

**Effects of *Entomophaga grylli* fungal pathogen on survivorship of *Camnula pellucida*
grasshopper populations along a moisture gradient**

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Abstract

Of interest to both the entomological field in disease dynamics studies and to the commercial agriculture industry in search of an effective biocide that will specifically eliminate target grasshopper pests, the *Entomophaga grylli* fungus is often a fatal plague to populations of *Camnula pellucida* clear-winged grasshoppers. While the life cycles of both organisms are understood, studies of the complex disease interactions between the two are limited. The objective of this study was to analyze survivorship of both healthy and *E. grylli*-infected *C. pellucida* populations over time and in response to variable water treatments, as propagation of the fungus is dependent upon moisture in the surrounding environment. Twenty-four nylon mesh cages (0.1m, basal area) were utilized in experimentation, twelve stocked with ten *E. grylli*-infected *C. pellucida* individuals each and twelve stocked with ten healthy *C. pellucida* individuals each. With four water treatment replicates per set of diseased or healthy cages, the controls, “low water” treatments (50 mL every other day), and “high water” treatments (100 mL every other day) were monitored by a soil moisture reader. After ten or nine days of counting healthy or diseased *C. pellucida* individuals, respectively, and characterizing *E. grylli* mortality of the cadavers, no significant differences in survivorship were exhibited on the basis of health or water treatment ($p > 0.15$) and the moisture gradient did not correlate with survivorship or disease mortality ($p > 0.15$). However, a significant interaction ($p = 0.043$) between survivorship and time in control cages suggests that earlier-dying *E. grylli*-infected individuals in diseased cages facilitated higher survivorship later in the diseased cages relative to controls by elimination of food resource competition, but near-total mortality ultimately occurred in both. Furthermore, mortality due to *E. grylli* among cadavers was significantly higher in diseased than in healthy cages ($p < 0.001$). Additional studies utilizing disease-free control populations and an experimental moisture gradient informed by current weather conditions could further contribute to knowledge about this important disease dynamic.

Key-words: biocide, *Entomophaga grylli*, *Camnula pellucida*

Introduction

The fungal pathogen, *Entomophaga grylli*, is both a natural cause of epizootics among North American grasshoppers and a potential agricultural biocide. Each year, grasshoppers destroy several billion dollars worth of crops worldwide (Bidochka & Khachatourians, 1990). Members of the Acrididae family, including grasshoppers and locusts, are among the most voracious pests known, with the fifth instars and adults capable of eating their own body weight in vegetation (2-3 g) daily (Alomenu, 1985). However, the residues of commonly employed insecticides, such as carbaryl bran, are frequently detrimental to other insects, small mammals, and humans who consume affected plants or the animals that feed on these plants (Cameron, 1963). Indiscriminate use of insecticides can also promote resistance among the target or another pest population, often rendering insecticide efficacy short-lived and self-defeating (Carruthers et al., 1988). Specific biocides should be developed commercially to target particular pests and minimize broader consequences for the surrounding ecosystem. Available evidence indicates such “microbial insecticides” are often harmless to humans and higher animals, by virtue of their restricted host ranges (Cameron, 1963).

Outbreaks of *Camnula pellucida*, the clear-winged grasshopper, can decimate rangeland fields of small grains and grasses, destroying areas as large as 2,000 square miles (Pickford, 1963). This species is particularly susceptible to infection by the *E. grylli* fungal pathogen, and suffers high rates of mortality (Pickford & Riegert, 1964). Resting spores of *E. grylli* germinate each spring, producing specialized asexual spores known as conidia which are ejected from the soil into the plant canopy where they infect grasshopper hosts by contact (Carruthers et al., 1988). Germination tubes facilitate fungal mycosis by penetrating the cuticle of the body wall through secretion of extracellular hydrolytic enzymes, including proteases and chitinases (Bidochka & Khachatourians, 1990). Inside the grasshopper host, *E. grylli* multiplies rapidly and digests body tissues to acquire

nutrients. Once infected, the grasshopper demonstrates sluggish movements and an inability to move or jump away when touched. As the disease progresses, the grasshopper slowly climbs local foliage in direct sunlight to increase body temperature by up to 10-15 degrees C above air temperature, which can create an intra-host environment above the thermal limit of *E. grylli* (Kemp, 1986). Although healthy and diseased hosts bask with equal frequency, this behavior may be an evolutionary adaptation to reduce *E. grylli* mortality among populations (Kemp, 1986). In the final stages of infection, the grasshopper displays symptoms of “Summit disease” in which it clings to the foliage with its front legs in a “death grip,” while slowly flexing its hind legs (Carruthers et al., 1997).

