

Relationship between Predation by Lycosidae spp. and the Fungal Pathogen

*Entomophaga* spp. in *Melanoplus borealis*

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## **Abstract**

Predator-prey interactions can be mediated by disease. The presence of a predator may decrease grasshopper movement and influences its location on the vegetation. Fungus-infected grasshoppers have been shown to increase activity and decrease defensive responses. My goal was to investigate this predator fungal disease- prey interaction. Grasshoppers in different enclosures were introduced to lethal or non-lethal spiders at different five-day intervals. Regular counts reveal mortality rates due to spider predation (*Lycosidae* spp.) and/or fungi (*Entomophaga* spp.) infection. Scan samples provide behavioral observations that indicate if grasshopper (*Melanoplus borealis*) defensive behavior (high preference and reduced activity) influences the prevention of fungal infestation.

Predator- fungal disease- prey interaction is significant and variable over time because the incidence of fungal infections is dependent on climactic conditions. We learned that this study was conducted on non favorable climate conditions for the fungus to develop. Only five out of 168 deaths were directly caused by fungus. Grasshopper mortality between treatments with and without spiders was the same, indicating that spider predation is compensatory. This indicates that food resources rather than predation or disease regulated grasshopper densities on 2006. *M. borealis* did not exhibit any behavioral changes in activity or height preference among different spider treatments. Grasshoppers in all treatments exhibited a preference for high and low locations in the vegetation.

## **Introduction**

Grasshoppers and spiders are diverse and abundant groups in grasslands. Interactions between spiders and grasshoppers have the potential to both directly and indirectly affect other species within a grassland ecosystem, altering grassland structure and function at several levels. Their relationship influences trophic cascades, such that indirect lethal and non-lethal impacts of spider predation on grasshopper herbivores can affect plant biomass (Danner and Joern, 2003). Predator-mediated interactions can play important roles in shaping prey-species characteristics, including growth rate, body size, physiological constraints, mating behavior, habitat use, movement patterns, escape behavior, abundance and morphology (Pitt, 2000). Fungal disease interactions also play important roles in shaping prey-species interaction, changing behavior and decreasing fitness of the individual (Arthurs and Thomas, 2001)

Frauendorf (2005) investigated the relationship between *Melanoplus borealis* and wolf spiders (Lycosidae spp.) in a small field at Undergraduate Notre Dame Environmental Research Center (UNDERC). She observed that grasshopper survivorship was density dependent. Survivorship was highest in enclosures with spider predators, due to a high incidence of death by a fungal pathogen of grasshoppers in enclosures without spiders.

Most predators do not routinely consume prey in proportion to their abundance, but select from a restricted range of sizes and/or stages of prey. It has been tested that spider predation contributes to mortality in a stage-size

dependent fashion (Oedekoven and Joern, 1998). Consequently, the timing of predation may be important to grasshopper survivorship. Early instar individuals typically experience the greatest mortality from predators. Death from starvation may be more likely in young nymphs, while adults may be at greater risk from other factors such as avian predation (Oedekoven and Joern, 1998). Herbivorous insects' overall performance can be negatively affected when exposed to non-lethal predation risk (Danner and Joern, 2003).

Fungal infection can also cause behavioral changes in grasshoppers. At least 10 genera of fungi are known to be entomopathogens of grasshoppers and locusts (Hostetter and Dysart, 1996). Entomopathogenic fungi have the greatest probability of exploitation as microbial control agents for managing grasshopper populations. Found among the most promising candidates, *Entomophaga* spp. has host-specific strains and are purported to be non-hazardous to non-target organisms (Hostetter and Dysart, 1996). Fungi are contact pathogens, they do not infect when the insect eats them, as do other pathogens. Rather, fungal infection may occur during the feeding process when conidia contact the mouthparts (Hostetter and Streett, 1996). Prior to death, infected grasshoppers climb onto the foliage; consequently the fungus spores can disperse more efficiently (Hostetter and Dysart, 1996). This behavior identifies individuals that have died from fungal infection as opposed to other causes.

Arthurs and Thomas (2001) concluded that infected locusts (*Schistocerca gregaria*) by a fungal entomopathogen (*Metarhizium anisopliae* var. *acridum*) may be more susceptible to predation before death is caused directly by disease.

