

# Host dynamics determine responses to disease: additive vs. compensatory mortality in a grasshopper–pathogen system

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**Abstract.** Disease is often expected to limit host populations, but diseases do not always dramatically reduce host numbers and often have no effect. The impact of a fungal pathogen (*Entomophaga grylli* pathotype 1) on grasshopper (*Camnula pellucida*) populations was studied in a field experiment. We tested whether the effects of disease on grasshopper survival were additive, with disease mortality summing with other mortality sources to determine the total population mortality rate; or whether they were compensatory, where disease mortality simply replaces mortality from other sources so that the total population mortality rate remains unchanged. We examined grasshopper survival in relation to differences in disease exposure, host density levels, and host developmental stage. The effects of disease varied with grasshopper developmental stage and density. Disease mortality increased by 60% at high grasshopper density compared to low-density treatments, and decreased when grasshoppers fully matured. Despite increased rates of disease mortality at high densities, the total mortality rate was not notably higher in diseased grasshoppers (87%) compared to disease-free counterparts at high densities (83%), indicating that a large percentage of disease mortality simply replaced mortality from food limitation. Additive responses were supported in early and late instars, with disease exposure resulting in decreased grasshopper survival. In contrast, the effect of disease on adults was inconclusive. Yet, the disease did not affect adult survival, suggesting that adult disease mortality is compensatory. Therefore, disease reduction of grasshopper populations (additive mortality) is more likely to occur during earlier developmental stages, when hosts are most vulnerable to disease, and at low host densities when food is abundant. Combined, our results emphasize the importance of host dynamics and food availability in how this host–pathogen system responds to disease. Accordingly, compensatory vs. additive mortality may need to be considered when examining how disease ultimately affects host population dynamics.

**Key words:** additive mortality; *Camnula pellucida*; compensatory mortality; density dependence; disease ecology; entomopathogen; *Entomophaga grylli*; grasshopper; host–pathogen interaction.

## INTRODUCTION

Since Anderson and May (1978) incorporated the study of disease into population ecology, a greater appreciation of how disease affects host population dynamics has emerged (Anderson and May 1980, Dobson and Hudson 1992, Murdoch 1994, Hudson et al. 2002). However, host populations will not be limited by disease if its effects are not “additive” with other sources of mortality. In some instances, disease mortality merely compensates for other mortality sources and plays no role in host population regulation (Holmes 1982). The relative importance of additive vs. compensatory mortality is a key concept in population ecology, especially when predicting the effects of harvesting (Burnham and Anderson 1984, Bartmann et al. 1992,

Kokko 2001, Pöysä 2004, Sparkman et al. 2011), predation (Oedekoven and Joern 2000, Wilmers et al. 2007, Belovsky and Slade 2010, Creel 2011, Ellis-Felege et al. 2012), and disease (Holmes 1982, Hudson et al. 2002, Jolles et al. 2006, Tobler et al. 2012) on population dynamics over time.

In this article, we refer to the term additive strictly in the context of population ecology (not be confused with additive genetic diversity or genetic variance in evolutionary ecology, as in Hughes et al. 2008). We define additive mortality as when one source of mortality adds proportionally to the total mortality rate. Thus, additive mortality is literally additive with other mortality sources and affects population processes. For instance, Jolles et al. (2006) found tuberculosis (TB) in prime-age African buffalo to be additive, since the disease killed adults that would have survived in the absence of TB, resulting in an equal increase in total population mortality. In contrast, compensatory mortality refers to a situation in which one source of mortality merely

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replaces mortality from another source so that the total mortality rate remains the same. For example, Errington (1946, 1956) observed that disease in muskrats had no effect on the total population mortality rate since disease killed less fit individuals who would have ultimately succumbed to starvation in the absence of disease. Simply put, disease mortality merely substituted for starvation mortality and did not affect muskrat population dynamics.

Whether diseases limit host populations (i.e., additive mortality) or have no effect on host population dynamics (i.e., compensatory mortality) remains unclear, since studies show that both responses occur in natural populations. Theoretical studies indicate that, if disease mortality is assumed to be additive, it can regulate both insect (Anderson and May 1980, Dwyer et al. 2000, 2004) and vertebrate populations (Heesterbeek and Roberts 1995, Roberts et al. 1995, Tompkins et al. 2002). This regulation may be to a stable level (via continuous mortality) or to a limit cycle (via periodic epizootics; Holmes 1982, Murdoch 1994, Dwyer et al. 2000). However, disease mortality in natural populations may not always be additive due to interactions between multiple sources of mortality (Burnham and Anderson 1984). The viewpoint that disease mortality is primarily compensatory was first derived from claims that disease mortality merely replaces mortality from starvation when food is in short supply (Errington 1946, Holmes 1982), and this response has been observed in nature (Errington 1956, Mech and Goyal 1993, Hawkins et al. 1997, Magle et al. 2012, Tobler et al. 2012). In addition, several studies have found predation may compensate for disease with sick individuals becoming easy targets for predators (Hudson et al. 1992, Murray et al. 1997, Joly and Meisser 2004, Laws et al. 2009). Finally, some studies suggest that population responses to disease can be either additive or compensatory, depending on density-independent and density-dependent processes (Jolles et al. 2006, Tompkins et al. 2011).