The interval from infection to death is approximately 7-10 days, but mortality can ensue sooner (Carruthers et al., 1988). Cadavers found still clinging to foliage can facilitate horizontal infection of neighboring grasshoppers by conidia (Carruthers et al., 1997). Under appropriate ambient conditions, including dew formation and reduced ultraviolet radiation in the morning, conidia can effectively transmit disease by contact or through the air if germination occurs within twenty-four hours of their overnight production (Carruthers et al., 1997). In addition to production of conidia, *Entomophaga grylli* pathotype I, the disease variant which infects *Camnula* and other Oedipodinae, the band-winged grasshoppers, is characterized by the production of resting spores, which are released into the soil for germination in subsequent years, under favorable environmental conditions (Ramoska & Soper 1988).

Because grasshopper outbreaks typically correspond with hot, dry conditions unfavorable for fungal development, the potential use of *E. grylli* as a biocide, which is not yet commercially available, has appeared limited thus far. Historically, industrial fungal biocides produced *in vitro* in the laboratory have been in the form of spore powder or wettable matter, with the limitation that

even short exposure to ultraviolet radiation decreases viability of *E. grylli* conidia (Bidochka & Khachatourians, 1990). In addition, proper timing of rainfall is essential to the spread of *E. grylli* among *C. pellucida*. In the absence of rain, cadavers dry quickly and sporulation of the fungus does not occur (Pickford & Reigert, 1964). Studies have indicated that heavy dews or even ranges of humidity between 60 and 80 percent are often too low to permit fungal sporulation (Cameron, 1963).

If rain falls shortly after symptoms of Summit disease are exhibited, an external waxy growth can sometimes be observed between the abdominal segments, and later on the intersegmental regions and sutures of the thorax and head of the grasshopper (Pickford & Riegert, 1964). These growths consist of numerous conidia-releasing tubes or conidiophores, which aid in transmission of the fungal pathogen by contact to uninfected grasshopper hosts. However, in dry climates such as western Montana, precipitation is often insufficient to facilitate conidia release, and diseased cadavers frequently produce resting spores, which ensure survival over periods of adverse environmental conditions and transmit the infection to nearby grasshoppers (Pickford & Riegert, 1964).

Because causal factors of natural, spasmodic fungal epizootics of North American grasshoppers are not well understood and there is a relative lack of formulation and large scale field trial with fungal biocides, the objective of this study was to examine the disease dynamics of *E. grylli* among populations of *C. pellucida*, common to the western Montana Palouse prairie. In addition, natural outbreaks of *E. grylli* often coincide with humid conditions, suggesting that weather plays an important role in the development and spread of the disease. In this study, it was hypothesized that: (1) cages stocked with *E. grylli*-infected *C. pellucida* individuals exhibit lower survivorship over time than cages stocked with healthy *C. pellucida*; and, (2) an increasing moisture gradient

corresponds to decreasing *C. pellucida* survivorship and increasing mortality due to *E. grylli*, as fungal growth is facilitated by ambient moisture.

Methods

Twenty-four nylon mesh cages (0.1 m²; basal area) were attached to a base of 7 cm wide aluminum edging, buried underground and secured with stakes (Belovsky and Slade, 1995). Each enclosure was placed over natural vegetation, including *Achillea millefolium*, *Bromus* spp., *Cirsium arvense*, *Lepidium* spp., *Linaria dalmatica*, *Pascopyrum smithii*, *Poa pratensis*, and other dominant grass and forb species. Experimental cages were placed in a grid separated by 2.5 m between each cage to prevent cross-contamination between healthy and diseased cages, and the cages were assigned treatments randomly (Belovsky and Slade 1995). Each cage was stocked with ten *C. pellucida* individuals collected with nets via 30 m x 30 m plot sweeps in Palouse prairie near Charlo, Montana. *Camnula pellucida* individuals were initially selected on the basis of developmental age, using only second, third, and fourth instars for the first and second stockings of cages (during the first three weeks of experimentation). Fifth instars and adults were used for the third stocking of cages (during the final ten days of experimentation, analyzed herein). Laboratory terrariums and jars with dandelions were utilized during transport, but cage stocking occurred within 24 hours after capture to minimize stress and mortality induced by handling.

Twelve “diseased” cages were stocked with *Entomophaga grylli*-infected *C. pellucida* individuals. Diseased *Camnula pellucida* individuals observed to be in a “death grip” body position on vegetation in their natural habitats were collected and stored in a humid environment, created by a Ziploc® storage container on a wet paper towel. Within twelve hours of *E. grylli* conidial sporulation on the diseased individuals, fungal residue was collected on a 1 µL sterile loop, dipped in water to

facilitate infection, and smeared on the pronotum of healthy individuals. Within twenty-four hours, these infected individuals were stocked in “disease” cages.

