What is the effect of herbivore saliva on the toxicity of fungal endophytes?

OR: How does simulated herbivory (mechanical defoliation & saliva application) affect the impact of the fungal endophyte, *Epichloe uncinata*, of Meadow fescue, *Schedonorus pratensis*, on the growth and survival of the house cricket, *Acheta domesticus*? A Bioassay

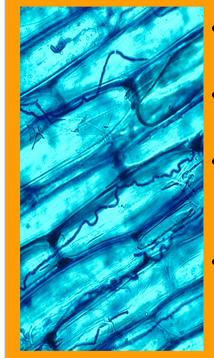
Michelle Binczyk SC/ BIOL 4000

Abstract

My research investigated 1) the effect of endophyte infection status (E+ and E-), on the survival and weight of an insect herbivore, the house cricket, *Acheta domesticus* and 2) the impact of simulated herbivory, including both defoliation and herbivore saliva application on endophyte functioning, measured via a bioassay with house crickets, *Acheta domesticus*.

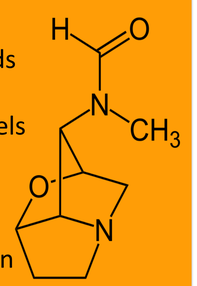
Introduction

What are endophytes?



- Endophytes are microorganisms living inside plants (Clay, 1998).
- Systemic endophytes are found throughout the host plant
- I studied the systemic fungal endophyte *Epichloe uncinata*, of Meadow fescue, *Schedonorus pratensis*
- Endophyte grasses are more robust and successful thanks endophyte uninfected grasses

Alkaloids



- Grass endophytes produce diverse alkaloids (Clay, 1988)
- Infected meadow fescue contains high levels of lolines (Justus et al. 1997)
- Lolines are are neurotoxic to insect herbivores
- Lolines decrease environmental stressors in the host plant

Defoliation

- Endophytes increase re-growth rate in clipped grasses (e.g Belesky, 1996).
- Clipping E+ grasses results in an increase in alkaloid levels (Bazely et al. 1997).

Hypothesis

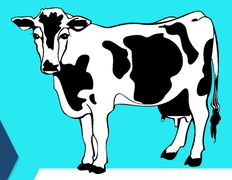
E+ treatments will affect the survival, consumption and weight of *Acheta domesticus*

Grass diets involving defoliation treatments will affect the survival, consumption and weight of *Acheta domesticus*

Grass diets involving saliva application treatments will affect the survival, consumption and weight of *Acheta domesticus.*

Methods

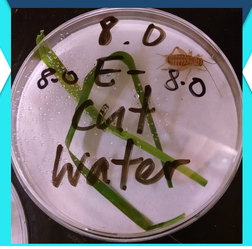
Generating endophyte-infected and uninfected grass plants for the experiment



Obtaining Herbivore Saliva

Herbivore saliva has the ability to combat alkaloids produced by endophytes (Tanentzap et al. 2014)

Bioassay to determine the effects of simulated herbivory on endophyte and host grass response



Statistical analysis

All statistical analyses were conducted on SPSS version 20

I used a three way ANOVA for data analysis

Grass Diet Treatments

Treatment combination	Grass genotype endophyte status	Grass genotype endophyte status
Cut + Saliva	Infected	Uninfected
Uncut + Saliva	Infected	Uninfected
Cut + Water (No Saliva)	Infected	Uninfected
UnCut + Distilled Water (No Saliva)	Infected	Uninfected

- 6 genotypes of *Schedonorus pratensi*
- 2*2*2 = 8 treatment combination
- Domestic cow, *Bos taurus* saliva was used

Bioassay

- n=120
- The crickets were weighed on the first day, and every second day
- Fed between 0.03 - 0.10 grams of grass every second day

Results

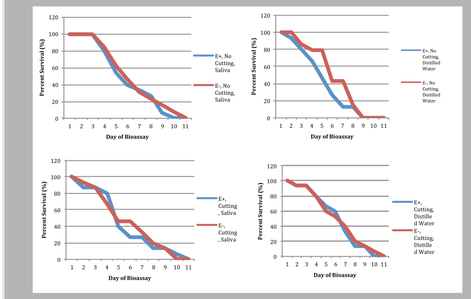


Figure 1: Percent survival of house crickets 8 different grass diets. The grass diets were paired based on the same cutting and saliva treatments. A: Compares E+ and E- for No Cutting and Saliva, B: Compares E+ and E- for No Cutting and Distilled Water, C: Compares E+ and E- for Cutting and Saliva, D: Compares E+ and E- for Cutting and Distilled Water.

Figure 2: A three-way anova for cricket consumption with three independent variables: presence of endophyte, application of saliva and cutting. The highlighted rows are the factors that were statistically significant.

Tests of Between-Subjects Effects					
Dependent Variable: Consumption					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.001[a]	7	8.463E-5	2.663	.014
Intercept	.003	1	.003	79.047	.000
Endophyte	.000	1	.000	4.641	.033
Saliva	5.800E-6	1	5.800E-6	.183	.670
Cutting	4.400E-7	1	4.400E-7	.014	.907
Endophyte * Saliva	1.121E-5	1	1.121E-5	.353	.554
Endophyte * Cutting	3.402E-5	1	3.402E-5	1.071	.303
Saliva * Cutting	.000	1	.000	11.294	.001
Endophyte * Saliva * Cutting	5.925E-5	1	5.925E-5	1.865	.175
Error	.003	108	3.177E-5		
Total	.006	116			
Corrected Total	.004	115			
a. R Squared = .147 (Adjusted R Squared = .092)					

Conclusion

- E+ diets significantly reduced the mean daily consumption of grass
- There was a trend (ns) to lower percent survival of the crickets fed E+ diets
- Significant effect of cutting on egestion
- significant saliva*cutting choice on consumption
- There is clear evidence of subtle saliva effects on dietary intake
- The mean cricket egestion (poop) was lower in the cut grass diets.
- Raising the question: why does cut grass cause constipation?