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An Herbivore's Thermal Tolerance is Higher Than That of the Ant Defenders in a Desert Protection Mutualism

G Fitzpatrick, G Davidowitz, JL Bronstein

University of Arizona, Tucson, Arizona, USA.

Corresponding author

Ginny Fitzpatrick
Department of Ecology & Evolutionary
Biology
University of Arizona
PO Box 210088, Tucson, AZ
85721 USA
E-Mail: ginfitz@email.arizona.edu

Abstract

In North American deserts, many species of cactus attract ants to their extrafloral nectaries; the ants actively defend the food source, and hence the plant, against herbivores. In thermally extreme environments, however, networks of positive and negative interactions like these are likely to be sensitive to the thermal limitations of each of the interacting species. We compared the thermal tolerance of a common phytophagous cactus bug, *Narnia pallidicornis* (Hemiptera: Coreidae), to that of the ants that defend the cactus *Ferocactus wislizeni* in the Sonoran Desert, USA. We used flow-through respirometry to experimentally determine the thermal limit of the herbivore and compared this to the thermal limits of the ant defenders, determined previously. In the field, we recorded herbivore frequency (proportion of plants with *N. pallidicornis*) and abundance (the number of *N. pallidicornis* per plant) in relation to ambient temperature, ant species presence and identity, and fruit production. We show that *N. pallidicornis* has a higher thermal tolerance than the four most common ant mutualists, and in the laboratory can survive very high temperatures, up to 43°C. Herbivore frequency and abundance in the field were not related to the daily high temperatures observed. Plants that were not defended by ants were occupied by more *N. pallidicornis*, although they showed no reduction in fruit set. Therefore, herbivory is likely to continue on fishhook barrel cacti even at high temperatures, especially those temperatures beyond the thermal tolerance of the ant defenders. The consequences of increased herbivory, however, remain unclear. Mutualisms are essential for ecosystem functioning; it is important to understand the thermal sensitivity of these interactions, especially in light of expected increases in global temperature regimes.

Introduction

Plants exhibit a variety of defenses against the constant threat of damage from herbivores. Mutualistic association with ant species is one such defense. In ant-plant protection mutualisms, ants are employed as guards against herbivores in exchange for rewards such as food and shelter (Beattie, 1985; Heil & McKey, 2003; Marazzi et al., 2013). Mutualisms between ants and plants are common across a variety of habitats (Rico-Gray & Oliveira, 2007; Oliver et al., 2008). Plants tended by ant defenders often have fewer herbivores, less damage from herbivory, and higher reproduction (Rudgers, 2004; Kersch & Fonseca, 2005; Chamberlain & Holland, 2009; Trager et al., 2010).

Plants and insects experience a variety of biotic and abiotic stresses depending on the environment in which they interact. These include intense competition in the tropics, widely fluctuating seasonality in temperate zones, and extreme temperatures in deserts and polar regions (Chamberlain & Holland, 2009). A commonly measured physiological response to temperature is thermal tolerance, the temperature beyond which an organism cannot perform life functions or dies. Depending on the environment, the focus may be on low (Sinclair et al., 2003) or high (e.g., Huey et al., 1992) thermal limits. Thermal tolerance has been frequently measured in insects (Sinclair et al., 2003) and plants (e.g., Nobel, 1982; Nobel, 2003), as well as in fishes (Becker & Genoway, 1979, reviewed by Beitinger et al., 2000), marine organisms such as bivalves (e.g., Peck et al., 2004) and corals (e.g., Berkelmans & van Oppen, 2006), and reptiles (e.g., Angil-

letta et al., 2009). However, there is scant evidence for how interactions among species, as opposed to species considered in isolation, are affected by extreme temperatures. Such studies are imperative to better understand how networks of species interactions, especially those in extreme environments, will respond to increasing temperatures expected as a consequence of global climate change.

In this study, we consider the relative thermal tolerances of ants and herbivores that interact within an ant-plant protection mutualism in the Sonoran Desert, USA. The fishhook barrel cactus, *Ferocactus wislizeni,* is protected by four common ant mutualist species. Typically, only one ant species occupies any given plant for a period of days and even months (Morris et al., 2005). There is a hierarchy of mutualist effectiveness among the ant species (Ness et al., 2006). The least-effective mutualistic ant species, *Forelius pruinosis*, is the most thermally tolerant and occupies hotter plants relative to those occupied by other ant species (Fitzpatrick et al. in review). Thus, rising temperatures might increase the frequency with which plants interact with this ant. Because mutualist effectiveness (as measured by success at herbivore removal) is positively associated with increased production of fruits and seeds (Ness et al., 2006), higher temperatures could have negative reproductive consequences for the fishhook barrel cactus, as well as for other extrafloral nectary-bearing plants in the Sonoran Desert that rely on herbivore protection from this same group of generalized ant defenders.