During the second and third stocking of cages, the number of healthy or diseased individuals added to healthy or diseased cages corresponded to ten less the number of surviving individuals one or three weeks after the initial stocking, respectively. Because of a natural *E. grylli* outbreak on the National Bison Range during a precipitation-heavy June, healthy cages not treated with *E. grylli* also required re-stocking at these time points. Healthy and diseased cages were re-stocked on subsequent days to minimize cross-contamination of the fungal pathogen.

After the third stocking with healthy or diseased *C. pellucida* individuals, cages were checked every morning for ten or nine days, respectively, beginning at 9-10 am to ensure a sufficiently elevated ambient temperature for grasshopper activity. In addition, in an attempt to eradicate conidia from prior stockings of *E. grylli*-infected *C. pellucida* individuals, JohnsonDiversey™ Virex TB Germicidal Cleaner (diluted 1:3 with water) was applied using paper towels on the nylon mesh cages. Ants, spiders, wasps, earwigs, and other insects were killed during cage checks to minimize experimental interference. Surviving grasshoppers were characterized as early or late instars or adults, and non-control cages were watered according to a “low” (50 mL) or “high” (100 mL) treatment every other day. Moisture treatments were applied using a 7.6-L *Gilmour*® portable spray jug at maximum pressure to ensure water pressure consistency across treatments. On each day prior to watering, a *HoldAll*® soil moisture reader (scaled 1-10) was used to measure the soil moisture of one cage per water treatment for both healthy and diseased cages (n = 6), selected by a random number generator.

Grasshopper behaviors such as sluggish movement and symptoms of Summit disease were noted, as applicable, to monitor fungal infection in the cages. Cadavers were removed from the

twelve healthy cages to minimize the natural spread of *E. grylli*. These cadavers from the healthy cages were collected in vials by cage number. Cadavers collected from the ground and not in the “death grip” position (diagnostic of *E. grylli* infection) were transferred to and stored overnight in a humid Ziploc® storage container to test for sporulation of *E. grylli*.

Mean percent survivorship was calculated for each treatment. Four replicates within treatments helped minimize differences among cages. Arcsine square-root transformed proportion of daily survivorship was analyzed using two-way and repeated measures ANOVA, linear regression, and non-linear regression. Chi-squared tests were used to analyze mortality due to *E. grylli*. A one-way ANOVA was used to test for significant differences between relative soil moisture across water treatments. Linear regression was used to correlate mean relative soil moisture with survivorship and mortality. All statistical analyses were conducted using SYSTAT® 11, and graphs were generated using both SYSTAT® 11 and Microsoft® Excel.

Results

Survivorship

C. pellucida counts on each day of the nine or ten-day period of analysis reflected declining percent survivorship over time, with 100% survivorship reflected in cages fully stocked with ten individuals on Day 1 (*Figs. 1-2*). Diseased cages monitored for nine days demonstrated converging survivorship regardless of water treatment on Day 9, while healthy cages monitored for ten days demonstrated a greater spread of survivorship by water treatment on Day 10.