They found that increased locomotion and bodily movement and reduced feeding and mating behavior until death (death  $\bar{x} = 11$  d after infection). Shortly before death infected locusts were observed to have a reduced escape capability from spider predation (Arthurs and Thomas, 2001).

Grasshoppers infected with fungal pathogens raise their body temperature by selecting warmer microhabitats in response to infection. Thermoregulation reduces mortality but does not completely eliminate the fungus from the infected hosts (Ouedraogo et al., 2004). This therapeutic effect is costly because it requires a direct increase in the metabolism. Furthermore, the need to regulate body temperature may reduce feeding opportunities of infected hosts (Ouedraogo et al., 2004). Anorexia can be observed in diseased insects as it may facilitate the body's attempt to raise and maintain a febrile temperature (Ouedraogo et al., 2004).

This study investigates the mechanisms by which spiders mediate grasshopper infection by the fungal pathogen *Entomophaga* spp. Three hypotheses may explain this interaction. First, I hypothesized that spiders reduce incidence of fungal pathogens in grasshoppers by preferentially feeding on infected grasshoppers. Infected grasshoppers behave differently than non-infected grasshoppers, which could make them more vulnerable or more conspicuous to predators (Arthurs and Thomas, 2001). Consequently, spiders kill these individuals, so the number of deaths from fungus is lower in presence of spiders. My second hypothesis was that spiders reduce the incidence of fungal pathogens in grasshoppers by reducing grasshopper activity. Healthy

grasshoppers are less active in the presence of predators like spiders (Danner and Joern, 2003; Pitt, 1999). Active prey is generally more vulnerable to predators because they are more easily spotted. If grasshoppers are less active, they may be less likely to encounter fungus and become infected. Third, I hypothesized that spiders reduce the incidence of fungal pathogens in grasshoppers by affecting where grasshoppers are located in the vegetation. In a previous studies, grasshoppers (*Melanoplus femurrubrum*) exposed to ground predators (*Eumeces septentrionalis*) spent more time higher in vegetation than those enclosures without ground predators (Pitt, 1999). By spending less time on the ground, grasshoppers may be less likely to encounter the fungus and become infected. In addition, conditions may be less humid in high vegetation, so that fungus may be less effective on grasshoppers that spend more time high in vegetation (Carruthers et al. 1992).

## **Methods**

### *Study Site and Organisms*

The study site was a grassland area, "Tick Site," located at UNDERC property in Gogebic Co., Michigan. Vegetation includes daisy (*Chrysanthemum* spp.), Kentucky bluegrass (*Poa* spp.), thistle (*Cirsium* spp.), cinquefoil (*Potentilla* spp.), clover (*Trifolium* spp.), goldenrod (*Solidago* spp.) and other unidentified grasses and forbs. I selected wolf spiders for this research because they are important predators of grasshoppers and abundant at my site. The grasshopper species *Melanoplus borealis* was selected for this study because it is the dominant

grasshopper species at the site and it has a ca. 29-day life span which is appropriate for the time this research is conducted (Frauendorf, 2005).

#### *Spider Traps, Grasshopper Collection*

Wolf spiders (Lycosidae spp.) were collected with pitfall traps and placed in cages. Third instar grasshopper nymphs (*Melanoplus borealis*) were collected with insect nets and sorted into enclosures (cages) with a 50/50 sex ratio. The cages (0.36 m<sup>2</sup>) were composed of aluminum screen and aluminum flashing dug into the ground. Stakes held the cage upright and binder clips held the upper opening closed. The cages were placed over natural vegetation. For a complete description of the enclosure, see Belovsky and Slade (1993).

#### *Treatments*

I established 28 cages with six grasshoppers per cage. I used seven treatments, each with four replicates: (1) zero spiders; (2) two spiders added day one; (3) two spiders (mouthparts glued) added day one; (4) two spiders added day five; (5) two spiders (mouthparts glued) added day five; (6) two spiders added day ten; and (7) two spiders (mouthparts glued) added day ten. The spiders were rendered non-lethal by applying beeswax to the chelicerae (Danner and Joern, 2003). This treatment removed the threat of direct predation while simultaneously retaining any indirect effects resulting from altered prey behavior in response to spider presence (Danner and Joern, 2003). This treatment was designed to test whether spiders are preferentially feeding on infected grasshoppers.

