Brianna McGuire

Mentor: Erica Kistner

The effect of external heat addition on internal temperature and pathogen elimination success in the Acridid grasshopper *Camnula pellucida*

University of Notre Dame Environmental Research Center-West

August 4, 2011

Abstract: Grasshopper internal temperature functions as a regulator of fungal growth. As anthropogenic climate change increases temperature, it is important to examine how grasshopper internal temperature will be likely to change with increased ambient heat exposure for its implications on grasshopper fitness and pest management concerns. 200 grasshoppers were kept in cages and were probed for their internal temperatures daily. Half of the grasshopper were infected with *Entomophaga grylli*, a natural pathogen susceptible to degradation at high temperatures. Half of each infection group was heat treated with a reptile heating lamp. All that died during the experiment and 40 that died after the experiment were dissected and pathogen load was tabulated. Though grasshopper temperature did differ significantly with ambient temperature, heat treatment, and the interaction between infection status, heat treatment, and ambient temperature, pathogen load was not as directly affected. This suggests that a larger sample size is needed for future study. Grasshoppers were also tethered outside, and infection status as well as ambient temperature were found to have significant effects ($p<0.005$) on grasshopper internal temperature. These findings suggest that grasshoppers will exhibit improved fitness under climate change predictions and will need to be managed with more than their naturally occurring fungal parasite.

Introduction:

As extreme temperature changes become more frequent due to anthropogenic climate change, the influence external temperature has on host-inducible fever in host-pathogen interactions bears not only effects on host fitness but also on pest management strategies (Elliott et al. 2002, Harvell et al. 2002). Many species of insects are known to

induce a behavioral fever to eliminate internal fungal pathogens (Elliott et al. 2002) while others are known to manipulate body temperature with typical thermoregulation to minimize the effects of internal pathogens, though not eliminate them (Springate and Thomas 2005). The insects of the order Orthoptera provide a wealth of well-studied thermoregulation effects on host-pathogen interactions (Carruthers et al. 1997). The Acridid grasshopper *Camnula pellucida*'s thermoregulating relationship with its internal obligate fungal parasite *Entomophaga grylli* provides an ideal model of this widely-observed phenomenon.

Camnula pellucida, or the clear-winged grasshopper, is widely distributed throughout the United States and Canada. It destroys approximately 1.25 billion dollars worth of rangeland and cropland per year in the state of Montana; the population assemblages of grasshoppers in the Western United States consume more grasses than all major ungulates of the North American West combined. Its demise is thus the subject of experimentation for those interested in pest control, especially those interested in biocontrol, given the infeasibility to apply pesticides to its considerable range.

This control over species proliferation is exercised by the fungus *Entomophaga grylli*. An obligate parasite, *E. grylli* infects a grasshopper by boring through its exoskeleton with modified spores called conidia, proliferating throughout the body in cell wall-less structures known as protoplasts, and finally regaining its cell walls to form hyphal bodies, at which point the grasshopper succumbs to the disease. In this final hyphal stage of the disease, the grasshopper exhibits a highly characteristic behavior: it climbs to the top of a blade of grass, wraps its limbs around said structure, and dies overnight (Carruthers et al. 1997). This spore-containing, wind-blown and elevated

grasshopper is likely to produce more conidia that is easily spread during the early summer months of June and July, and produces more resting spores during the months of August and September. Given the characteristic death grip associated with the fungus, it is easy implicate it in a grasshopper's death in a laboratory. Despite the clear detrimental effects this fungus has on the grasshopper, many grasshoppers can live with some percentage of fungus in their bodies provided that prevailing abiotic conditions allow; i.e., that sufficient thermoregulation is possible.

However, high internal temperature is hardly achievable with adjustments in metabolism: it requires some means of harnessing external heat, namely through basking (Carruthers et al. 1992). Grasshoppers have been observed to bask extensively in laboratory after exposure to the disease, limiting the extent and development of the disease (Carruthers et al. 1992). Because adequate heat is necessary for the grasshopper to thermoregulate to around the temperature of 35 degrees Celcius, the threshold for destruction of the fungus (Carruthers et al. 1992), as well as limit to eliminate the effects of the disease, and because most of the area most vulnerable to grasshopper outbreaks (the Western United States) has been undergoing extreme temperature fluctuations over the past ten years, fluctuations in temperature will affect the year-to-year host-pathogen disease ecology (IPCC 2007 Data, Erica Kistner EPA Star proposal 2011). With the possibility of an expanding habitat area for the clear winged grasshopper and a possibility of a degraded ideal habitat for its fungal pathogen, there is an ecological impetus to evaluate the role of external temperature change on grasshopper/pathogen relationships. Thus, to gain the precision and control in-laboratory manipulation as well as the context and relevance of an in-field study, this study will attempt to bolster the scientific

literature with a three-part study that will focus on in-lab internal temperature measures, in-field internal temperature measures, and dissections of laboratory-studied grasshoppers.