In this article, we experimentally test whether disease mortality is additive (i.e., limits host populations) or compensatory (i.e., has no effect on host populations) using a grasshopper–fungal pathogen system. This concept may be tested by comparing diseased and disease-free host populations (Scott and Dobson 1989). For instance, if disease mortality is additive with other sources of mortality, then diseased populations should exhibit lower survival rates compared to disease-free populations (Hudson et al. 2002). In contrast, if disease mortality is compensatory, then survival rates in diseased and disease-free populations would not ultimately differ. To date, little work in natural environments has investigated the conditions that determine when the effects of disease on a host population are additive or compensatory (see Jolles et al. 2006 for one such investigation).

Two host factors are often considered important in determining when disease mortality is additive vs.

compensatory. First, age or developmental stage of the host individuals influences infection rates, pathogen development, and fecundity rates, as well as host resistance to the disease (Mackauer 1973, Dwyer 1991, Hudson et al. 2002). For example, young hosts often exhibit higher disease mortality rates compared to mature hosts with greater resilience (Wantanabe 1987, Carruthers 1988a, Hajek and Roberts 1991). As a result, disease mortality may be additive in juveniles and mostly compensatory in adults, with disease killing weak adults who would have died anyway (Hudson et al. 2002). Second, host density can influence disease establishment (Scott and Dobson 1989, Grenfell and Dobson 1995, Fuller et al. 2012) and disease transmission (Murdoch 1994, Arneberg et al. 1998, Langwig et al. 2012). This density-dependent feedback is often exhibited in insect diseases that drive predictable multiyear population cycles and can produce strong additive responses (Wallner 1987, Dwyer and Elkinton 1993, Fuller et al. 2012). However, other diseases have little to no effect on host numbers even at high host densities, suggesting that disease mortality is compensatory (Holmes 1982). This makes intuitive sense given that intraspecific competition often increases with population density, resulting in disease mortality simply replacing mortality from starvation (Errington 1946). Whether disease mortality is compensatory with food limitation may also depend on the intensity of intraspecific competition, which is often determined by population density (Errington 1956, Mech and Goyal 1993, Hawkins et al. 1997).

Insects, such as grasshoppers, are a good model system for addressing which host factors influence whether disease mortality is additive or compensatory. The effects of diseases on insect populations vary spatially and temporally (Altizer et al. 2006), indicating that these diseases are additive in some instances and compensatory in others. Insects provide a logistically feasible field experimental system to address this question due to their small size and abundance. In addition, insects have important applied value because they are often pests and their diseases are sometimes employed as biocontrols. For example, entomopathogens are common pathogens of insects that can exhibit massive epizootic outbreaks that dramatically reduce insect populations (Dempster 1963, Hajek and St. Leger 1994) and have been used for biocontrol of several insect pests, including grasshoppers (Goettel et al. 1995).

Entomopathogen outbreaks may even play a central role in the population dynamics of a number of insect species (Hajek and St. Leger 1994). However, entomopathogen life history is often complex and can be affected by both density-independent and density-dependent processes. For instance, Liebhold et al. (2013) found that an introduced entomopathogen infecting gypsy moths was largely density-independent resulting in additive mortality regardless of host density. In contrast, a related entomopathogen, *Entomophaga grylli* pathotype 1, infecting the pest grasshopper

*Camnula pellucida* (Goettel et al. 1995), is viewed as density dependent (Carruthers et al. 1997). Nevertheless, epizootics that drastically reduce grasshopper populations (i.e., additive mortality) usually occur under favorable weather conditions for peak pathogen reproduction and transmission leading to large numbers of fit grasshoppers dying of infection (Pickford and Riegert 1964), a density-independent event. Furthermore, this entomopathogen may play a more compensatory role in unfavorable years when pathogen prevalence and infectivity is low, resulting in less fit individuals dying from disease instead of starvation or predation (Holmes 1982). Unfortunately, it is difficult to tease apart interacting sources of mortality, since the presence of pathogens generally cannot be controlled in the field.

Here, we developed a model system of replicated, enclosed field populations of the host grasshopper that can be exposed to and protected from *E. grylli*. In addition, grasshopper density and developmental stage was varied. We examined all deceased individuals for traces of *E. grylli* to confirm that disease was a major contributor to total grasshopper mortality. To distinguish between additive and compensatory responses, we used parametric survival models and Akaike information criterion (AIC) model selection to assess whether disease exposure best explained experimental grasshopper survival across varying host densities and developmental stages (Burnham and Anderson 2002).