Each cage was surveyed two times per week to monitor grasshopper survival and to look for grasshoppers that die from fungal infection. I also recorded the death and cause of death of each grasshopper or spider individual, temperature, weather, and any special conditions. Weather data were collected from UNDERC weather station online data.

### *Behavioral Observations*

I conducted scan samples in each cage to determine the proportion of grasshoppers active and the height of grasshoppers on vegetation or the screen during five random days. I recorded grasshopper activity every 30 sec. for 3 min. Every cage was observed at least twice on every scan sample day (12 observations per scan sample day) .Two minutes before recording were necessary to spot grasshoppers in the cages. Recorded grasshopper activity included the proportion feeding on the vegetation, moving on the vegetation, stationary on the vegetation, moving on the screen and stationary on the screen. This data allowed me to test for difference in grasshopper behavior and height in vegetation in response to spider presence.

### *Data Analysis*

The data were analyzed with SYSTAT 11.0 (Systat Software Inc., Point Richmond, CA). A Two-way ANOVA tested the relationship between the proportion of surviving grasshoppers (dependent variable) and both treatment and time (independent variables). Behavioral data were analyzed using individual one-way ANOVA to test the proportion of active grasshoppers (dependent variable) among treatments (independent variable). Finally, I ran a two-way

ANOVA to analyze grasshopper height preference. The count of surviving grasshoppers is the dependent variable, height categories (0-10, 10-20, 20-30, >30 cm) and spider treatment were the independent variables. An additional T-test tested significance of relative humidity and temperature difference between 2005 and 2006.

## **Results**

### *Survival*

Grasshopper survival decreased over time (Figure 1). The proportion of grasshoppers surviving did not vary significantly with treatment ( $df = 6$ ,  $F = 0.55$ ,  $P = 0.76$ ), but did vary significantly over time ( $df = 1$ ,  $F = 607.70$ ,  $P < 0.01$ ; Figure 1). The interaction term was not significant ( $df = 6$ ,  $F = 0.39$ ,  $P = 0.88$ ), indicating that the decrease in survival over time did not vary significantly by treatment.

### *Behavior*

Spider treatment did not have a significant effect on behavior, as grasshoppers did not change their behavior with the presence of spider predators (Table 1). Grasshoppers subjected to different treatments were equally active. Data show that grasshoppers preferred to be 30 cm or higher on the screen or on the vegetation (Figure 2); the ground (0-10cm) was the next preferred height followed by 20-30cm and the last preferred height was 20-30cm high ( $df=3$ ,  $F=24.52$ ,  $p<0.01$ ). However, there was no significant difference in height of grasshoppers among spider treatments ( $df=2$ ,  $F=1.316$ ,  $p=0.272$ ; Table 2).

I found only five grasshopper deaths from fungus during this research project. Three of these deaths were observed on cages near to each other. Two out of the five cages had non-glued spiders and three were the glued spider treatment (Figure 4). These spiders were added five or 10 days after cages were stocked (Figure 3). UNDERC weather station data indicates an average air temperature of 17.33°C (0.36 °C - 33.33 °C) and a relative humidity of 71.68% (24.91% - 99%) for the last half of May and the entire June of 2005. The temperature average for 2006 was 16.82 °C (-0.13 °C - 32.3 °C) and relative humidity averaged 65.88% (17.19%-99%). Temperature (df =1103; T=2.397; P=0.017) and relative humidity (df=1103; T=9.146; P<0.01) were significant between 2005 and 2006 (Figure 4 and 5).

## **Discussion**

My data suggest that predation is compensatory. Grasshoppers died with time independently of the treatment they received. Compensatory mortality refers to a process where additional risk of death from one factor is balanced by subsequent decreased mortality from other causes in such way that the overall mortality of the population does not change (Oedekoven and Joern, 2000). This means that spiders are removing individuals (grasshoppers) that may have died from other causes such as old age or disease. This suggests that *M. borealis* populations at my site are limited by available vegetation (bottom-up processes) as opposed to spider predation (top-down processes). In a similar study with grasshoppers (*Melanoplus sanguinipes*) and wolf spiders (*Tarentula kochi*),

Schmitz (1993) observed the same bottom-up control of food chain structure. In his study, grasshopper survival related to amount and quality of the vegetation and not to presence of predators (Schmitz,1993). Oedekoven and Joern (2000) obtained the same results when ambient food levels were compared with and without spider predation, spider predation was compensatory. Differences in individual grasshopper quality may influence survival, e.g., grasshopper quality is impaired by fungal infection. Arthurs and Thomas (2001) observed high rates of predation on locust (*Schistocerca gregaria*) infected by the mycoinsecticide (*Metarhizium anisopliae* var. *acridum*) before they were killed directly by the pathogen.

