

The effect of shading on the disease ecology of the fungal pathogen *Entomophaga grylli* and its host the clearwinged grasshopper (*Camnilla pellucida*)

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Zuleika Cruz Ramos

Advisor: Erica Kistner

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Abstract

Climate change is making precipitation and temperature patterns change and because of that most ecosystems have been affected. In grasshoppers case very low temperature, below normal summer ones, increase the possibility of infection with fungus. Grasshoppers tend to do thermoregulation, which is very important for their biological functions, fitness and development. *Camnula pellucida* use incident solar radiation to increase internal body temperature above ambient levels. To begin normal activities temperature required is between 67-95 °F. *Entomophaga grylli* is one of more than 100 of the genus that attack a variety of insects. This study investigated how shade affects the development of fungal *Entomophaga grylli* that tend to reduce populations of clearwinged grasshoppers. The analysis of the data revealed that there was not a significant relationship between survivalship and treatment day by day, which mean that shade does not have any effect on the grasshoppers survival and temperatures (°F) were hotter in the East shade treatment overall, which was no expected.

Introduction

The clearwinged grasshopper, *Camnula pellucida* is distributed widely in North America including the northern mixedgrass prairie (Figure.1). Is a severe pest of small grains and grasses, it has a preference for bluegrasses (e.g., Sandberg bluegrass (*Poa secunda*) , Kentucky bluegrass (*Poa pratensis*) and Wheatgrasses (e.g., western wheatgrass (*Pascopyrum smithii*) and crested wheatgrass (*Agropyron cristatum*) (Pfadt 1994) In its natural habitat, the clearwinged grasshopper consumes small amounts of forbs.

Camnula pellucida use incident solar radiation to increase internal body temperature above ambient levels, they prefer temperatures near 40°C (104 °F) for optimal development. (Carruther, Larkin, Firstencel and Feng 1992) For begin normal activities like; feeding, starting migration, molting and mating in adult

stages temperature required is between 67-95 °F. When temperature is greater than 40°C (104 °F) grasshoppers show signs of overheating. More than four hours exposure to temperatures above 35°C (95 °F) is damaging to the fungal *Entomophaga grylli*, which usually tend to cause high mortality on the clearwinged grasshopper population (Pickford and Riegert 1963). *Entomophaga grylli* is one of more than 100 of the genus that attack a variety of insects. (McLeod 1963) Most reports show a relationship between the outbreak of the fungus and warm, humid conditions, cloudy, wet weather for one or more weeks promotes fungal development (Steinhaus 1949)

Climate change is making precipitation and temperature patterns change and because of that most ecosystems have been affected. In grasshoppers case very low temperature, below normal summer ones, can increase the possibility of infection with the fungus (Carruther, Larkin, Firstencel and Feng 1992). Therefore episodic outbreaks of *E. grylli* are associated with cooler summers temperatures and prolonged periods of increased precipitation (Pickford and Riegert 1963). For grasshopper communities, densities are often correlated with the previous year's temperature and precipitation (Belovsky and Slade 1995). Therefore thermoregulation is very important to grasshoppers given that temperature affects biological functions, fitness and development. (Belovsky and Slade 1986).

We are looking to see what is the effect of shading on the development of the grasshopper fungus *E. grylli* in *Camnula Pellucida* individuals. I hypothesize that grasshoppers will survive better in areas of direct sunlight because they can increase their body temperatures enough (over 35 °C) to overcome the fungus (Carruthers et al 1992) and control treatment should show lower temperatures because does not have shade.

Methods

The research will take place at the University of Notre Dame Environmental Research Center (UNDERC West) located within the National Bison Range namely the Triangle (Figure. 2) where considerable previous grasshopper research has been conducted (Belovsky and Slade 1986, 1993, 2000). Study site is dominated by

Kentucky bluegrass, western wheatgrass and some forbs including Canada thistle (*Cirsium arvense*) and Yarrow (*Achillea millefolium*).

Three different treatments using the grasshoppers fungus *E. grylli* will be applied randomly in a three transects grid for 12 of 24 cages. Cages will be separated by 2.5 m within transects to prevent cross contamination of the fungus (Belovsky and Slade 1995). We will repair the cages and cover patch holes as needed to avoid the possibility of grasshoppers escaping and disinfect all the healthy cages with Virex TB Cleaner ® to avoid crossed contamination coming from the cages.