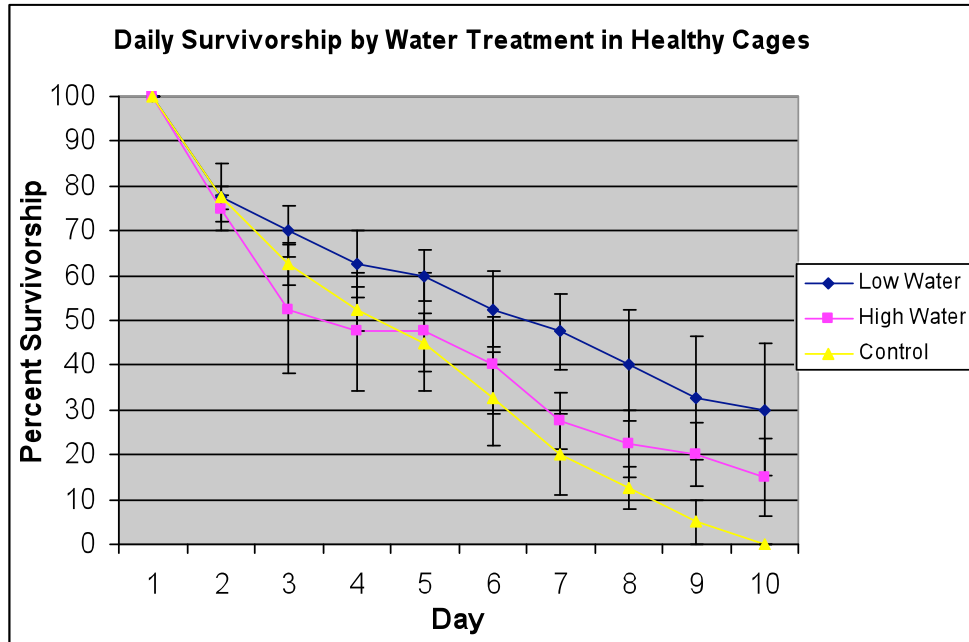


Fig. 1

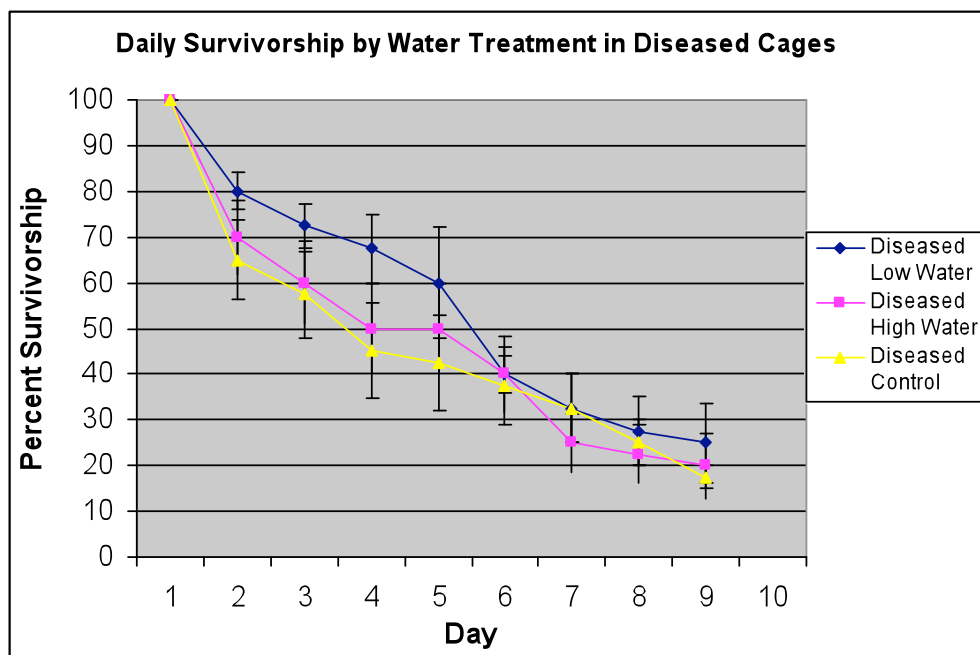


Fig. 2

Figures 1-2. Daily survivorship of *C. pellucida* populations, with cages grouped by water treatment. N = 4 for each water treatment in either a healthy (Fig. 1) or diseased (Fig. 2) cage. Survivorship is reported as a percent (+/- SE), with 10 individuals stocked per cage on Day 1.

Analyses by two-way ANOVA's during the first five days and the second four days of experimentation suggested that neither health of cages nor water treatment were significant factors on proportion of survivorship of *C. pellucida*, arcsine square-root transformed. The same number of days (five followed by four) for both healthy and diseased cages were analyzed to ensure equitable comparisons. For the first five days, $F(1, 24) = 0.008$, $p = 0.932$ for health and $F(2, 24) = 1.355$, $p = 0.283$ for treatment, with no significant interaction between health and treatment. For the second four days, $F(1, 24) = 1.969$, $p = 0.178$ for health and $F(2, 24) = 1.648$, $p = 0.220$ for treatment, with no significant interaction between health and treatment.

Analyses by repeated measures ANOVA's on healthy and diseased cages of each water treatment, using eight repeated measures (from Days 2-9), indicated that health of cages was not a significant factor on proportion of survivorship over the nine-day period ($p > 0.15$ in control, low water, and high water treatments). However, among diseased and healthy cages, controls exhibited a significant interaction between health of cages and time in days ($F(7, 8) = 2.312$, $p = 0.043$).

Linear regression analyses of proportion of survivorship, transformed by natural log, over a nine or ten-day period for each cage treatment (diseased or healthy cage and control, low, or high water treatment) did not yield any significant correlations ($p > 0.15$, $n = 36$ or 40 for diseased or healthy cages, respectively). Non-linear regressions correlated the proportion of daily survivorship with each cage treatment. R-squared values and parameters from the best-fit lines of the form $y = A * e^{Bx}$ reported in Table 1. Based on analysis of parameters A and B, no significant differences in survivorship were exhibited over the entire time period for any cage treatment, within 95% confidence. Exponential decline in survivorship over time for each cage treatment is evident in Figures 3-5, grouped by water treatment.