Grasshopper mortality caused by the fungus this year is low compared with last year's data (Fraudendorf, 2005). Fungus is more likely to develop in warm and humid years. This 2006 climate was colder and less humid than 2005, significantly limiting fungus development. Clearly, the variation in climate varies predator-prey-disease interactions.

In this study, grasshoppers subjected to different spider treatments were equally active on the screen or vegetation, which could indicate that grasshoppers were not aware of the presence of spiders. Grasshoppers can respond to spiders and can sense the presence of predators (Danner and Joern, 2003; Pitt, 1999). Grasshopper-to-spider density could have been too low to observe significant differences in activity patterns. In addition, spiders could be spending most of their time underground and hunting at night. Grasshopper-to-spider density on other studies that have found behavioral changes have widely

varied from nymph to adults population, 3-5 adult grasshoppers/m<sup>2</sup>, 30 nymphs/m<sup>2</sup> to one spider, opposed to my 16 nymphs/m<sup>2</sup> to two spiders (Danner and Joern, 2003; Oedekoven and Joern, 2000; Oedekoven and Joern, 1998). Perhaps the time of day when the observations were made is an important factor on grasshopper behavior. However, for this study, I did not take time into account.

*Melanplus borealis* could not have been the most suitable specimen for our study because of its short life span and because of this time limit they feed and stay active independently of spider presence to ensure reproductive success. Prey responses to predators can result in reduced growth and developmental rates, increased mortality from starvation or decreased fecundity, which this species may not afford (Danner and Joern, 2003).

Spiders are generalist predators, preferring to feed on grasshopper nymph stages when grasshoppers are more vulnerable (Oekoven and Joern, 1998). Because this species (*Melanplus borealis*) is short-lived, it grows fast, reducing grasshopper early instars in a short amount of time. Consequently, spiders may prefer to feed on other vulnerable insects on the cage rather than adult grasshoppers unless adult grasshoppers are unfit, which seems to be the case. I found adult grasshoppers on the cages since the first count that was made (three days after stocking the cages with grasshoppers). By the third count, ten days after stocking, the majority of individuals were adults, which indicates that for my first behavior observations, seven days after stocking, most of the grasshoppers were adults.

Data do not support my last two hypotheses because height and behavior did not significantly change between different treatments and fungus deaths were too few to develop a trend. To further support the mentioned ideas, height preference, another type of behavior, was not significant among grasshopper treatments. There was a preference of the higher and lower height on all the cages for thermoregulation. However, this height observation could have been impaired by the observer's ability to spot grasshoppers on the cages. A grasshopper located high on the gray screen or on the brown ground offers contrast between grasshopper green and pinkish colors and background colors which makes them easier to spot. Variable host plant quality may also influence predator-prey interactions by changing the spatial and temporal distribution of the prey (Oedekoven and Joern, 2000). Forbs are the tallest and preferred food for many grasshoppers including *Melanoplus borealis* (Schmitz, 1993). The time of the day when I conducted behavioral data may have influenced their position on the vegetation.

The first hypothesis (that spiders reduce incidence of fungal pathogens in grasshoppers by preferentially feeding on infected grasshoppers) was the best supported hypothesis given that there was no difference in behavior, unless that, too, changes over time. Frauendorf's (2005) mortality data seemed to follow this possible explanation because grasshoppers that had no spider predation died at a faster rate than those ones under predation pressure. Fungal infection was not controlled in cages that did not have spiders. However, incidence of death from fungus was not sufficient to support this hypothesis. No death from fungus was

reported in the cages without spiders, two out of five fungi deaths were on cages with a non-glued spider. But all fungal deaths were recorded in cages that reserved spider treatment when grasshoppers were adults already. For this reason, I believe that spiders, instead of eating grasshoppers, preferred other insect species available in the cage. This hypothesis should be further investigated.