The cages will be set up over a 0.1 m² basal area of vegetation containing a variety of the dominant grasses and forbs species. Each cage is secured in the ground and attached to a base of 7 cm wide plastic edging, (Belovsky and Slade 1995). Shade treatments will be randomly assigned to 16 of the 24 cages (i.e., 8 cages with east shading and 8 cages with west shading).

Each of the 24 cages will have ten *Canulla pellucida* grasshoppers in late developmental stages (fifth instars and adults). Both treatment cages are going to be stock on different days to prevent crossed contamination of the fungus. Twelve cages will have grasshoppers infected by the fungus *E. grylli* and the other 12 serving as the uninfected controls. We will monitor both the infected and healthy grasshoppers every day by calculating mortality rates for all treatments. We will remove cadavers from the healthy cages to prevent contamination and also to test them in the lab and make sure they die by the fungal. Temperature is going to be read randomly for 6 cages (one of every treatment) everyday using Krestrel 3000 ® temperature reader.

Statistics:

To analyze the data we will run six Non-linear Regression, one for every treatment, looking the survival proportion day by day since the last re-stocking date. We will also running a pair of T-Test to compare temperature reads by shade direction.

Results

After re-stock the third time, I had a total of 7 days of survival and temperature data. I ran six Non-linear regressions, one for every treatment, looking the survival proportion day by day since the last re-stocking date. I also ran a pair of T-Test to compare temperature reads by shade direction. I compared the non-linear regression parameter A and B with 95 percent confident for every West, East and control treatment (Figure. 3) and I found that all the values overlap which means there were not a statically significant relationship. Although all the Non-linear for the healthy cages treatments show less mortality (Figure. 4, 5, 6), which is what we were expecting. The paired of T-test comparing treatments show statically significant relationship between West Shade and East Shade ($p = 0.0085186$) and between West Shade and Control treatments ($p = 0.0049621$). Besides it was not a statically significant relationship between East Shade and Control treatments ($p = 0.5908231$) (Figure. 7)

Discussion

Clearwinged grasshoppers (*Canulla pellucida*) tend to die by drought resulting in starvation or by the *Entomophaga grylli* infection. The most common sing of the fungus infection is that the cadavers are usually found clinging atop vegetation. (Bidochka and Khachatourians 1990) In this case most of the cadavers were found clinging in the vegetation or in the cages, the ones found in the ground use to have their legs crossed, called death grip, which indicated that some die from the fungal but then fell to ground. The individuals that did not die from the infection could die by other factors as stress, crowding and higher temperatures. Although a 0.1 m^2 patch is maybe not enough space to support ten adults' grasshoppers, namely when lack of vegetation and competition for food is a fact. Once infected their tend to reduced fecundity and food consumption activity.(Bidochka and Khachatourians 1990)

As expected grasshoppers mortality were less in the healthy cages because most of them did not get infected with fungal. Even due it was not possible for the grasshoppers reach their optimal temperature required to prevent or kill the fungal because this summer temperature were cooler than expected. Shade treatment did not

show an effect on the grasshoppers survival but that does not mean that the idea is wrong, the problem in our case were contamination, we were putting infected grasshoppers in our cages without know about it because it takes 5-7 days to them for star showing symptoms or die. Even the individual in the control cages could not reach their maximum temperature and kill the fungal.

Control cages were supposed to show the higher temperatures but in our case temperature were hotter for East Shade treatments because in the afternoon which is the hotter part of the day the sun is hitting West site, the cages that have West shade are protected from the sun at that time, that is why they are cooler in the morning. We summer sky stay cloud free all afternoon maximum temperature may occur sometime between 3:00 pm and 5:00 pm. (Ahrens 2007) Even when every cage was supposed to cool down by radiational cooling at night they still can have some heat residuals in the morning. In radiational cooling both ground and air above cool by radiating infrared energy (Ahrens 2007) Overall our results still show that the disease have a big impact on the grasshoppers survivalship which was the expected results

Improvements:

For better results is a good idea to collect the eggs, keep them in the refrigerator, disinfect them with Virex TB Cleaner ® and leave them in a pristine area and once they are big enough put them in the cages. (Carruthers et al 1992) Although better shading, pad should cover than a 60 percents of the cages. Also to avoid crossed contamination, healthy cages should be more separated from the disease ones, always making sure that they going to have the same kind of vegetation, slope and conditions. HOBO® Data Loggers for record temperature measures 24/7 can be really helpful but they are expensive. Finally I think insect immunity is a underexplored research area that is why is really important to improve knowledge and keep doing Science.