We examined two key hypotheses. The first hypothesis was whether disease mortality would be additive when grasshoppers are immature and compensatory when they are mature. Early and late instars are highly susceptible to fungal infection (Carruthers et al. 1988a, Carruthers et al. 1997) and should exhibit high disease mortality rates compared to adults, which often exhibit very low rates of disease mortality (Kistner and Belovsky 2013). In turn, the few adults that do die of infection should consist of less fit individuals destined to die with or without the presence of disease. Such compensatory responses have been documented in grasshoppers, with poor foragers dying of starvation in the absence of birds or from predation when birds were present (Belovsky and Slade 2010). The second hypothesis was whether disease would be additive at low densities and compensatory at high densities. Reduced population size from disease should increase future per capita survival of remaining individuals by decreasing intraspecific competition for food (Oedekoven and Joern 2000, Jolles et al. 2006). Given that intraspecific competition for food (Belovsky and Joern 1995) and disease transmission increase with grasshopper density (Carruthers et al. 1988a), disease mortality in high-density populations should replace mortality from food limitation (Errington 1946).

#### METHODS

This study was conducted at the National Bison Range, Moiese, Montana (47°21.040 N, 114°10.190 W),

at an elevation of 832 m. This site is primarily Palouse prairie dominated by  $C_3$  grasses. *Poa pratensis* (L.) and *Elymus smithii* (Rybd.) are the dominant grasses, while *Aster falcatus* (Lindl.), *Achillea millefolium* (L.), and *Erigeron* sp. are the most common forbs. The grammivorous clearwinged grasshopper, *C. pellucida*, (Orthoptera, Acrididae) is common at the site and is univoltine, overwintering as eggs that hatch in late May through early June (Pfadt 1994). *Entomophaga grylli* pathotype 1, unofficially known as *Entomophaga macleodii* (Humber, unpublished data; Casique-Valdez et al. 2012), is one member of a species complex of obligate grasshopper fungal entomopathogens (Goettel et al. 1995). Pathotype 1 is endemic to North America and only infects grasshoppers in the subfamily Oedipodinae (Carruthers et al. 1997). This pathogen is known to periodically dramatically decrease populations of *C. pellucida* (Pickford and Riegert 1964) and is common at this site (Kistner and Belovsky 2013).

The life cycle of this entomopathogen and its grasshopper host are complex. *Entomophaga grylli* pathotype 1 overwinters as dormant spores that germinate in the spring and infect grasshoppers by contact (Carruthers et al. 1988a). Inside the grasshopper, the fungus multiplies rapidly and digests the grasshopper's tissues to develop and replicate (MacLeod et al. 1966). The infection leads to death within 7–10 days (Carruthers et al. 1997). Just before dying, the infected grasshopper climbs to the top of a plant where it dies grasping the foliage. This posture is characteristic of grasshopper mortality from *E. grylli* and can be easily recognized (Pickford and Riegert 1964). The cadaver can produce resting spores that transmit the disease in the next year, or conidia which can transmit the disease in the current year (Carruthers et al. 1997). Conidia are highly vulnerable to high temperatures, low humidity, and intense sunlight (Carruthers et al. 1988b), and tend to be produced under cool, humid conditions, while resting spores are produced under hot, dry conditions (Carruthers et al. 1997).

Grasshopper populations were established in aluminum window-screen cages placed over natural vegetation (with basal area of 0.5 m<sup>2</sup> and height of 1 m). Each cage had aluminum flashing at its base that was buried in the ground to prevent insects from entering or leaving while excluding grasshopper predators. The cage was secured by wire to wooden stakes, and cage tops were closed using binder clips that allowed easy access but prevented grasshoppers from escaping (Belovsky and Slade 1995). Cages were placed over similar vegetation (quantity and type) in a grid with at least 1.5 m between adjacent cages. Prior to stocking cages with grasshoppers, the cages were wiped with diluted VIREX II 256 Germicidal Cleaner (Diversey, Surtevant, Wisconsin, USA), and PT Infuse Systemic Fungicide (Bonide Products, Oriskany, New York, USA) was sprayed on the ground and vegetation in each cage to eliminate *E. grylli* resting spores or conidia.

The three treatments used were disease exposure (infected grasshopper cadaver present or absent in cage); grasshopper density (stocked with 10 or 20 individuals per cage); grasshopper developmental stage (stocked with early [second and third] instars, late [fourth and fifth] instars, or adults). Experimental densities reflect observed high and low densities at our site (Belovsky and Slade 1995). We used a  $2 \times 2 \times 3$  fully crossed design with four replicate cages for each treatment combination, for a total of 48 cages. Treatment combinations were randomly assigned to cages. Disease exposure was provided by tying an infected grasshopper cadaver (one fifth-instar female per cage) on a wire 0.6 m above the ground, which mimics infected individuals that climb up into the vegetation before dying (Carruthers et al. 1997).