Grasshopper-fungus-spider interactions are very interesting and apparently variable over time. For future projects, time should be taken into account, and climate should be meticulously monitored. The use of another less short-lived grasshopper species may also demonstrate a better relationship between instars and predation.

Table 1: One-way ANOVA results testing behavior-treatment relationship.

Different active behaviors (dependent variables) show a non-significant relationship with treatment. Df (for all dependant variables) = 2

Dependent variable	F	P value
Feeding in the vegetation	0.552	0.581
Moving on vegetation	0.841	0.440
Stationary on vegetation	0.067	0.935
Moving on the screen	0.905	0.415
Stationary on the screen	1.308	0.284

Table 2: Grasshopper height preference among treatments. Grasshopper height preference is independent of treatment. Df (for all heights) = 2

Height	F	P value
0-10cm	0.184	0.833
10-20cm	1.827	0.177
20-30cm	0.293	0.748
>30cm	0.489	0.617

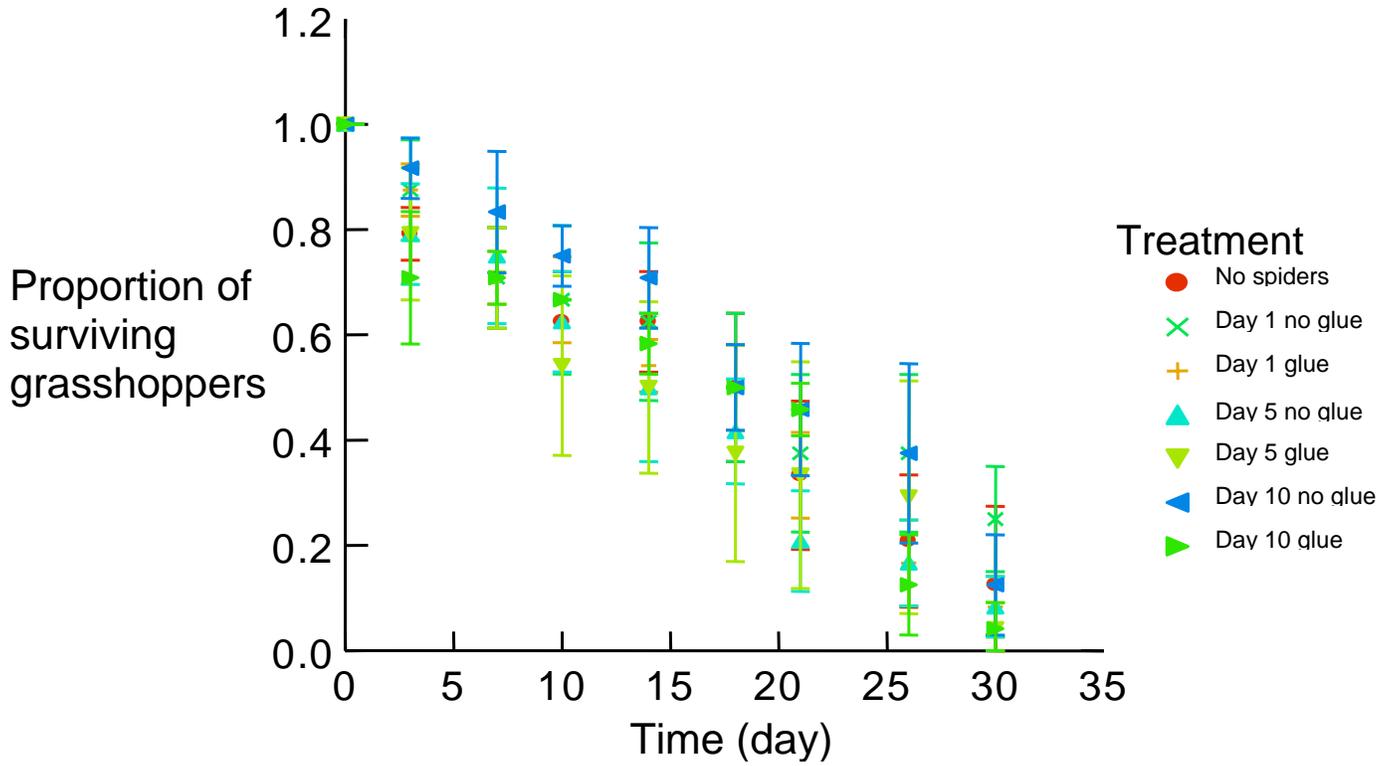


Figure 1: Proportion of grasshopper survivors (with SD error bars) vs. time, separated by treatment type. Survivorship significantly decreased with time.