I hypothesize that the addition of a supplemental heat source to ambient grasshopper conditions will increase grasshopper internal temperatures. Grasshoppers that are heat-treated will exhibit a lower pathogen load than those that are not exposed to a heat lamp. Ambient temperatures will also have an effect on grasshopper internal temperature.

With regard to grasshopper internal temperatures in the field, I hypothesize that grasshoppers tethered at lower heights will exhibit significantly lower internal temperatures than grasshoppers tethered at higher temperatures. I also hypothesize that grasshoppers infected with *E. grylli* will exhibit higher internal body temperatures than those uninfected with the fungus. I finally anticipate that grasshopper body temperature will co-vary with ambient temperature.

Finally, with regard to dissection data, I hypothesize that grasshopper cadavers recovered over the course of the in-lab experiment will exhibit differing pathogen loads depending on their exposure to heat and their exposure to fungus. I hypothesize that there will be a significantly higher number of *E. grylli* protoplasts and hyphal bodies as heat exposure decreases and as fungus exposure increases.

Materials and Methods:

This experiment was carried out on a residence in an agricultural area of Charlo, MT. Most grasshoppers were collected from the National Bison Range and its environs.

In-Field Tethering Observations:

Fourth and fifth instar grasshoppers were either tethered to the ground using fishing line tied around their pronotum or placed in enclosures 24 inches above the ground. Their temperatures were taken using a Barnant thermometer with an Omega probe and adaptor once before placing in the designated treatment area, then once one hour after placement to allow for in-field thermoregulation. Each grasshopper's sex and developmental stage in instars was recorded, as well as average wind speed, maximum wind speed, and ambient temperature using a Kestrel datalogger. This procedure was performed between 2-3 PM Mountain time over three days, with a total of 18 infected grasshoppers tethered and 18 uninfected grasshoppers tethered. A regression analysis of this data was performed to determine the rate of change of internal temperature with regard to ambient temperature. A t-test was performed to determine the differences in regression intercepts.

In-Lab Daily Temperature Readings:

Aquaria with dimensions of 12"x20"x10" (those of a standard 10 gallon aquaria) were built using 6 mm metal roof flashing and insect netting attached with Liquid Nails and duct tape. Cages were kept stocked with 10 grasshoppers each, though for the last two days of the experiment natural mortality was observed due to insufficient catching to maintain stocking. Ten cages were placed in two sites: one designated for healthy grasshoppers, and one designated for infected grasshoppers. These two sites were both east facing and well ventilated. They were separated by approximately 400 m to prevent cross-contamination, and temperature recorders changed gloves and clothing between measurements. Half of all cages at each site received 12-14 hours of solar-imitation insulation from ReptiSun 10.0 UVB Desert Lights. To prevent light pollution and

possible heating of cages designated as unheated, non heat-treated cages were hung near windows in cradles made of nylon rope and secured to the roof using a staple gun.

Temperatures were measured of one randomly chosen grasshopper from each cage at three different intervals throughout the day using a Barnant Type J thermocouple thermometer and an Omega Type 2 Hypodermic 21 gauge probe. No grasshopper was measured over two consecutive measuring periods, and no grasshopper was measured more than three times total. The probe was inserted under the grasshopper pronotum until a pressure differential was noted by the operator. The grasshopper was held for a maximum of fifteen seconds, or less time if the temperature stabilized. If the grasshopper's temperature was increasing or decreasing by more than 0.4 degrees Celsius per second. This time limit on temperature measuring was done to ensure that the operator's body temperature did not affect the temperature of the grasshopper.

A three-way repeated measures ANOVA was performed using this data on SYSTAT 13.

In-Lab Dissections:

Grasshopper cadavers were removed from all in-lab cages each morning and frozen immediately. They were later thawed out and the abdomens sliced of in the middle. Pressure was applied from the back of the abdomen to the sliced end to remove and hemolymph or fungal deposits. Any clumps that partially extruded from the abdomen were pulled out and removed for staining. One drop of Dalynn Lacto-Fuchsin stain, which specifically detects fungal cells, was applied to each slide. Samples were examined under Olympus compound microscopes, and protoplasts and hyphal bodies were counted.