Grasshoppers stocked in cages were obtained in two ways. In cages without an infected grasshopper cadaver, grasshoppers were obtained by rearing eggs collected from captive individuals in the previous year (see Carruthers et al. 1992) to ensure that they were not exposed to the fungus prior to stocking. Too few eggs could be collected to stock all treatment combinations; therefore, these individuals were used in cages not exposed to the disease (no infected grasshopper cadaver), and field-caught individuals were used in cages exposed to the disease (infected grasshopper cadaver present). Field-caught individuals were collected in the same field as the parents of lab-reared individuals to ensure all experimental grasshoppers were from the same population. Field-caught individuals were also observed in terraria for 10 days prior to stocking to minimize the use of injured or diseased individuals. We tested for the effect of grasshopper source (egg-reared vs. field-caught) by comparing survival of disease-exposed, field-caught, late instars ( $n = 4$  cages) to disease-exposed, egg-reared, late instars ( $n = 4$  cages) in our  $0.5\text{-m}^2$  experimental cages stocked with 10 grasshoppers each. We found that survival was unaffected by grasshopper source ( $\chi^2 = 0.11$ ,  $df = 1$ ,  $P = 0.74$ ) and that the final proportion of surviving individuals did not differ (0.32 egg-reared vs. 0.35 field-caught). Therefore, the use of both egg-reared and field-caught grasshoppers in this study is unlikely to be confounding our results.

Survival of grasshoppers in the cages was monitored from July to September in 2011, when lower temperatures at night began to kill the grasshoppers. Cages were stocked when particular grasshopper developmental stages were abundant. Early and late instars were stocked in early July and adults in August. Individuals in cages and cadavers were counted every two days (Belovsky and Slade 1995). Cadavers clinging high in the vegetation were noted as mortality from *E. grylli* infection, as this behavior is uniquely characteristic of the disease. These individuals were left to continue disease transmission, which can occur over time when temperature/humidity is appropriate (Saw-

yer et al. 1997). All other cadavers (mainly those on the ground) were collected, frozen, and later stained with Lacto-Fuchsin (AEML, Pompano Beach, Florida, USA) to examine for the fungus' hyphae, conidia, and spores under a microscope (Sánchez-Peña 2005). Given that only 5% of cadavers removed from the cages were infected with *E. grylli*, it is unlikely that our cadaver removal impacted overall transmission rates. Only individuals whose cause of death could be determined (19% of all experimental individuals were never recovered) were included in the mortality analysis. Mortality due to the disease was the sum of cadavers observed clinging high in the vegetation plus the number of other cadavers found to be infected.

We employed generalized linear modeling (GLM) with a binominal distribution and logit function to test the effect of disease exposure, host density, and developmental stage on the number of disease-induced deaths (R Development Core Team 2013). We constructed parametric survival models with a Weibull distribution and used disease exposure, density level, and developmental stage as predictive factors (R Development Core Team 2013, Therneau 2013). We chose to use parametric survival models as opposed to semi-parametric approaches, such as Cox proportional hazards models, because they depict population-level responses to disease rather than an individual's risk of dying from disease (Crawley 2013). Individual survival models were also constructed for disease-exposed and disease-free populations. Finally, survival models were employed across all three developmental stages. These parametric survival models examined whether differences existed in the dynamics of the response by treatment over time (Wilson et al. 2002, Crawley 2013). We ranked models containing all subsets of predictors by AIC function (R Development Core Team 2013). Support for each model was evaluated by calculating the delta AIC  $\Delta_i$ , where models with  $\Delta_i < 2$  are considered to have substantial support; and Akaike weights  $w_i$ , which describe the weight of evidence that model  $i$  is the best model from the set of alternative models (Burnham and Anderson 2000). To test a priori hypotheses regarding additive vs. compensatory responses to disease, we compared the  $\Delta_i$  and  $w_i$  of models with and without the disease exposure variable. If models containing the disease variable are supported, then we conclude disease is acting in an additive manner. If models without the disease exposure variable are more strongly supported, then we conclude disease is compensatory and does affect grasshopper population dynamics. Alternatively, if both models are equally supported, then we cannot definitely say whether disease is additive or compensatory. These models were conducted on the first 42 days of the experiment for early and late instars and the first 22 days for adults, the period before *E. grylli* mortality ceased, vegetation (food) senesced, and cold temperatures caused mortality. All analyses were conducted in R v. 3.0.1 (R Development Core Team 2013).