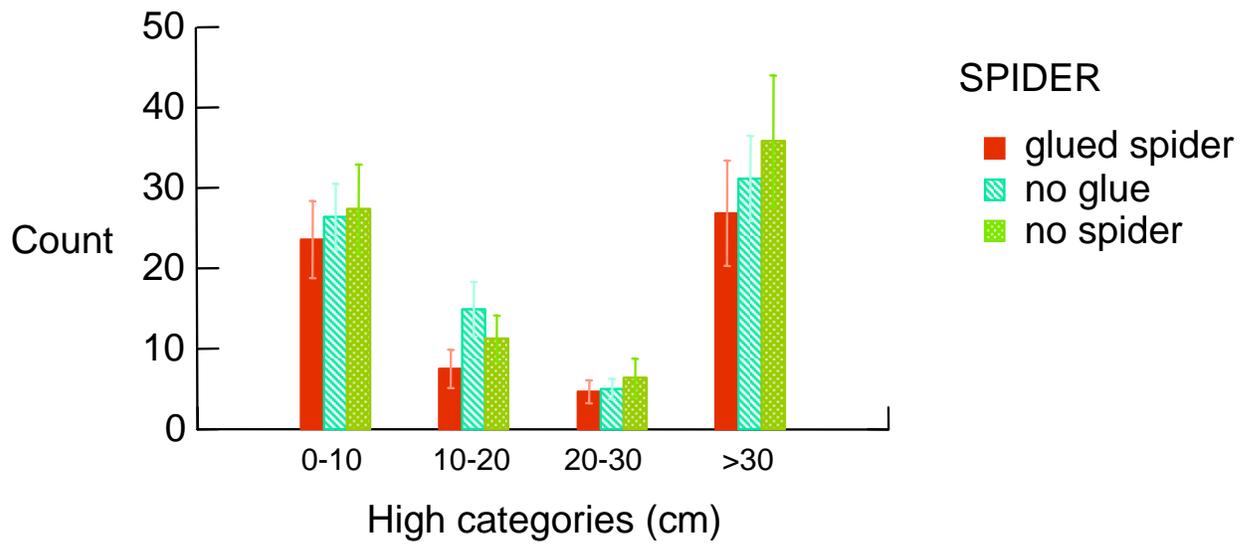


Figure 2: Grasshopper height preference. Grasshoppers preferred to be higher in the cage independent of the spider treatment. (SE error bars)

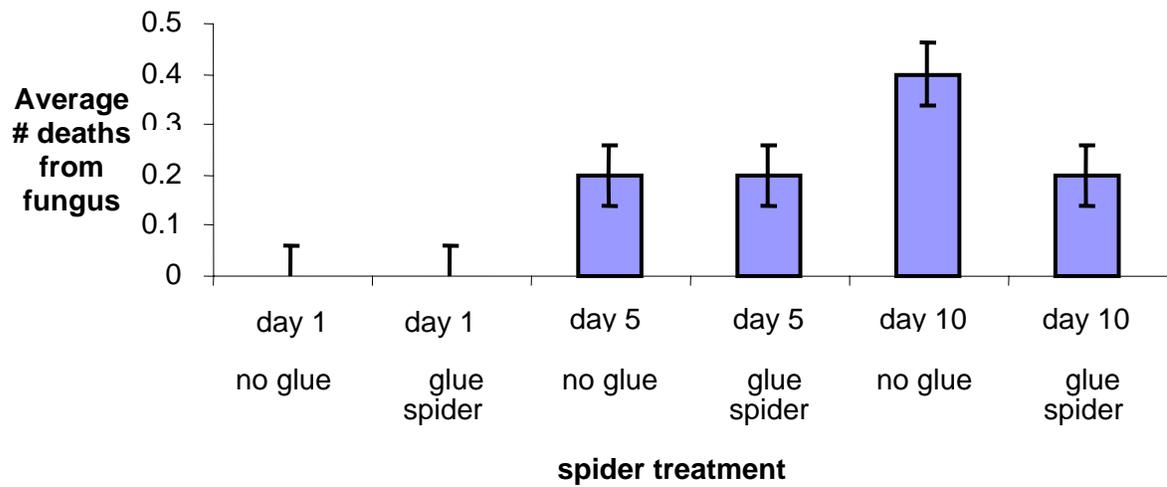


Figure 3: Average number of deaths in this study vs. spider treatment.