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Appendix



Figure. 1 Clearwinged grasshopper distribution in North America.



Figure 2. Triangle, research area at the National Bison Range

Health	Treatment	Estimate A	Parameter A Lower	Parameter A Upper	Estimate B	Parameter B Lower	Parameter B Upper	R-squared
1	3	1.182	0.986	1.378	-0.167	-0.211	-0.123	0.719
1	1	1.079	0.904	1.254	-0.118	-0.154	-0.081	0.597
1	2	1.137	0.979	1.296	-0.131	-0.164	-0.098	0.711
2	3	1.224	0.979	1.469	-0.300	-0.380	-0.220	0.783
2	2	1.061	0.720	1.402	-0.250	-0.364	-0.137	0.555
2	1	1.210	0.979	1.441	-0.271	-0.342	-0.200	0.779

Figure. 3 Regression results for every of the six treatments day by day.

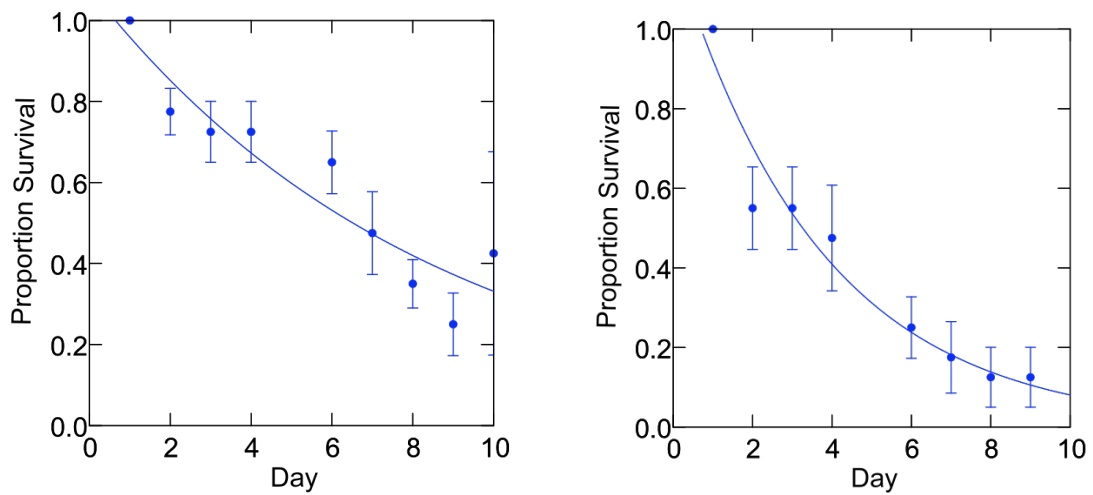


Figure 4. Healthy and Disease Control Survival Regressions Day by Day

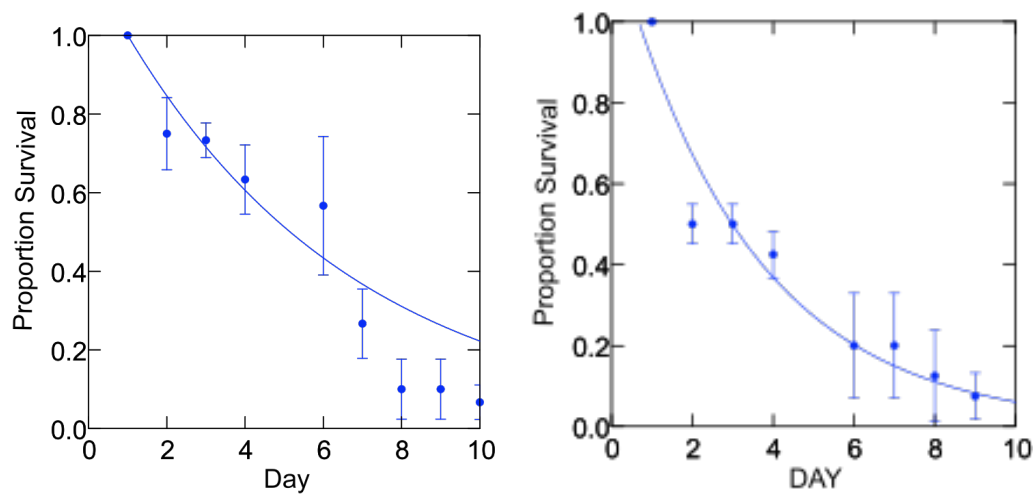


Figure 5. Healthy and Disease West Shade Survival Regressions Day by Day

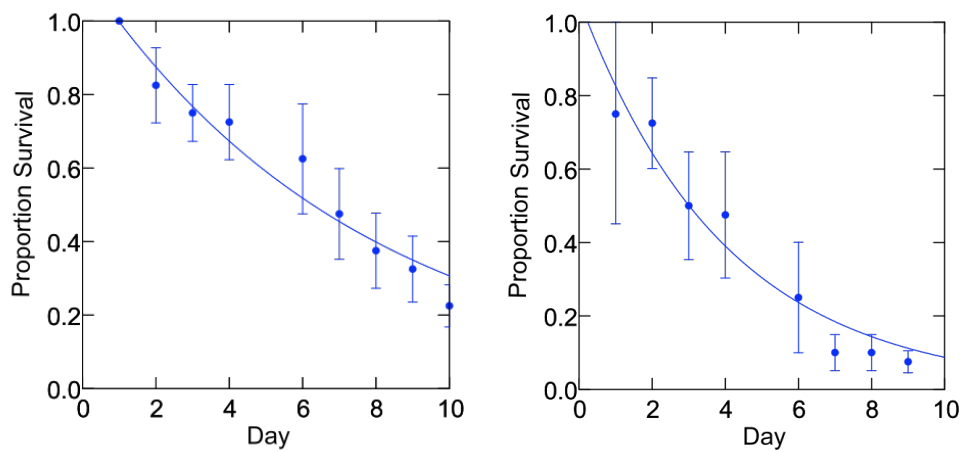


Figure 6. Healthy and Disease East Shade Survival Regressions Day by Day

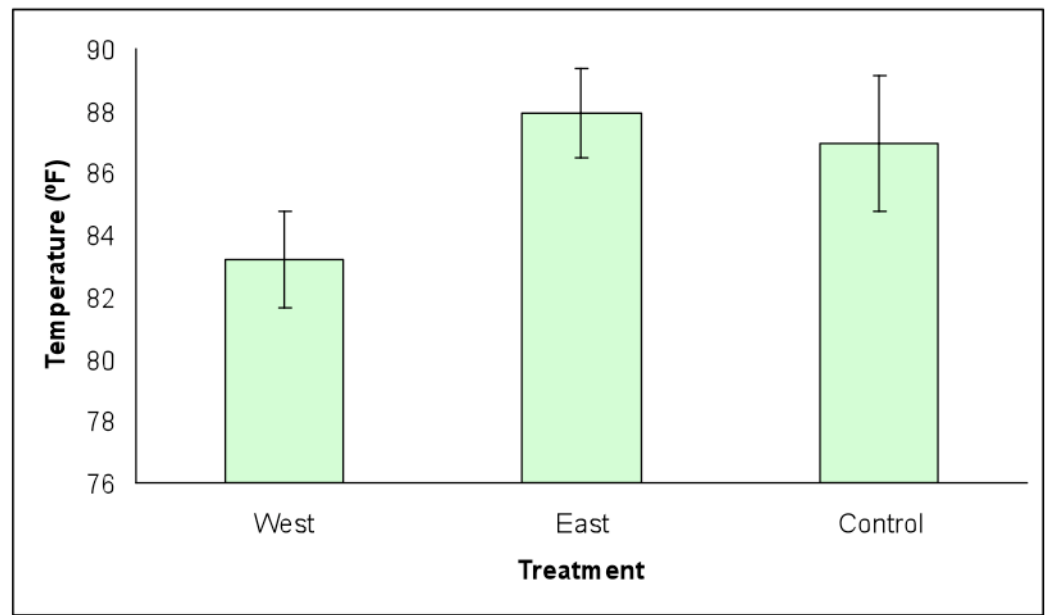


Figure 7. Shade effect on the Average Temperature.