However, this scenario does not take into account whether or not herbivores themselves are responsive to temperature. If they are more tolerant of high temperatures than are the mutualistic ant defenders, then the consequences for plants may well be severe. However, if herbivores are less tolerant of high temperatures than are the ants, then the effects of rising temperatures might be mitigated: although less-mutualistic ants would dominate in a warmer world, herbivores would be less abundant there. To distinguish between these two possibilities, it is necessary to have data on thermal tolerances of both ant mutualists and the herbivores they deter. We are unaware of the existence of such data for any ant protection system. We chose to focus on one abundant herbivore, the cactus bug *Narnia pallidicornis* (Hemiptera: Coreidae), which feeds upon the reproductive tissues of *F. wislizeni*. We asked: (1) how does *Narnia* thermal tolerance compare to that of *F. wislizeni*'s ant mutualists, and (2) how does *Narnia* abundance and frequency change in response to environmental factors, including ant mutualist identity?

Materials and Methods

Study System

The fishhook barrel cactus, *Ferocactus wislizeni,* is found between 0 and 1500 m in elevation throughout the Sonoran and Chihuahuan Deserts, from Mexico into Arizona,

New Mexico, and Texas, USA. Flowering begins in late July and continues through mid-October, with a peak in late August (McIntosh 2002). The extrafloral nectaries are positioned around the crown of the plant surrounding the fruit. Cacti can live more than 40 years, and individuals produce extrafloral nectar year-round from the time they are 1-2 years old (Fitzpatrick unpublished data); this creates a reliable, long-term resource for foraging ants (Lanan & Bronstein 2013).

This study was conducted at the Desert Laboratory in Tucson, Arizona (32°13' N, 111°05' W), a 370 ha reserve consisting of Sonoran Desert scrub. We used two study plots within which the mutualism between *F. wislizeni* and its ant mutualists has been extensively studied (Ness et al., 2006; Ness et al., 2009; Morris et al., 2005; Lanan & Bronstein, 2013; Fitzpatrick et al. in review; Fitzpatrick & Bronstein unpublished data). Observational data were collected in the field once a month from January through September 2011. Laboratory experiments were conducted from August-October 2012. August-October is the time of year when *F. wislizeni* produces buds and flowers and should be most vulnerable to attack by herbivores that attack these water-rich, delicate tissues. Average high temperatures from August-October range from 29 to 36°C, with extreme temperatures above 44°C (for Tucson, 1981 to 2010; National Oceanic and Atmospheric Association). This time overlaps with the end of the monsoon season, with average monthly rainfall of 3-5 cm.

Four native ant species make up >90% of ant occupants of *F. wislizeni* at this study site: *Forelius pruinosus, Solenopsis aurea, Solenopsis xyloni,* and *Crematogaster opuntiae*. Typically, a single ant species occupies a given plant at a given time. A percentage of the plants are naturally unoccupied by ants, which changes with the season. There is predictable seasonal turnover in the most abundant species across the site (Morris et al. 2005). *Crematogaster opuntiae* is most frequent on *F. wislizeni* (measured as the proportion of plants on which a species is active) during winter and spring months. *Solenopsis aurea*, *S. xyloni,* and *F. pruinosus* are most frequent during summer and autumn (Morris et al. 2005, unpublished data). All four ant species were observed frequently on cacti during this study.

There is a clear hierarchy of mutualistic effectiveness among the ant species (Ness et al. 2006). *Solenopsis xyloni* is most effective at removing herbivores (measured as per capita aggressiveness towards surrogate herbivores intentionally placed on the plant), more so than *C. opuntiae* and *S. aurea*, which are equivalently effective. All three are more effective than *F. pruinosus*. *Forelius pruinosus* was also the least dominant species, as measured by Colley dominance rankings, which reflect the proportion of staged confrontations won (Lanan et al. in review). The dominance hierarchy was: *S. xyloni > S. aurea > C. opuntiae > F. pruinosus*.

The most frequently observed herbivores on the plants are cactus bugs, *Narnia pallidicornis* (Hemiptera: Coreidae; subsequently, *Narnia*), phytophagous insects that specialize on the phloem of cacti, preferring to feed on reproductive

structures such as fruits (Miller et al. 2006; Miller 2007). *Narnia* are iteroparous, with two generations per year. Adults overwinter in debris at the base of plants (Miller et al. 2006). Juveniles are flightless and develop on a single host plant. On *F. wislizeni*, *Narnia* are often observed in the ribs of the cactus and are attacked by ants when they approach the extrafloral nectaries where the ants are foraging (Fitzpatrick, personal observation).

Herbivore thermal tolerance

Individual *Narnia* were collected from the study plots on the day of the trial in which they were used in October of 2012. Three individuals were collected each day for three days. Individuals were weighed before and after the experiment using an analytical balance (Mettler Toledo NewClassic ML). Mass ranged from 64.6-128.0 mg prior to the experiment and from 28.7-76.9 mg after death. Individuals were placed in 10 ml syringes in a climate-controlled chamber (Pelt 5, Sable Systems International) and allowed to acclimate for 15 min. The rate of CO_2 production (VCO_2) was measured using flow-through respirometry (FoxBox, Sable Systems International). Air was scrubbed of CO_2 and water vapor with a flow rate of 100 ml/min. Data were collected and analyzed using Expedata (Sable Systems International). All values of metabolic rate were mass-specific and used individual mass measured prior to the experiment, as above. The temperature of the climate chamber (32, 36, 40, 42, 44, 46, 47, and 48°C) was increased at 60-min intervals, with 5 min of baseline and 15 min of recording for three individuals at each temperature. One of the syringes in the chamber did not record properly, so data for only two individuals per trial were collected. We decreased the temperature interval at higher temperatures to attempt to capture the exact temperature at which individuals ceased CO_2 production. The experimental temperature gradient simulated typical temperatures experienced in the hottest months in the field.