Table 1

<i>Non-Linear Regression Analysis of Survivorship by Cage Treatment</i>						
Health of Cage	Water Treatment	R-Squared	Parameter A	95% Confidence Interval for A	Parameter B	95% Confidence Interval for B
Healthy	Control	0.952	1.316	1.152<x<1.481	-0.253	-0.294<x<-0.212
Healthy	Low Water	0.931	1.069	0.899<x<1.238	-0.125	-0.161<x<-0.0890
Healthy	High Water	0.900	1.154	0.939<x<1.370	-0.203	-0.256<x<-0.149
Diseased	Control	0.929	1.109	0.923<x<1.295	-0.199	-0.249<x<-0.150
Diseased	Low Water	0.956	1.188	1.032<x<1.344	-0.168	-0.203<x<-0.132
Diseased	High Water	0.935	1.148	0.965<x<1.332	-0.198	-0.244<x<-0.151

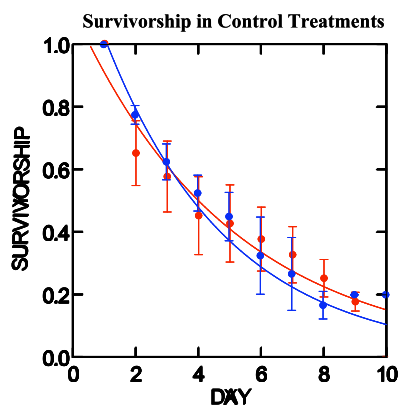


Fig. 3

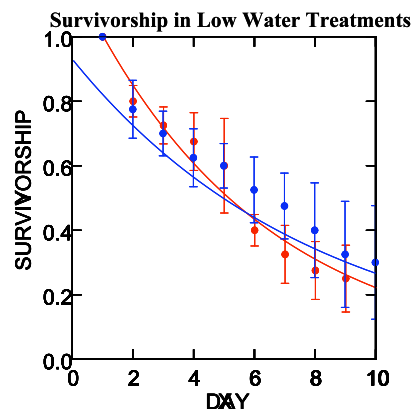


Fig. 4

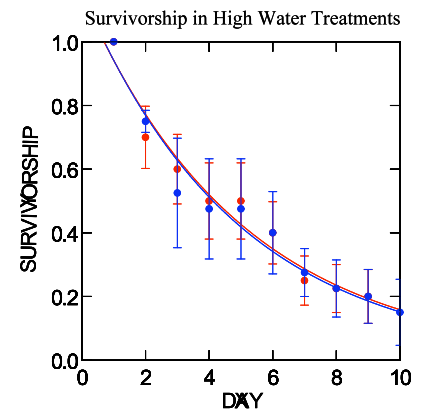


Fig. 5

— Diseased Cages
— Healthy Cages

Figures 3-5. Daily survivorship of *C. pellucida* populations in diseased (red) or healthy (blue) cages over a 9 or 10-day period, respectively. $N = 4$ for each water treatment in either a healthy (Fig. 1) or diseased (Fig. 2) cage. Survivorship is reported as a percent (+/- SE), with 10 individuals stocked per cage on Day 1.

E. grylli Mortality Among Cadavers

Over the nine-day period of cadaver counts (Table 2), mortality due to *E. grylli* was significantly higher in diseased cages than in healthy cages ($\chi^2 = 28.677$, $p < 0.001$, d.f.= 1, chi-square test). In the first five days of this period of cadaver counts, mortality due to *E. grylli* did not vary significantly between diseased and healthy cages ($\chi^2 = 0.817$, $p = 0.366$, d.f.= 1, chi-square test). During the last four days of this period of cadaver counts, mortality due to *E. grylli* was significantly higher in diseased cages than in healthy cages ($\chi^2 = 22.024$, $p < 0.001$, d.f.= 1, chi-

square test). The proportions of mortality due to *E. grylli* among all cadavers collected over the nine-day period in healthy cages and observed in diseased cages is grouped by water treatment in Figure 6.