## RESULTS

Mortality from *E. grylli* peaked in late July and totally disappeared by late August. The majority of fungal deaths (72%) occurred within the first two weeks after disease exposure, and less than 6% of these deaths occurred after an individual transitioned between developmental stages (early instar to late instar or late instar to adult). Early- and late-instar treatments initiated in July exhibited twofold more disease mortality than adult treatments that began in August. The number of deaths from disease was significantly affected by disease exposure, host density, and developmental stage (Appendix A). Only one grasshopper (<2%) died from the disease in an unexposed cage while 20% of individuals in disease exposed cages died from disease (70 deaths). On average, the number of grasshopper deaths from *E. grylli* increased by 60% at high grasshopper density compared to low grasshopper density treatments (Fig. 1).

Grasshopper survival was affected by disease exposure ( $\chi^2 = 40.44$ ,  $df = 1$ ,  $P < 0.001$ ) and developmental stage ( $\chi^2 = 76.74$ ,  $df = 2$ ,  $P < 0.001$ ), with significant interactions between disease exposure and developmental stage ( $\chi^2 = 76.74$ ,  $df = 2$ ,  $P < 0.001$ ) as well as disease exposure and density ( $\chi^2 = 4.50$ ,  $df = 1$ ,  $P = 0.034$ ; Appendix B). Consequently, diseased and disease-free grasshoppers responded differently to density and developmental stage treatments. For disease-free grasshoppers, both developmental stage and density level affected survival ( $\chi^2 = 51.711$ ,  $df = 2$ ,  $P < 0.001$ ;  $\chi^2 = 51.752$ ,  $df = 1$ ,  $P < 0.001$ , respectively). In contrast, only developmental stage affected survival of disease-exposed grasshoppers with density level having no significant impact ( $\chi^2 = 46.86$ ,  $df = 2$ ,  $P < 0.001$ ;  $\chi^2 = 1.57$ ,  $df = 1$ ,  $P = 0.21$ , respectively). These same trends are seen in the survivorship plots (Fig. 2). Disease decreased survival time of early instars by 54.2% and late instars by 50.3% ( $\chi^2 = 23.04$ ,  $df = 1$ ,  $P < 0.001$  and  $\chi^2 = 14.18$ ,  $df = 1$ ,  $P < 0.001$ , respectively) regardless of starting densities ( $\chi^2 = 0.544$ ,  $df = 1$ ,  $P = 0.544$ ;  $\chi^2 = 0.501$ ,  $df = 1$ ,  $P = 0.501$ , respectively). Survival of adults was unaffected by disease exposure ( $\chi^2 = 0.494$ ,  $df = 1$ ,  $P = 0.8803$ ) or density level ( $\chi^2 = 0.020$ ,  $df = 1$ ,  $P = 0.8863$ ).

Of the 13 candidate models fit to the entire data set, the top two models indicated that disease exposure was an important predictor of grasshopper survival (Appendix C). The combined  $w_i$  for the top two models indicated a 96% probability that the best approximating model for our experimental data contained disease exposure. However, the top two models also contained developmental stage and disease exposure by developmental stage interaction. Since each development stage responded differently to disease, we modeled each developmental stage separately to provide a more thorough assessment of additive vs. compensatory responses in our experimental system (Table 1).

For early instars only, models containing disease exposure captured ~100% of the total weight evidence

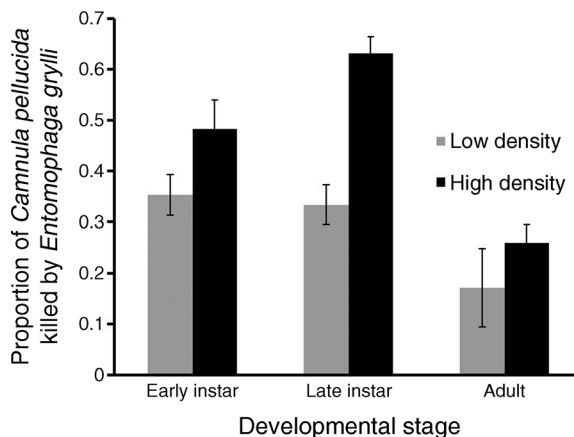


FIG. 1. Proportion of *Entomophaga grylli*-exposed grasshoppers that died from infection (mean  $\pm$  SE) at low densities ( $n = 10$  individuals/cage) and high densities ( $n = 20$  individuals/cage) for three developmental stages. Cages had a basal area of  $0.5 \text{ m}^2$  and height of  $1 \text{ m}$ . We conducted glm analyses on 168 cadavers for which cause of death was known. We could not determine cause of death for another 115 individuals, so they were not included. Disease mortality rates decreased when grasshoppers fully matured and increased with density level.