Results:

In-Lab Internal Temperature Measurements:

Hypothesis I was largely supported by these results: grasshopper internal temperature did significantly increase with a warm supplemental source of heat ($p<0.001$, Table 4) and also increased based on ambient temperature ($p<0.001$, Table 4). Although infection status alone did not serve as a statistically significant factor in influencing grasshopper internal temperature, its interaction with ambient temperature and its interaction with time of day significantly affected grasshopper internal temperature.

In-Lab Dissection Data:

Hypothesis II was not supported by the data generated by this part of the experiment. Protoplasts did not significantly differ in number as a function of light treatment nor of infection status (Figure 4 and Table 1), nor did hyphal body counts (Figure 5 and Table 2).

In-Field Tethering Observations:

Grasshopper internal temperature was found to significantly depend on infection level and ambient temperature (Figure 1), $p<0.005$. Height of tethering had no statistical effect on grasshopper internal temperature. This indicates that grasshoppers situated in any part of a grassland under two feet tall achieve body temperatures that are reflective of not only the ambient temperature but also reflective of the grasshopper's pathogen load.

Discussion:

The results from the three experiments denote instances of complementarity, but beg for a more complete study to provide a full picture. In-field tethering data, while significant, was of a modest size. It was also not the most contextually full picture

possible from a tethering study: only two heights were employed, and as *Camnula pellucida* is a pest and would likely be found in denser, taller grasses, more tethering heights and sites are necessary to develop a full picture of this.

The in-lab dissection data, though not significant, suggests that small amounts of heat exposure may the delay the development of *E. grylli*, though not its growth. Heat exposed, infected grasshoppers exhibited a greater average number of protoplasts, while infected grasshoppers not exposed to heat exhibited a greater average number of hyphal bodies. As hyphal bodies are more complex versions of the disease, it is possible that heat retards fungus development but does not prevent its proliferation, leaving a grasshopper at risk of death from early stage fungus accumulation. Achieving significance in this portion of the study was prevented by a wide range of hyphal body and protoplast counts. This wide variation in infection amount may be due to cross contamination. Manual wiping of the thermometer was the only cleaning method employed by this study, as the probe could not be dipped in a cleaning solution. A few renegade conidia or spores may have adhered to the probe or to the thermometer itself and exposed healthy grasshoppers to the fungus. Furthermore, airborne conidia can be extremely prevalent during times of sporulation (Sanchez Pena 2005), and may have simply floated into the healthy site, as it is located near a field and only insulated from the elements by insect netting.

In-lab temperature data supports the first set of hypotheses. Heat-exposed grasshoppers did exhibit higher temperatures, but only when interacting with ambient temperature and time of day. Thus, external heat added a small but measurably significant improvement to how grasshoppers heat their bodies. However,

thermoregulation in its strictest sense was not observed during the in-lab experiments. Although some grasshoppers did clearly bask in the light of the heat lamp, their metal cages conducted at least some small amount of heat in the frame of the cage. As it was difficult to divine whether or not a grasshopper sitting in a relatively obscure corner was thermoregulating like its clearly basking cage-mate, this experiment was not suited to behavioral analysis. Further studies could include a cage with a heat gradient, be it a line conducting heat outwards across the floor of the cage to its edges, or differential heating in different parts of the cage, to observe how grasshoppers move throughout the cage. This would provide the behavioral data to prove that thermoregulation is truly occurring.

This study indicates that heat additions have at least some biological effect on grasshopper pathogen load, and at its most effective influences a grasshopper's internal temperature to the point that it can eliminate its fungal pathogen host. Given the recent 10-year trends in drier summers in the Eastern United States, with lower rainfall and lower humidity, it would appear that grasshopper range and population expansion can go more and more unchecked by soil reserves of conidia. However, extreme cold weather events are also becoming more prevalent, indicating that grasshopper growth may not go entirely unchecked in the Western United States.

Tables and Figures:

Figure 1: Grasshopper Temperature as a function of infection status and height in grass.

This provides a visualization of the following two figures, which give regression values for rate of change of internal temperature with respect to ambient temperature.

