E. grylli and *Camnula pellucida*

August 4

2012

Menzie Yezak

INTRODUCTION

This summer I examined the occurrence of *E. grylli* in *Camnula pellucida* grasshoppers across varying habitats within and near the Nation Bison Range. In addition I investigated whether occurrence of *E.grylli* was higher in areas where proportions of *Camnula* to other grasshopper species were high. I also ran an experiment looking at removal rates of *E.grylli* killed cadavers placed at each field site mimicking natural conditions.

The more we understand the disease the more accurate of a model we can create. The model will ultimately lead to predicting *Camnula* outbreaks. Farmers and ranchers could know a *Camnula* outbreak is going to happen before it invariably does and hurts their livelihood. This research is important because it has the potential to significantly reduce the amount of pesticides some farmers and ranchers use. E. grylli can regulate grasshopper pest populations and is a good natural alternative to pesticides.

Camnula pelucida is the common clear winged grasshopper. An insect is a pest. Not all grasshopper species are pests. Pest or not, grasshoppers are important because they are the chief consumer of grass in the National Bison Range as well grasslands in the surrounding area. Grasshoppers also have an important ecological role as prey by animals and humans.

This project's scientific merit comes about by observing disease infection in a completely natural non-lab habitat. Since there have been few studies on fungal diseases, this research has important implications for the disease ecology in conjunction with pest management.

BACKGROUND/LITERATURE REVIEW

Grasshoppers have important roles including fertilizing soil with their droppings and providing food for birds, lizards, and other animals (Belovsky and Slade 1993, 1995). They can be used to test chemicals and medicines for they have similar nerves to humans, and they are a food source for humans in some countries.

Entomophthora grylli Fres. is one of over 100 species of the genus that attack wide variety of insects including the grasshopper *Camnula pellucida* (MacLeod 1964). *E.grylli* has been identified in Africa, Asia, Australia, Europe, North America, and South America (Carruthers et al 1997). There is a relationship between the fungus and wet, warm, and humid conditions

(Pickford 1964). The pathogen develops within its host without confrontation with the organisms' immune system (Cory et al 2009). An *Entomophthora grylli* infected host dies approximately one week after initial exposure to the pathogen. Behavior changes happen 1-2 days before the grasshopper's death. Near death they are slothful, unable to hop away when touched (Pickford et al 1964). The cadavers are always found in a specific position. Before they die they slowly climb up foliage where they cling to the top, and perish (Pickford et al 1964). Under adequate conditions the fungus is capable of sporulating within hours of killing the host. *E.grylli* protoplasts begin to develop during the incubation period of the pathogen. When the host is near death the protoplasts begin to develop cell walls forming hyphal bodies which in turn give rise to either conidia or resting spores (Carruthers et al 1997). The weather after their death elects what happens next. If it is dry and hot they quickly desiccate, if rain falls the *Camnula* cadaver gets a waxy growth visible through its abdominal segments (Pickford 1964). If the weather is warm and moist, the cadavers, if not preyed upon right away, either produce resting spores or conidia. Conidia are the pathogen traveling like dust through air, known as aerial conidia. If the conditions are right, the pathogen can stay vital and infect others for up to 48 hours. Though, the aerial conidia generally do not stay vital for so long due to solar radiation, dry air, and high temperatures (Carruthers et al 1997). If conidia lands on a non-host insect it is capable of making secondary and tertiary aerial conidia (Carruthers et al 1997). Conidia infect a host by landing on them and produce a germ tube that penetrates their body (Carruthers et al 1997). Resting spores are the fungal pathogen enclosed in a thick wall waiting for the warm humid conditions necessary for it to germinate. Conidia and resting spores can be carried by other organisms without infecting them. Cadavers also spread the disease by being foraged by ants which then become carriers that leave dormant spores in soil that later germinate when conditions are suitable.

RATIONAL

Does density and abundance of *E.grylli* in *Camnula* grasshoppers change across different habitats?

We predict that habitats with higher proportions of *Camnula pellucida* will exhibit greater levels of E. grylli infection.

Does density and abundance of *E.grylli* increase as the proportion of Camnula to all non-*camnula* hoppers increases?

We predict that it does.

Will the scavenger rate of cadavers differ between three different habitats?