(Table 1). Therefore, disease exposure is a strong predictor of early-instar grasshopper survivorship, indicating an additive response to disease. For late instars, the combined  $w_i$  for the top three models indicated a 99.6% probability that the best model contained disease exposure; we conclude that late-instar disease mortality is additive (Table 1). Finally, the overall effect of disease on adult grasshoppers was inconclusive (Table 1). There was similar support for a density ( $\Delta_i = 0$ ) and disease exposure effect ( $\Delta_i = 1.49$ ).

## DISCUSSION

A central focus in disease ecology is determining under what conditions a disease may limit host populations (Holmes 1982, Grenfell and Dobson 1995, Hudson et al. 2002, Tompkins et al. 2011). In a field experiment, we tested whether disease mortality is additive vs. compensatory using disease-exposed and disease-free grasshopper populations. Our results indicate that the effects of disease on a host population can be very complex, depending on season, developmental stage (or age) of host individuals, and host density. In the context of our experiment, these factors interact to produce disease limitation (additive mortality) under some conditions and no disease limitation (compensatory mortality) under other conditions.

We found that *E. grylli* loses its ability to cause host mortality as the summer progresses. This trend makes intuitive sense given that virulent fungal conidia are highly vulnerable to heat, desiccation, and UV exposure (Carruthers et al. 1988b). Furthermore, the observation that <2% of grasshoppers contracted the disease in cages where a diseased cadaver was not present indicates that transmission is spatially limited. Consequently,

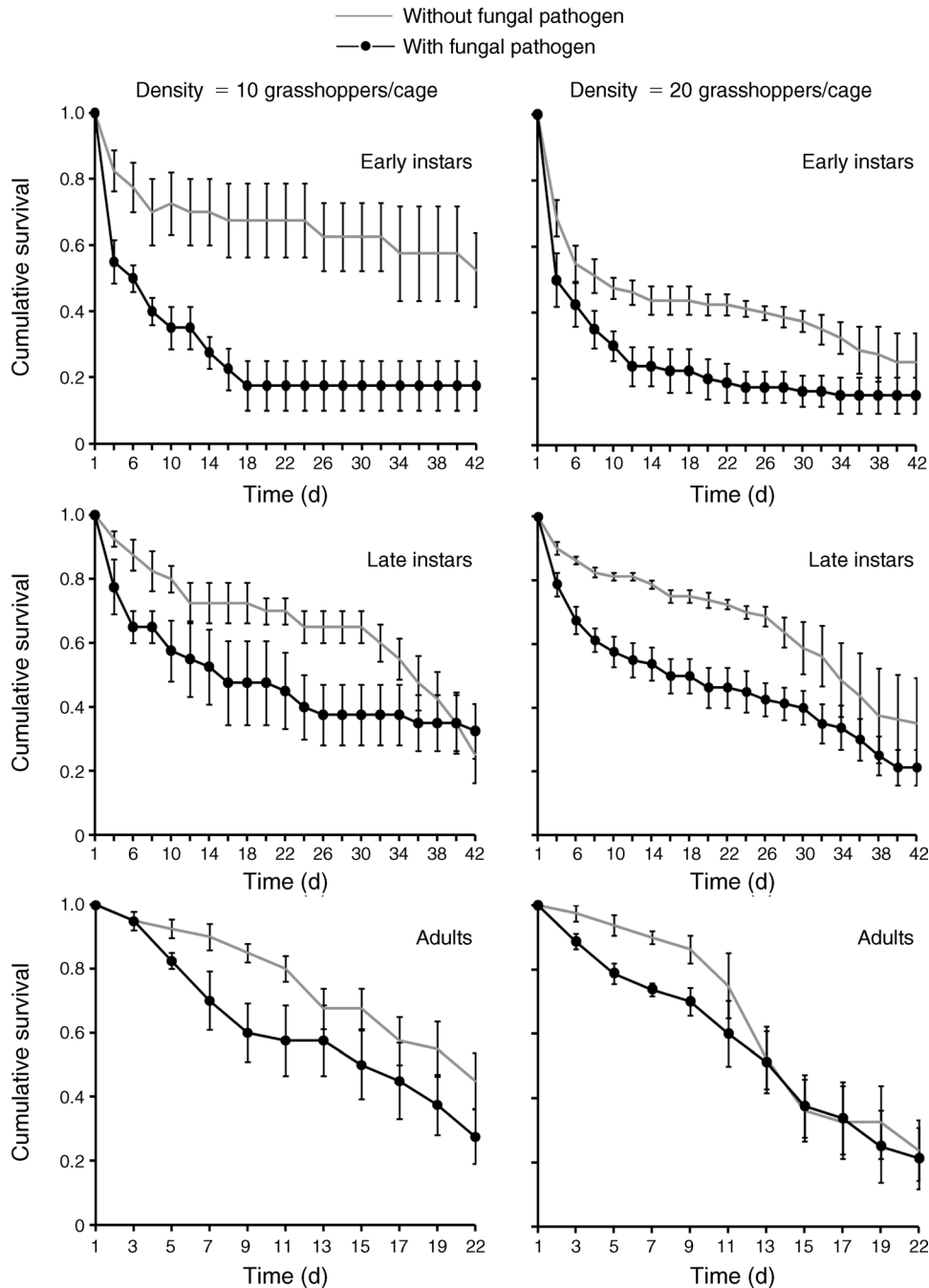


FIG. 2. Grasshopper survival (mean  $\pm$  SE) of pathogen-exposed and pathogen-absent populations across different density and developmental stage treatment combinations. Densities and cages are as described in Fig. 1.

within-year disease transmission becomes highly restricted over the summer.