Table 2

<i>Cadaver Counts and Characterization of Mortality for Chi-Square Analysis of Overall Period</i>			
	Healthy Cage	Diseased Cage	Total
<i>E. grylli</i> Mortality	24	50	74
Natural Mortality	71	25	96
Total	95	75	170

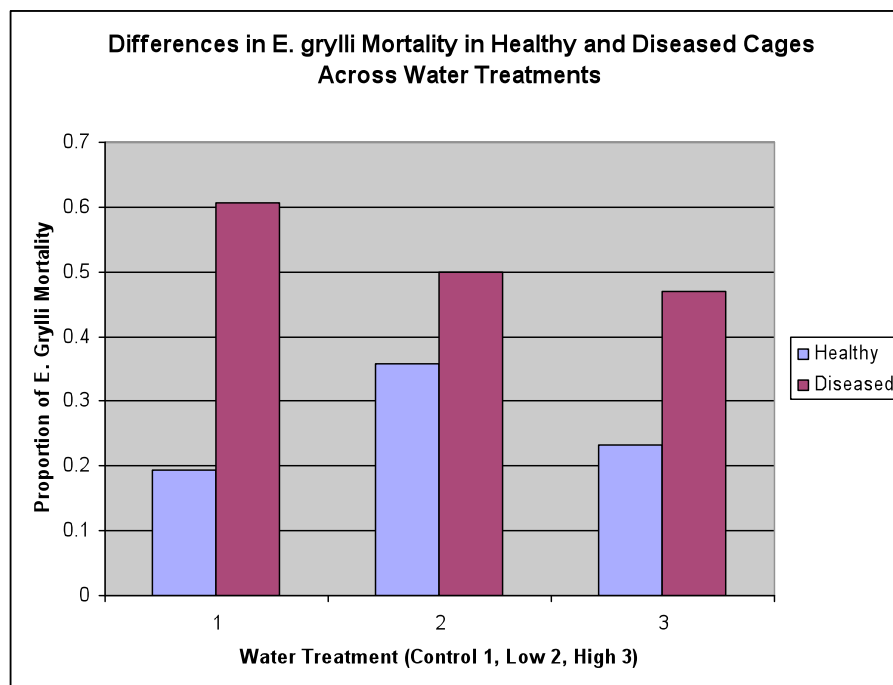


Figure 6. Proportion of total cadavers characterized by *E. grylli* mortality per cage treatment (diseased or healthy cage and control, low, or high water treatment).

Effects of Relative Soil Moisture

Mean soil moisture was calculated (+/- SE) for each water treatment across the five days on which measurements were conducted (Days 2, 4, 6, 8, and 10). Controls and high water treatments were significantly different ($p < 0.001$) by ANOVA and Tukey's post-hoc analysis (Fig. 7).

Relative Soil Moisture by Water Treatn

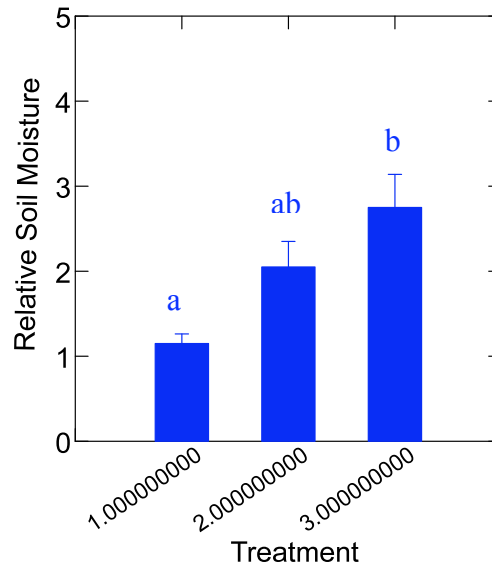


Figure 7. Mean relative soil moisture (+/-SE) across five alternate days of soil testing for each water treatment of healthy and diseased cages.

Regression analyses of proportion of survivorship, arcsine square-root transformed, in all cages over the first five days, the second five days, or throughout all experimentation did not reflect any significant correlations with mean relative soil moisture of the three water treatments ($p > 0.15$). In addition, regression analysis of proportion of mortality due to *E. grylli*, arcsine square-root transformed, in all healthy cages throughout the ten-day experimentation period did not reflect a significant correlation with mean relative soil moisture of the three water treatments ($p > 0.15$). Likewise, regression analysis of proportion of mortality due to *E. grylli*, arcsine square-root transformed, in all cages (both healthy and diseased) throughout the ten-day experimentation period did not reflect a significant correlation with mean relative soil moisture of the three water treatments ($p > 0.15$).