Scavenger rates should be higher in areas with higher ant densities. We also predict that e. grylli killed cadavers will persist

Answering these questions will provide knowledge of where a higher abundance of these pest hoppers are as well as where the disease is common. We studied how quick cadavers disappear after their tragic fungus induced death. The answers to these questions contribute to our knowledge of this pathogen's life history in different habitats. That information will serve another study focusing on disease transmission, which is something we know little about because few studies have focused on it. This research is a precursor to being able to predict major outbreaks of *Camnula pellucida* and the *E.grylli* disease. That knowledge can lead to a significant reduction of pesticide use on our local farms and ranches. Decreasing or eliminating pesticide use reduces chemical runoff into our water systems and chemicals in our food.

MATERIALS AND METHODS

During my time at UNDERC West I worked to execute two connected grasshopper projects:

1) **Determine host densities levels and natural levels of *E. grylli* in *C. pellucida* populations at varying locations on the National Bison Range over time**.

2) **To examine how quickly *E.grylli* infected grasshopper cadavers are removed by predators after death**

Data collection for the densities levels includes sampling two different ways once a week for four weeks. Our goal was to learn the proportion of *Camnula* to all other grasshoppers at our three sites. Our three sites were Gary Belovsky's yard, triangle at the National Bison Range, and Erica Kistner's home on Locust Lane. At each site an area of approximately 100m squared was sampled using a catch-effort technique (Belovsky and Slade 1995). Surveys were taken starting late-June and ending in mid-July. The time span was selected to match with known periods of *E. grylli* infections.

The ratio of *Camnula* to other grasshoppers was found by hunting grasshoppers with nets. The first 50 grasshoppers caught and killed in a jar of water were kept and analyzed. 50 grasshoppers were always caught with the exception of the first survey when grasshoppers had not hatched yet. The catching technique used involves holding the net, sweeping it across your body's width at ankle height, quickly turn the net's rod, then doing the same thing the other way. This sweep is done quickly over and over. This technique can be used while running. At the lab the grasshoppers were separated by species and

instar. All non-*camnula* hoppers were disposed of. The *Camnula* were dissected. We cut off their thorax, spread their insides on a slide, stained them with Lacto Fuschsin, then analyzed under a compound light microscope for presence or lack of *E.grylli.* This is in order to answer the question, "Is *E.grylli* occurrence heightened when the ratio of Camnula to all other grasshopper species increases?"

To examine how quickly *E.grylli* infected grasshopper cadavers are removed by scavengers after death we placed cadavers that were undoubtedly infected with *E.grylli* along a 15 meter transect. The cause of death for these hoppers is known because they were raised in lab conditions and exposed to the pathogen. 15 flags marked points along the transects. At each flag one cadaver was placed on wire in the upper level of vegetation and another on the ground right beneath. The transects were checked daily for a week or until all cadavers are gone. I witnessed cadavers being eaten by both black flies and ants.

At each of the three sites temperature and precipitation was recorded constantly by 502 USB data loggers. They take temperature and humidity levels once every hour. This data was not used due to lack of weather differences at my sites.

Data Analysis

For my cadaver experiment I used the Kaplan Meier Survivorship analysis test three times. Once for top vs bottom at the Charlo site (15-15), another for top vs bottom at the UNDERC West site (15-15), and again for Charlo's site vs the UNDERC West site (30-30).

For my surveys I used a two-way MANOVA to look at the effect the week had on disease and *Camnula* proportions and the effect site had on disease and *Camnula* proportions.

Predicted Results

I predict that occurrence of *E.grylli* will increase as the proportion of *Camnula* to other species of grasshoppers increases.

I predict that cadavers will disappear faster than my allowed week and that the cadavers set on the ground will disappear first because ants are well known foragers of grasshopper cadavers and it is easier for them to scavenge on the ground.