Host developmental stage clearly affected disease mortality rates. The disease inflicts high mortality rates in early- and late-instar individuals while adults experience significantly less mortality (Fig. 1). The main reason for this pattern is very straightforward. As a grasshopper develops, its exoskeleton becomes thicker

and harder, making penetration by the fungus more difficult (MacLeod et al. 1966, Hajek and St. Leger 1994). Differences in exoskeleton structure led us to observe that 62% of early-instar individuals swabbed with an aqueous suspension of *E. grylli* conidia in the lab died from the disease, while only 11% of adults died under the same conditions (E. J. Kistner, unpublished data). In addition, adult grasshoppers are present when

TABLE 1. Summary of model selection statistics evaluating variation in grasshopper survival across the three developmental stages.

Developmental stage	Model	Log-likelihood	AIC	$\Delta$ AIC	Akaike weight
Early instars only	<b>DE</b>	-572.1	1150.15	0	0.694
	<b>DE, DEN</b>	-571.9	1151.79	1.64	0.306
	DE, DEN, DE $\times$ DEN	-571.7	1169.72	19.57	0.000
	DEN	-583.4	1170.98	20.83	0.000
Late instars only	<b>DE</b>	-664	1333.99	0	0.495
	<b>DE, DEN, DE <math>\times</math> DEN</b>	-662.5	1335.03	1.04	0.2889
	<b>DE, DEN</b>	-663.8	1335.53	1.54	0.212
	DEN	-670.9	1345.72	11.73	0.001
Adults only	<b>DEN</b>	-468.3	940.65	0	0.567
	<b>DE</b>	-468.1	942.14	1.49	0.269
	DE, DEN	-468.1	944.12	3.47	0.100
	DE, DEN, DE $\times$ DEN	-467.5	945.03	4.38	0.063

Notes: Models are ordered from best to worst. Boldface text in the "Models" column depicts the selected models based on Akaike information criterion (AIC). Multiple models are bolded when one model cannot be unambiguously selected (i.e.,  $\Delta$ AIC  $\leq$  2). Abbreviations are DE for disease exposure, a categorical variable with two levels (i.e., pathogen vs. no pathogen); and DEN for density, a categorical variable with two levels (i.e.,  $n = 10$  vs.  $n = 20$  individuals).

field conditions are least favorable for conidia propagation (Carruthers et al. 1988a, 1997).

In our study, the effect of host density on disease mortality is unequivocal. The disease produces greater mortality as the grasshopper population density increases (Fig. 1). Since fungal conidia are highly vulnerable to environmental conditions and transmission is spatially limited, *E. grylli* greatly benefits from close proximity among host individuals, which increases with density (Carruthers et al. 1988a, Dobson and Hudson 1992, Murdoch 1994, Arneberg et al. 1998, Hudson et al. 2002). Yet, diseased grasshoppers at higher density did not exhibit lower survival compared to low-density diseased grasshoppers despite density-dependent disease mortality (Fig. 1, Appendix B). Conversely, higher starting densities decreased survival time of disease-free grasshoppers by 17%. We suspect that this trend arises from the interplay of density and intraspecific competition for food. As grasshopper populations at our site are food-limited, higher grasshopper densities will lead to greater intraspecific competition for food (Belovsky and Slade 1995). Therefore, individuals dying from the disease make more food available to each surviving individual, so that disease mortality becomes compensatory with food limitation (Oedekoven and Joern 2000). Similar observations have been obtained for predation effects on grasshopper populations (Belovsky and Slade 1995, Belovsky and Joern 1995, Oedekoven and Joern 2000, Belovsky and Slade 2010). Taken together, our results indicate that both density and developmental stage affect the population dynamics of disease-free grasshoppers while developmental stage is the most predictive factor in determining survival of disease-exposed grasshoppers, with density level interacting with disease to help determine the strength of this response (Appendix B).

Disease exposure led to additive responses in our early- and late- populations (Table 1). The magnitude of these responses was determined by both host density and developmental stage. For instance, early instars exhib-

ited additive disease mortality, but host density was also affecting grasshopper numbers as indicated by density's inclusion in the second most supported model ( $\Delta_i = 1.64$ , Table 1). Survivorship plots show that additive responses to disease were stronger in low-density compared to high-density treatments (Fig. 2). It is likely that *E. grylli* disease killed some early instars that would have died anyway from other causes (Holmes 1982). Yet the outcome of this disease-grasshopper interaction was ultimately limiting (i.e., additive) with disease increasing the total population mortality rate by killing mostly fit individuals (Jolles et al. 2006).