Discussion

Survivorship

Based on the plots of daily survivorship by treatment over time which demonstrated changes in survivorship approximately halfway through the experiment (*Figs. 1-2*), the nine days of daily counting tested by analysis of variance were divided into two periods (five days then four days) to determine significant differences on the basis of health or water treatment. In contrast with the hypotheses that diseased cages would exhibit lower survivorship over time than healthy cages and that increasing the water treatment of the cage would correspond to decreasing survivorship of *C. pellucida*, analysis of variance testing indicated that health and water treatment were not significant factors affecting survivorship. Low survivorship in healthy cages can likely be attributed to natural *E. grylli* infection in the area from which the source populations for the healthy cages were collected. Mortality due to *E. grylli* among healthy-cage cadavers supports this assertion (Table 2). While the National Bison Range area in which data collection occurred receives an average of 0.5-1.5 inches of monthly precipitation, June 2010 was marked by area precipitation ranging between 50-100% above the mean ("Montana Daily/Monthly"). Even in July, the month of experimentation, precipitation either met or exceeded the monthly mean by 33% ("Montana Daily/Monthly"). Because of the historical understanding of atmospheric moisture as the most important abiotic factor in the propagation of fungal diseases of insects (Carruthers et al., 1997), the processes of *E. grylli* spore production, germination and penetration of *C. pellucida* cuticles were likely enhanced by the increased precipitation in this region of western Montana, making it difficult to obtain an *E. grylli*-free control population.

In addition, Figures 1 and 2 indicate differences in *C. pellucida* survivorship between diseased and healthy cages on the ninth day of monitoring. Regardless of water treatment,

survivorship of *C. pellucida* in diseased cages converges between approximately 18% and 25% (+/-SE), while water treatment seems to differentiate survivorship among *C. pellucida* in healthy cages to a greater extent. Control cages exhibit approximately 5% (+/-SE) *C. pellucida* survivorship, and cages applied with low or high water treatments exhibit approximately 33% (+/-SE) or 20% (+/-SE) *C. pellucida* survivorship. Because diseased cages are characterized by ten *E. grylli*-infected individuals from the start of experimentation, it is plausible to assume that all will eventually die of the disease regardless of water treatment, as reflected by the converging of percent survivorship values. However, not all individuals are infected from the start of experimentation in healthy cages, suggesting that individuals with access to more nutrients (from more robust, watered cage plants) will exhibit increased survivorship. From this logic it follows that the “high water” treatment should exhibit the highest survivorship on Day 9, which could still be exhibited if experimentation is conducted on a larger sample size. However, because greater ambient moisture facilitates the spread of *E. grylli*, the *C. pellucida* in this experiment’s “high water” cages may have suffered greater disease mortality, counterbalancing the positive effect of moisture on the cage plants. Thus, the “low water” treatment may have served as the optimum balance between the spread of *E. grylli* and abundance of vegetative nutrients.

Also in contrast to the prediction that diseased cages would exhibit lower survivorship over time, analysis by repeated measures ANOVA indicated that health of the cage did not significantly affect survivorship over time for any water treatment. However, the significant interaction between health of control cages and time suggests that there is a significant difference in the time at which deaths occur (*Fig. 3*). Diseased cages exhibit lower survivorship than healthy cages during the first half of the period analyzed due to *E. grylli*, but then survivorship in diseased cages plateaus and diminishes less rapidly than the steadily declining survivorship in healthy cages. The compensatory

effect results in similar survivorship at the end of the period, but the cause of mortality is different. In both healthy and diseased cages, food resources are a limiting factor and survivorship diminishes exponentially as vegetative nutrients are depleted. However, in diseased cages, early mortality of *C. pellucida* individuals due to *E. grylli* reduces competition for food resources, facilitating higher survivorship than in healthy cages during the second half of the period analyzed. Eventually, all cages are marked by nearly total mortality, but for healthy cages the driving factor was primarily the limitation of food resources, while for diseased cages the driving factor was primarily *E. grylli*. No significant interaction between survivorship and time was found in the cages applied with low or high water treatments, suggesting that the added water may have masked any potential differences in timing of mortality by providing greater vegetative nutrients (Figs. 4-5).

***E. grylli* Mortality Among Cadavers**

After characterization of cadavers by body positioning to determine if the cause of mortality was *E. grylli*, significantly more diseased cadavers were found in diseased cages than healthy cages over the nine-day period of cadaver counts. Because diseased cages were stocked with ten *C. pellucida* individuals with known *E. grylli* infection, this result is logical. However, because *E. grylli* mortalities were also found in healthy cages, this observation may support the claim that secondary infection of grasshopper hosts can occur by contact with conidia from sporulating cadavers in the same vicinity (Carruthers et al., 1997). By chi-squared analysis, mortality due to *E. grylli* was not significantly different between diseased and healthy cages during the first five days of counts, likely because all the weakest *C. pellucida* individuals in healthy and diseased cages, infected with *E. grylli* either naturally in their source habitat or in the lab, died during the initial period. However, in the second four days of counts, mortality due to *E. grylli* was significantly higher in the diseased cages, supporting the previous deduction that mortality in diseased cages at

the end of the period was due to *E. grylli*, but mortality in healthy cages at the end of the period was due to diminishing food resources.