Results

The tests for the cadaver experiment were

1. Top vs Bottom, Charlo site p=0.661
2. Top vs Bottom, UNDERC West site p=0.355
3. Charlo site vs UNDERC West site p=0.103

The MANOVA ran with survey data said

1. Week had no significant effect on *camnula* proportions (F-Ratio=3.904 p=0.073 df=3)
2. Week had no significant effect on disease (F-Ratio=2.121 p=0.199 df=3)
3. Site effected *camnula* proportions (F-Ratio=22.544 p=0.002 df=2)
4. Site had no effect on disease (F-Ratio=2.353 p=0.176 df=2)

DISCUSSION

All of my cadaver data is insignificant. All of the cadavers disappeared very quickly no matter where they were placed. I did notice the adults, especially the ones suspended in the tops of vegetation lasted longer than the smaller sort. There was not enough data to produce a significant p-value.

At the beginning of the summer I believed that more *Camnula* would mean more E. grylli. According to my data that is wrong. I now know that *Camnula* are specific about where they live. For example I never found *Camnula* at the site on the National Bison Range which is a dry, crunchy, thistly, pasture-like plot. I did find tons of them at both other sites which are yards with green soft grass that is watered regularly. Their living location preference just so happens to match that of *E. grylli*. The yards offer the hot moist environment fungus needs to stay viable. I have confidence that that is why my data is insignificant. E.grylli and *Camnula* thrive in the same environment. My one significant result of site effecting *Camnula* proportions was only so because we found no *Camnula* at the National Bison Range.

Camnula pellucida environment preference brings them to the giant lush constantly irrigated fields of farmers. Farmers want them dead, but we don't want the chemicals of pesticides on food crops. An E. grylli model will be beneficial to everyone.

As for future studies, I do not think these experiments could be improved much. The data was clean and plentiful, it just didn't say what we thought it would, and that is alright.

ACKNOWLEDGEMENTS

I want to thank my mentor, Erica Kistner**.** She designed my project and taught me much more than I thought I would learn this summer. She has helped me finish work I'm excited about. I will have opportunities this fall to present research and I'm glad to share this. I would also like to thank the authority figures: Gary Belovsky, Page Klug, and Stef Strebel. I don't stay out of trouble and I know I caused them unnecessary stress, and for that I'm sorry. I have to thank farmer Dan for showing us everything Moiese, Montana has to offer. I'll forever remember him for his hospitality and words of wisdom.

WORKS CITED

Belovsky, G., & Slade, J. (1995). Dynamics of two montana grasshopper populations:relationships among weather, food, abundance and intraspecific competition. *Springer International Association for Ecology*, *101*(3), 383-396. Retrieved from http://www.jstor.org/stable/4220899

Carruthers, R.I., Ramos M.E., Larkin T.S., Hostetter D.L., Soper R.S. 1997. The entomophaga grylli batko species complex: it's biology, ecology, and use for biological control of pest grasshoppers. *Memoirs of the entomological society of Canada.* West Beltsville Maryland.