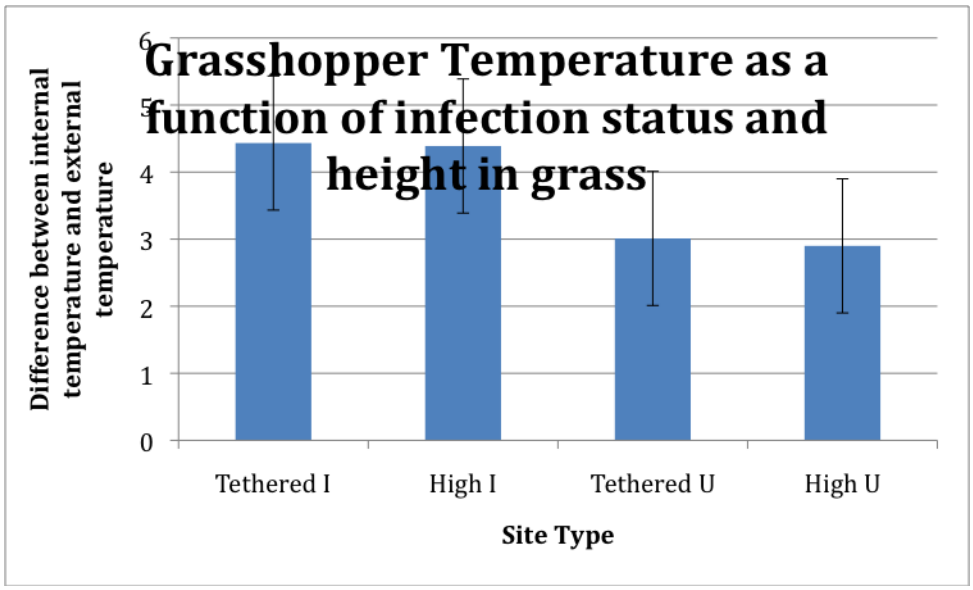

Figure 2: Regression Line for the difference between body temperature and ambient temperature as a function of ambient temperature in uninfected grasshoppers. As can be clearly seen, as uninfected grasshoppers experience an increase in ambient heat, they slow their rate of change of internal temperature increase ($p<0.005$).

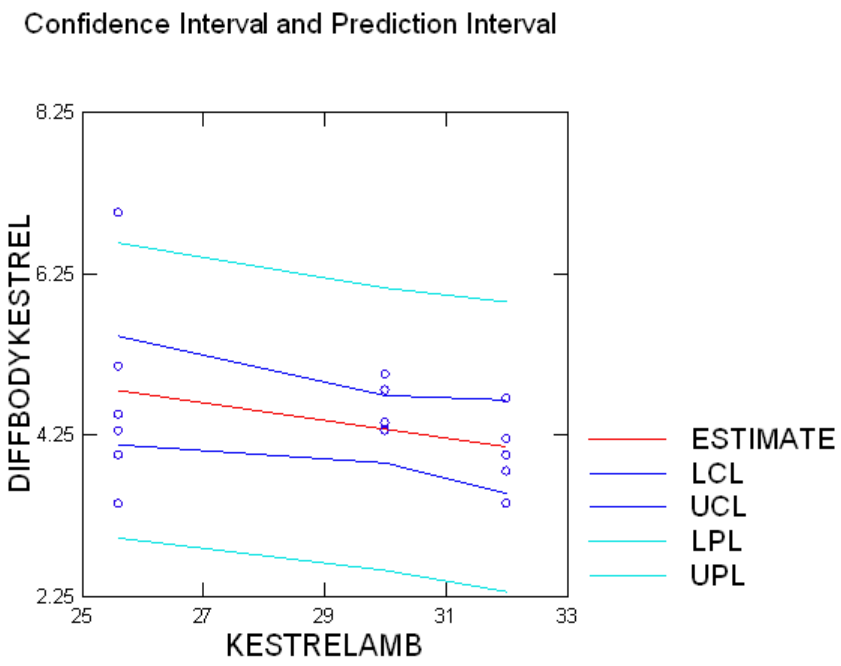

Figure 3: Regression line for the difference between body temperature and ambient temperature as a function of ambient temperature in infected grasshoppers. As can be seen, as infected grasshoppers experience an increase in ambient heat, they augment their rate of change of internal temperature increase ($p<0.005$).