It should also be noted that with increasing application of water (relative to controls), the proportion of mortality due to *E. grylli* diminished (*Fig. 6*). Although this is a non-significant trend and water treatments were also not significantly correlated with survivorship, it is possible that in this experiment, the effect of moisture on vegetative growth was stronger than the effect of moisture on the infectivity of *E. grylli*. If increasing amounts of applied water promoted greater growth of plants from which *C. pellucida* could perch, this adaptive behavior could raise body temperature above the optimal conditions for *E. grylli* (Kemp, 1986) and diminish mortality due to *E. grylli* in diseased cages. Although the nine-day period in question was too short for such differences in growth to occur, water treatments were applied during the first two phases of experimentation, causing the watering of cages to span for approximately one month overall.

Effects of Relative Soil Moisture

Because controls were significantly different in mean relative soil moisture from “high water” treatments, some degree of moisture gradient was maintained in this experiment (*Fig. 7*). During regression analysis, the period of experimentation was again divided into two parts to determine if water treatment on alternate days would begin to take an effect on *C. pellucida* survivorship over time as the soil retained water. However, in contrast with the hypothesis, relative soil moisture was not significantly correlated with *C. pellucida* survivorship or mortality due to *E. grylli*.

In the period of experimentation analyzed (July 20-29, 2010), two major thunderstorms occurred during the nights prior to morning counts. As few as four hours are necessary for rehydration and sporulation of *E. grylli* pathotype I, so these storms likely increased disease

prevalence across all cages (Carruthers et al., 1997). Furthermore, as previously discussed, greater-than-average precipitation in western Montana during the months of June and July could have masked any differences between controls and cages treated with water. Because of the elevated soil moisture during the summer of 2010 in this area, low and high water treatments should have been increased accordingly to investigate differences in survivorship along a more defined moisture gradient. Again, because fungal growth is facilitated by ambient moisture, high precipitation likely facilitated *E. grylli* transmission in all cages and in the habitats of the source populations.

Future Studies

Many temporal, spatial, and genetic factors regulate the disease dynamics of *E. grylli* in conjunction with the *C. pellucida* life cycle, and few statistical correlations have consistently linked biotic and abiotic factors with population levels (Carruthers et al., 1988). However, this reality should not preclude scientific efforts toward understanding these complex interactions, and several additional improvements could be made to this study.

First, a larger sample size should be employed to reduce variability. Also, if appropriate ambient conditions can be created in a laboratory setting, hand-raising grasshoppers from the egg life stage could ensure access to an *E. grylli*-free healthy, control population of *C. pellucida*. In addition, because cages were stocked three times, healthy cages were likely contaminated with resting spores and even short-lived conidia. By virtue of their size (ca. 28 by 38 μ m) and shape, airborne *E. grylli* conidia ejected from grasshopper cadavers cannot disperse more than a few meters in still air; however, because cages were placed just 2.5 meters apart and treatments were randomized, conidia could have been transmitted between cages (Carruthers et al., 1997). Greater spacing between diseased and healthy cages must be implemented.

Although diluted Virex TB Germicidal Cleaner was used to wipe the inside and outside of the nylon mesh cages, Virex could not be applied to the vegetation (potentially consumed by *C. pellucida*) or the ground, two main source areas of *E. grylli* infection. Thus, greater care must be taken to prevent the introduction of *E. grylli* to the healthy cages. In addition, the re-stocking of cages should entail the removal of any surviving individuals in the cages to prevent older, weakened individuals from altering results or even transmitting *E. grylli*.

Furthermore, although analysis of all floor cadavers collected from healthy cages did not demonstrate overnight sporulation on cadavers, some brown residue was observed on the moistened paper towels beneath cadavers. This staining, perhaps indicative of resting spores, supports the suggestion that *E. grylli* produces resting spores as the host population nears adulthood and eventually death in the late summer (Carruthers et al., 1997). Rather than release transient conidia, it is more advantageous for *E. grylli* to produce long-lived resting spores within grasshopper body cavities such that a more abundant, future generation of hosts can be infected under more optimal conditions. Thus, because of the difficulty of assessing *E. grylli* infection from resting spores rather than conidia, experimentation during the late spring and early summer would be a more ideal time frame.

In all, much is left to understand about the disease interaction between the *E. grylli* fungal pathogen and its grasshopper hosts, particularly *C. pellucida* populations prevalent in western Montana. In particular, additional evidence regarding the specific ambient conditions in which *E. grylli* induces mortality is necessary to understand how epizootics of the disease affect *C. pellucida* populations. The applications to agricultural biocide development and entomological study are far-reaching.

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