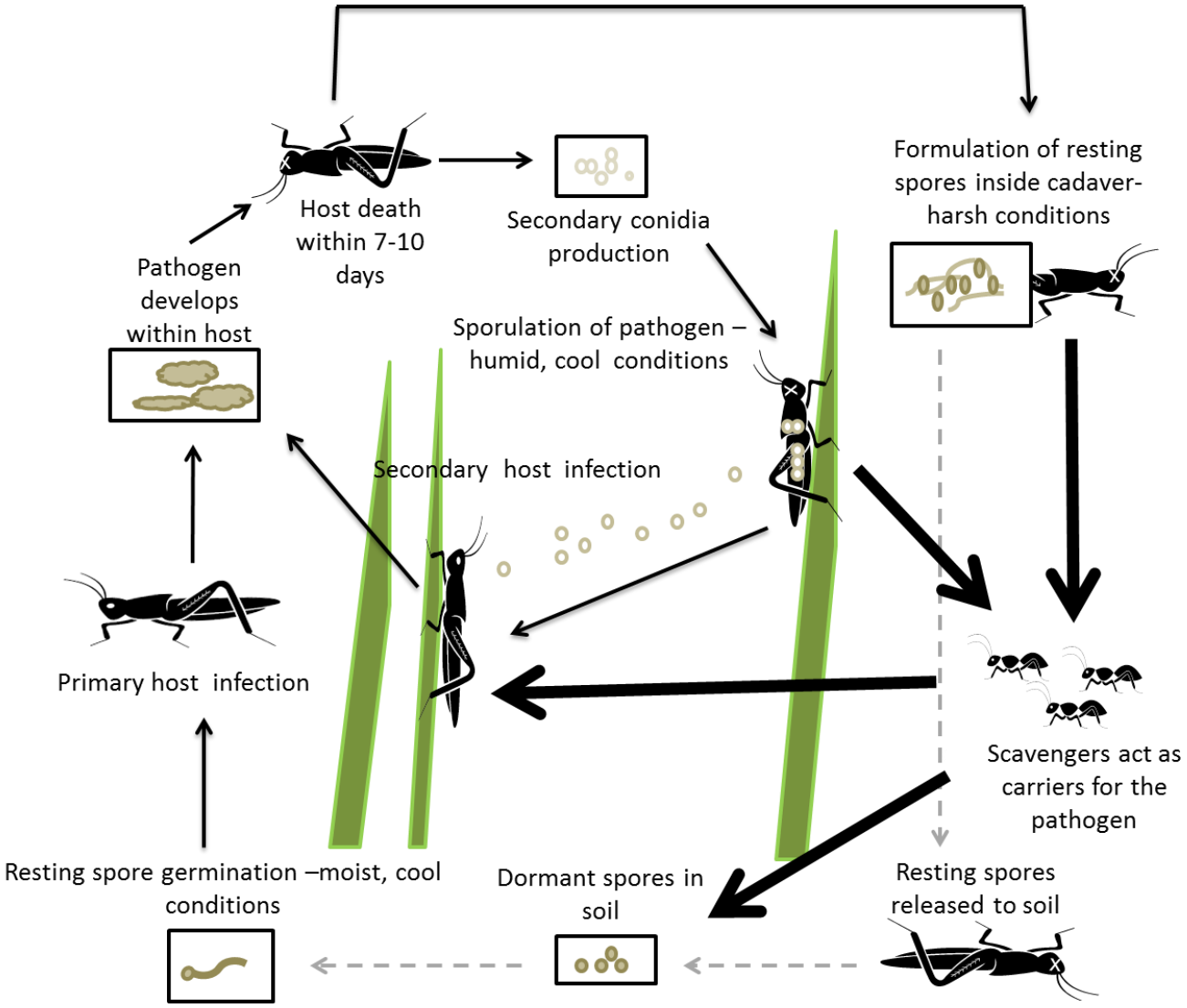
Cory J.S., Ericsson J.D. 2009. Fungal entomopathogens in a tritrophic context. *BioControl* 55: 75-88

Pickford R., Riegert P.W. 1964. The fungous disease caused by *entomophthora grylli* Fres., and its effects on grasshopper populations in Saskatchewan in 1963. *The Canadian Entomologist.* 96: 1158-1166.

Sawyer A.J., Ramos M.E., Poprawski T.J., Soper R.S., Carruthers R.I. 1997. Seasonal patterns of cadaver persistence and sporulation by the fungal pathogen *entomophaga grylli* batko infecting *camnula pellucida* (scudder). *Memoirs of the Entomological Society of Cananda.* 171: 355-374.