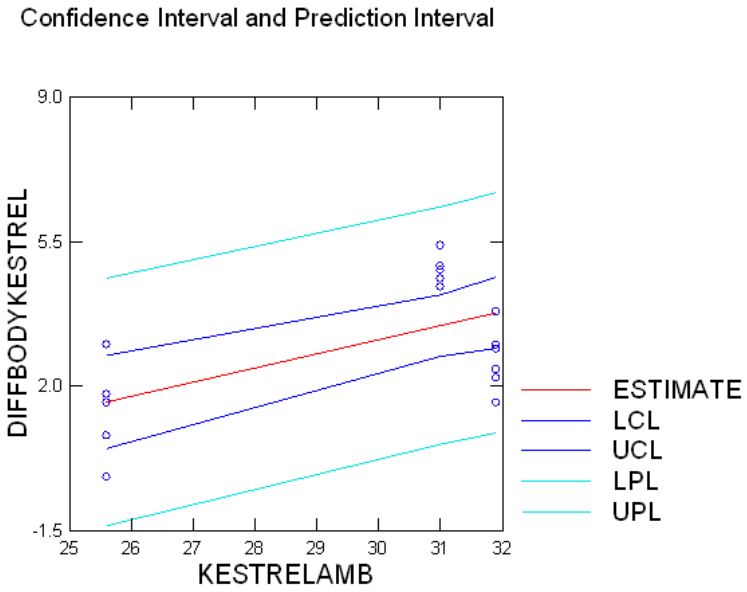

Figure 4: Output reflecting relative abundances of protoplasts in grasshopper cadavers as a function of infection status and light treatment.

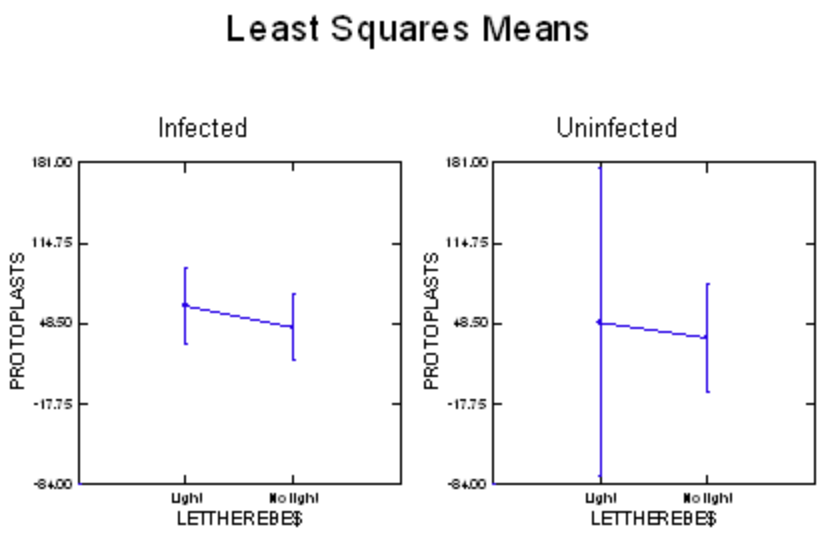

Table 1: Analysis of Variance output describing the relationship seen in Figure 4.

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
LETTHEREBE$	755.419	1	755.419	0.191	0.665
TREATMENT$	404.228	1	404.228	0.102	0.751
LETTHEREBE$*TREATMENT$	24.369	1	24.369	0.006	0.938
Error	174,435.583	44	3,964.445		

Figure 5: Output reflecting relative abundances of hyphal bodies in grasshopper cadavers as a function of infection status and light treatment.

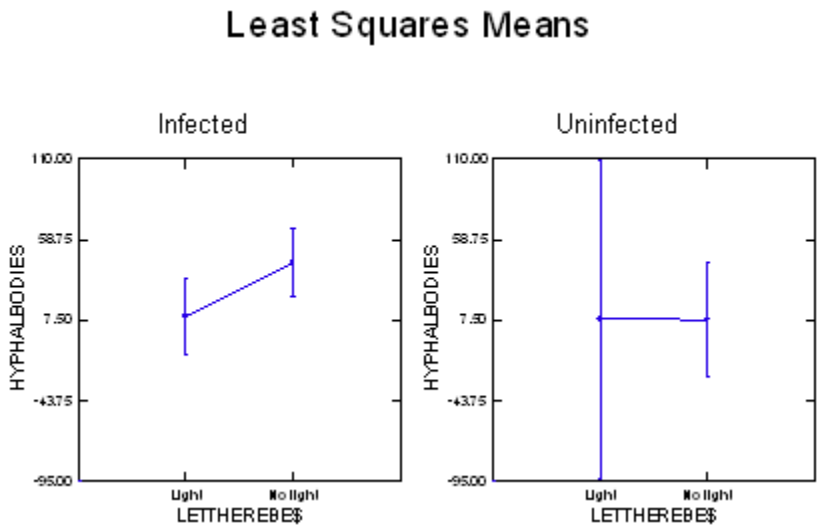

Table 2: Analysis of Variance output reflecting the relationship in Figure 5.

Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
LETTHEREBE$	952.929	1	952.929	0.379	0.541
TREATMENT$	1,183.202	1	1,183.202	0.471	0.496
LETTHEREBE$*TREATMENT$	980.975	1	980.975	0.390	0.535
Error	110,616.302	44	2,514.007		

Analysis of Variance					
Source	Type III SS	df	Mean Squares	F-Ratio	p-Value

Figure 6: Grasshopper internal temperature minus ambient temperature as an effect of an interaction of time of day and light treatment, statistically significant (P<0.001).

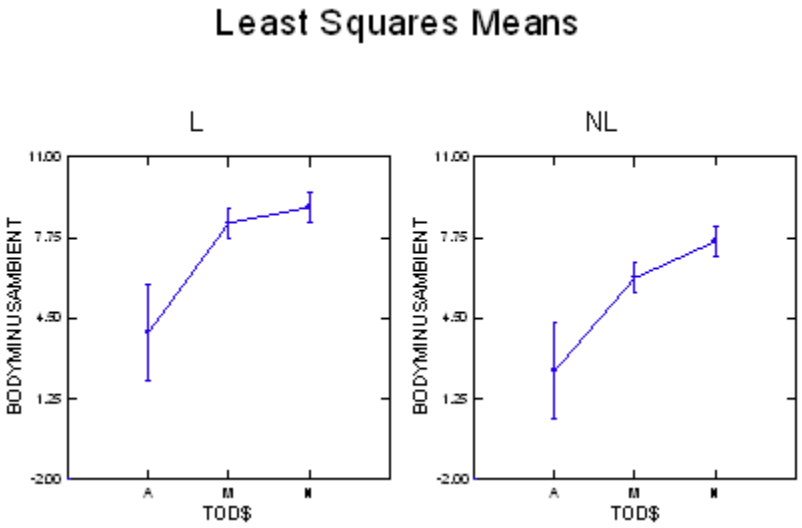

Table 3: Three way repeated measures ANOVA output that shows the significance of Light treatment, time of day, the interaction between time of day and infection status and the interaction between infection status, light treatment, and time of day on grasshopper internal temperature.

Between Subjects					
Source	SS	df	Mean Squares	F-Ratio	p-Value
HEALTH$	0.523	1	0.523	0.084	0.773
TREATMENT$	217.361	1	217.361	35.056	0.000
TOD$	544.701	2	272.351	43.925	0.000
HEALTH$*TREATMENT$	1.291	1	1.291	0.208	0.650
HEALTH$*TOD$	115.503	2	57.752	9.314	
TREATMENT$*TOD$	18.651	2	9.325	1.504	0.233
HEALTH$*TREATMENT$*TOD$	61.168	2	30.584	4.933	0.011
Error	297.616	48	6.200		

Acknowledgements:

I would like to thank first and foremost the Hank Family Endowment for the opportunity to do this research, as well as the administrators of UNDERC. I would like to extend a heartfelt thanks to my mentor, Erica Kistner, as well as my fellow students Diana Saintignon, Tara Hill, Kacey Gergely, and teaching assistant Rebecca Flynn for their invaluable assistance over the course of this project. Thank you all very much.

Works Cited

Springate, Simon, and Matthew B. Thomas. 2005. Thermal biology of the meadow grasshopper, Chorthippus parallelus, and the implications for resistance of disease. *Ecological Entomology.* 30 (6): 724-732.

Carruthers, Raymon, Timothey Larkin, and Heidi Firstencel. 1992. Influence of thermal ecology on the mycosis of a rangeland grasshopper. *Ecology* 7(1): 190-204.

Sanchez Pena, Sergio. 2005. *In vitro* production of hyphae of the grasshopper pathogen *Entomophaga grylli* (Zygomycota: Entomophthorales): Potential for production of conidia. *Florida Entomologist.* 88(3): 332-335.

IPCC 2007 Data, accessible online at: http://www.ipcc-data.org/ar4/gcm_data.html. Last modified 16 May 2011.

Carruthers, Raymond, et al. 1997 Seasonal patterns of cadaver persistence and sporulation by the fungal pathogen *Entomophaga grylli* (Fresenius) Batko (Entomophthorales: Entomophthroaceae) infecting *Camnula pellucia* (Scudder) (Orthoptera: Acrididae). *Memoirs of the Entomological Society of Canada.* 171: 355-374.

Erica Kistner, personal communication