Grasshopper mortality is greater on no glued spiders' added day 10. (SE error bars)

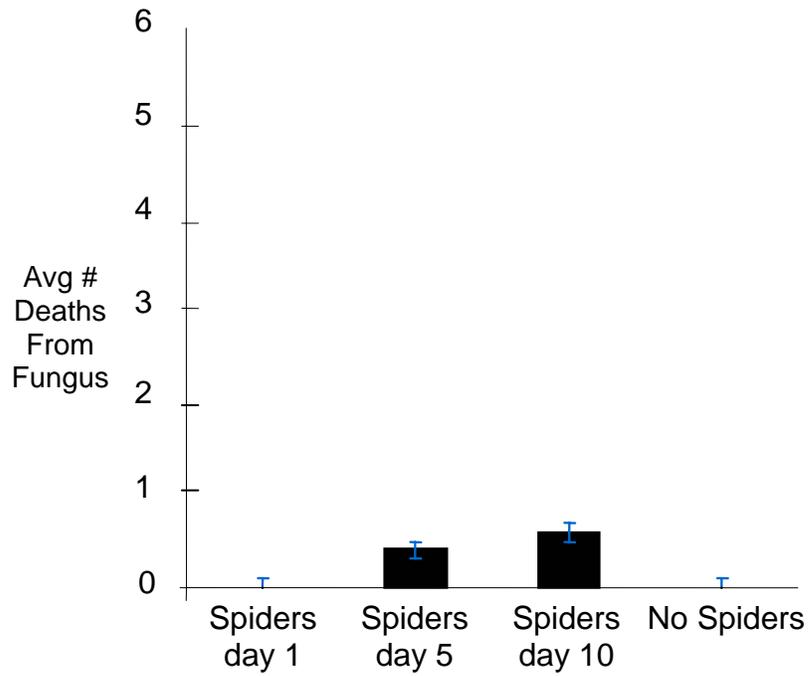


Figure 4: Average number of deaths in this study vs. time spider treatment was added. Grasshopper only died from fungus when spiders were added on day 5 and 10. (SE error bars)

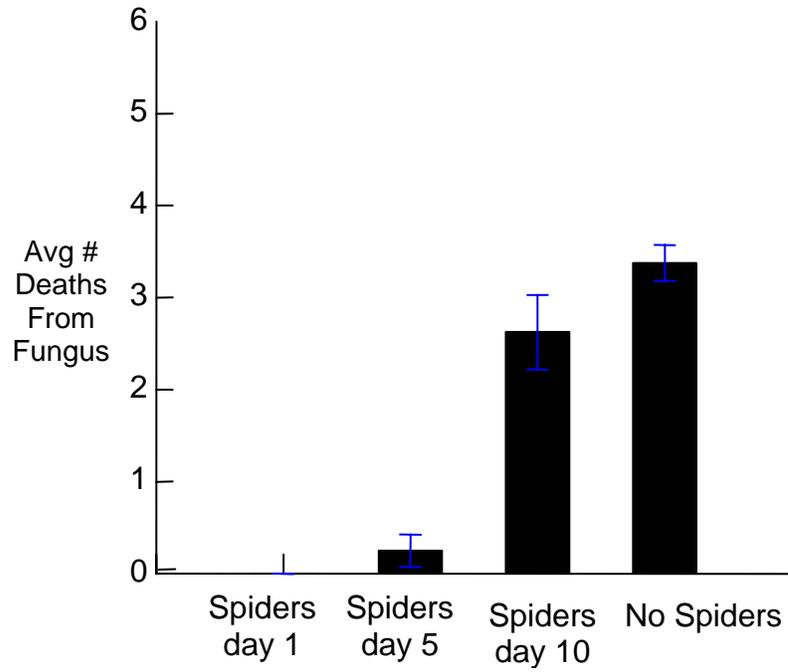


Figure 5: Average number of deaths 2005 vs. time spider treatment was added.

Fungus deaths increase without spider presence (Frauendorf, 2005).

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