inoculum treatment was included to maximize the opportunity for a mix of smut fungus mating types, to allow for a sexual stage and the formation of an infective dikaryotic mycelium, as is the case with the iconic corn smut. However, we found that infections were more likely when the teliospore source was from a single host plant. Assuming that teliospores from within a plant derive from one fungal race, this suggests that a sexual phase may not be required for infection. Indeed, work by our group with *S. ellisii* in culture, and microscopic examination, suggest that a sexual phase does not exist. Our results also indicate that the inoculum load of a genetically compatible smut race is important for infection; the teliospore mixture included only 1/10 the amount of teliospores collected from the infected maternal parent.

We also have shown that these grasses are infected systemically within a shoot, but that some shoots on an infected plant may be uninfected. Therefore, it is likely that the infection takes hold early in development of a new tiller and is retained during its growth. When inoculation is at the early plant stage (seeds and seedlings) this is more likely to result in whole-plant systemic infections, but inoculation through a wound on a mature plant is more likely to result in localized, single tiller infections.

Fungal detection – Our results for PCR fungal detection showed that we can detect *S. ellisii* DNA in tiny amounts even when mixed with *A. virginicus* DNA, and our tests of infected plant DNA do indeed detect the pathogen. We have promising results suggesting that the fungus overwinters in the root crown and then moves through the plant to the flowering shoot. We will repeat this work on our tagged plants as the growing season progresses and flowering shoots develop, and again on overwintering plants. This technique has promise for detecting cryptic infections, in which the fungus is present in the plant, but the host can tolerate its presence without showing outward signs of disease. The method also will allow us to determine as rapidly as possible if experimental inoculation in a greenhouse study causes infection.

Ecological/evolutionary studies of natural plant-pathogen interactions face challenges due to a lack of developed techniques for particular species, since each study system is unique (unlike crop systems). The techniques we are developing are crucial for further study of pathogen interactions with this ecologically important grass species, but also can be models for other natural plant-pathogen study systems.

Acknowledgements: This research was funded by a 2009 SURF from the American Society of Plant Biologist and the Office of Academic Affairs at The College of New Jersey, through the Mentored Undergraduate Summer Experience (MUSE) program. Special thanks to undergraduates Lauren Frazee and Joshua Breslin for their help and support.