FIGURES

Figure 1



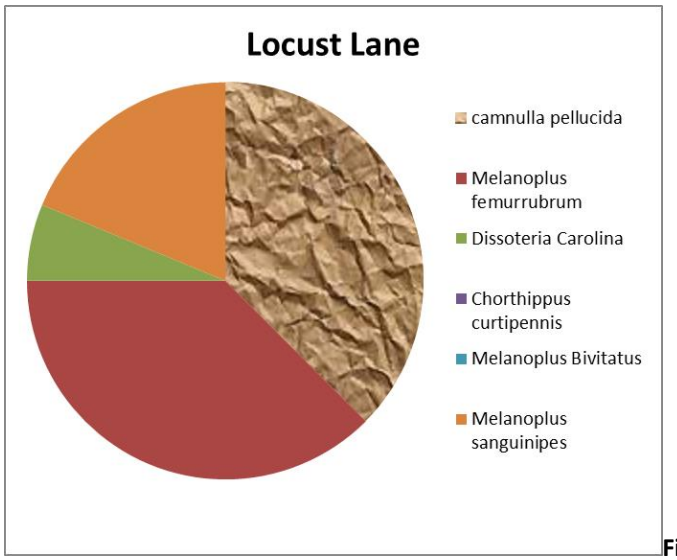
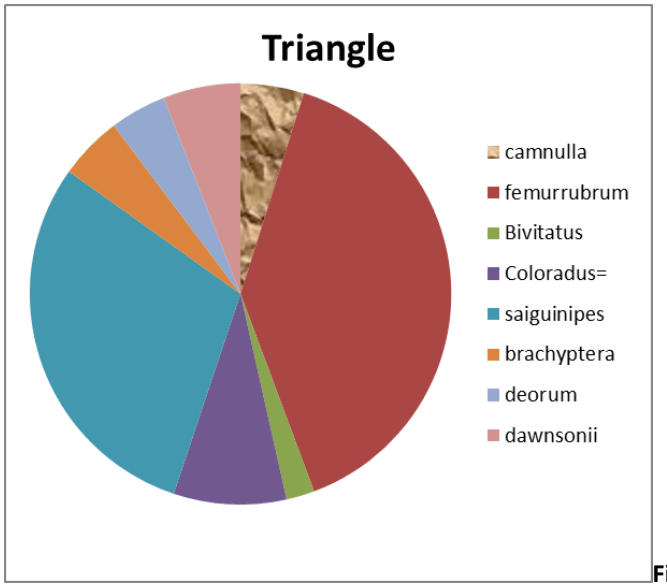