Late instars also exhibited additive disease mortality, but this response was weaker than that of early instars (Fig. 2, Appendix B). This outcome is the result of disease interacting with density level (Table 1), which increased late-instar survival time by a factor of 1.47. This response is unsurprising, given that grasshopper food requirements increase as they mature over the summer (Belovsky and Joern 1995, Belovsky and Slade 1995). In addition, food supply (vegetation) for the grasshoppers at our study site decreases over the summer, as it is consumed by herbivores and senesces due to desiccation (Belovsky and Slade 1995). Thus, disease does not limit late-instar grasshoppers to the same extent as early instars, since later developmental stages are under stronger food limitation and reduction of diseased individuals enhances survival of the remaining grasshoppers (Oedekoven and Joern 2000).

While AIC model selection indicates that the effect of disease on adults is inconclusive (Table 1), low disease mortality rates and the survivorship analysis suggest that disease is compensatory (Figs. 1 and 2, Appendix B). Disease mortality in adult grasshoppers comprised only 24% of the total deaths due to disease. Consequently, there was no significant effect of disease exposure on overall adult survival ( $P = 0.8803$ ) as disease mortality mostly replaced mortality from starvation (Belovsky and Slade 2010). Furthermore, the model containing only density was 2.12 times more

likely to be the best explanation for adult survival compared to disease exposure only (Table 1). In summary, we found that disease has the greatest impact on grasshoppers when vulnerable early developmental stages and food are abundant.

Our results have applied value for pest control. For example, in order for pest managers to employ *E. grylli* as a biocontrol, application would be most advantageous at low densities of early-instar individuals when food is most abundant. There is evidence that some diseases can be effective at controlling insect pests at low densities (Webb et al. 2004, Liebhold et al. 2013). However, concerns over grasshopper damage often occur when grasshoppers are abundant, at later stages of development, and when vegetation is scarce (Belovsky and Joern 1995). Thus, effective use of this fungal pathogen as a biocontrol requires foresight given that disease limitation in this system is highly contextual (Goettel et al. 1995).

Through a field experiment, we have shown that disease mortality can be additive and limit host populations (Anderson and May 1980, Heesterbeek and Roberts 1995, Roberts et al. 1995, Tompkins et al. 2002), but also result in compensation by substituting for mortality caused by competition for food (Errington 1946, 1956, Holmes 1982, Mech and Goyal 1993, Hawkins et al. 1997, Magle et al. 2012, Tobler et al. 2012). Each can occur under certain environmental and demographic conditions, and the picture of how disease affects host populations is complex (Jolles et al. 2006, Tompkins et al. 2011). Our results suggest that disease mortality is more likely to be additive when host densities are low and when vulnerable early instars are present. While high host densities are often associated with additive disease mortality (Anderson and May 1980, Carruthers et al. 1988a, Dobson and Hudson 1992, Murdoch 1994, Arneberg et al. 1998, Hudson et al. 2002), we found that host numeric responses to disease became more compensatory at higher densities as disease mortality replaced mortality from food limitation (Holmes 1982). Therefore, food-limited host populations are more likely to exhibit compensatory disease mortality especially at high densities when intraspecific competition is enhanced (Oedekoven and Joern 2000). Our study provides mechanistic insights that may help predict when grasshopper pests will be limited by disease, and we hope lead to a better understanding of population and disease ecology.

However, these results must be approached with caution given that we only examined predator-free population responses to disease and food limitation at one site over the course of a single year. Natural grasshopper populations vary temporally and spatially with multiple interacting variables influencing population size (Belovsky and Joern 1995) including predators, food abundance, and other pathogens (Laws et al. 2009, Oedekoven and Joern 2000, Tompkins et al. 2011). To better understand the role entomopathogens play in host

population dynamics, surveys on natural host populations should be conducted alongside manipulative experiments. Future studies should also consider the potential interactions of predators, food availability, and pathogen limitation of host numbers.

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## SUPPLEMENTAL MATERIAL

### Appendix A

Analysis of the factors affecting disease mortality rates of grasshopper hosts using generalized linear modeling (GLM) with a binomial distribution ([Ecological Archives E095-225-A1](#)).

### Appendix B

The effects of disease exposure on grasshopper survival across density levels and developmental stages ([Ecological Archives E095-225-A2](#)).

### Appendix C

Summary of model selection statistics evaluating variation in grasshopper survival across developmental stage, density level, and disease exposure variables ([Ecological Archives E095-225-A3](#)).