Figure 2



Figure

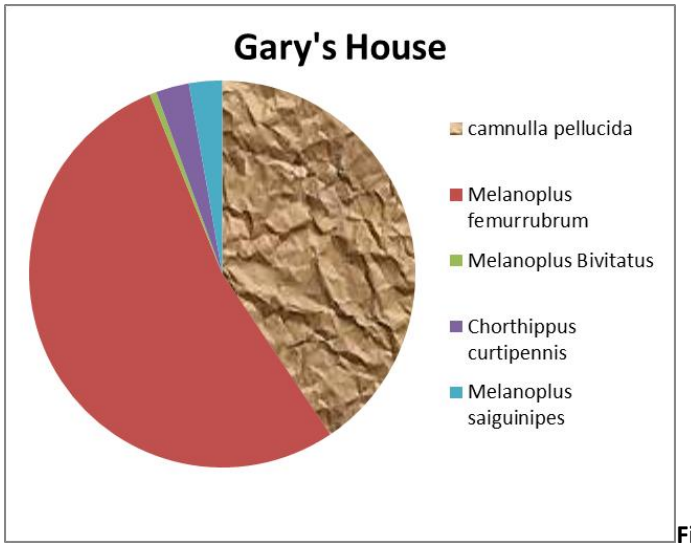


Figure 4

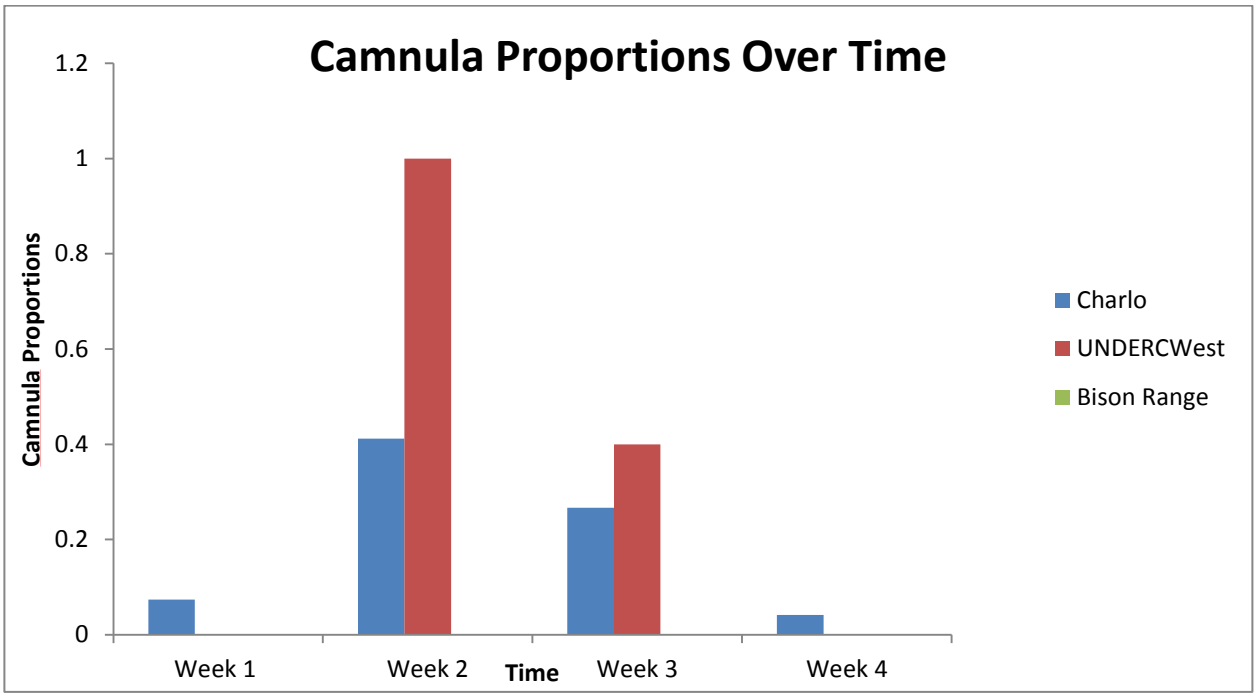


Figure 5

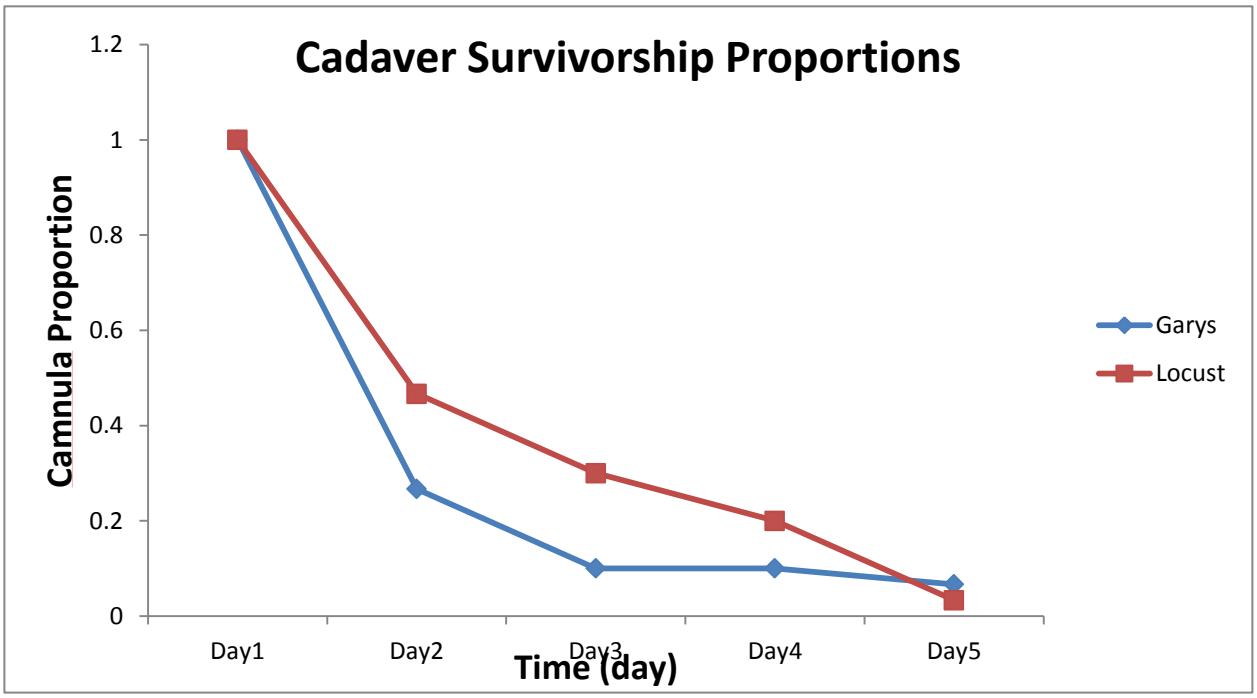


Figure 6