Narnia frequency and abundance

Narnia abundance in the field was measured as the number of individuals observed on long-term study plants throughout 2011. Approximately 182 cacti were regularly included in a census carried out monthly, although the number fell slightly over the study period due to mortality. Observations were typically made between 0600-1100, when herbivores and ants were known to be most active (Fitzpatrick unpublished data). Herbivore frequency was calculated as the proportion of cacti that had at least one *Narnia* present.

Thermal tolerance comparison

To compare the thermal tolerance of *Narnia* to that of the four ant mutualists, we calculated the predicted critical

thermal maximum from the recorded surface temperature of cacti using the linear relationship between ambient and surface temperature in the field. The critical thermal maximum temperatures of the ant species were determined using a water bath by Fitzpatrick et al. (in review) as the temperature above which 50% of ants died. This commonly used method is described by Cerda and Retana (e.g. 1997; 2000). The water bath method for determining thermal tolerance could not be used for *Narnia* because of the low abundance of individuals in the field; the minimum number of individuals necessary for this method was not available for the experiment. Thus, flow-through respirometry was more appropriate for measuring the thermal tolerance of *Narnia*. Plant surface temperature and ambient temperature were recorded in the field using an Omega 871A digital thermometer with type K thermocouple. We then calculated the positive linear relationship between plant surface temperature and ambient temperature by comparing the observed temperatures recorded.

Statistical Analysis

The thermal tolerance curve for *Narnia* was plotted using the means and standard deviations of VCO_2 during the 15-min sampling interval averaged across individuals for each temperature. Sample sizes ranged from 2-6 individuals at the different temperatures, for a total of 35 measurements. The relationship between ambient temperature and plant surface temperature was determined using previously recorded temperatures in the field. To determine *Narnia* frequency and abundance in response to biotic and abiotic environmental factors in the field (time of year, daily high temperature, fruit production, and ant species attendance), we performed an ANOVA to compare values across months. We performed a simple linear regression to determine the relationship between the number of *Narnia* per plant and the number of fruit per plant. These data were log (base 10) transformed to meet the assumptions of linear models.

Results

Herbivore thermal tolerance

There was a curvilinear relationship between mean VCO_2 of *Narnia* and experimental temperatures, peaking at 43°C, then decreasing sharply with further temperature increases (Fig. 1). CO_2 production ceased in all individuals at 48°C. After correcting for the difference between ambient temperature (T_a; based on the method for measuring herbivore thermal tolerance) and surface temperature (P_{st}; based on the method for measuring ant thermal tolerances), and using the relationship between the two as observed in the field ($P_{st} = 1.49T_a - 8.16$), *Narnia* thermal tolerance was higher than for any of the four ant mutalist species (Table 1).
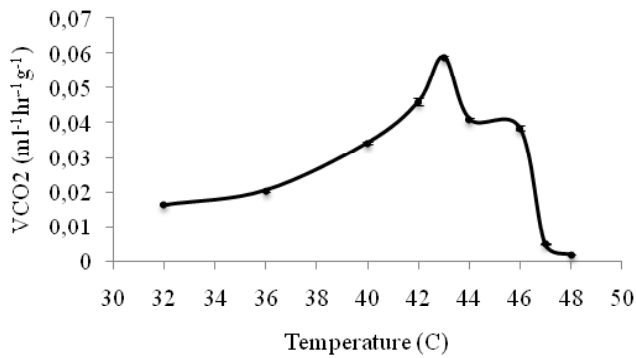
Fig 1. The thermal tolerance curve of *Narnia pallidicornis*. Error bars are averages of the average standard deviation across individuals and in some cases are smaller than the symbol.

Table 1. Thermal tolerance of four common ant mutualists and one herbivore of *Ferocactus wislizeni*. Mutualist effectiveness rank (High, Moderate, Low) is based on the hierarchy as determined by Ness *et al.* (2006). Mean plant surface temperature (P_{st}) was calculated from measurements in the field. Critical thermal maximum values were determined using laboratory experiments and then transformed for comparison using the relationship between ambient temperature (T_a) and plant surface temperature (P_{st}) as determined in the field ($P_{st} = 1.49T_a – 8.16$). Asterisks indicate values that were calculated using this formula describing the relationship between T_a and P_{st}.

Species	Mutualism Role	Mean Ta Termal Max. ºC	Mean Pst Termal Max. ºC (SE)	Mean Pst. ºC (SE)
S. xyloni	High	*37.7	48.0 (0.54)	43.3 (1.6)
C. opuntiae	Moderate	*38.8	49.6 (0.48)	38.9 (1.6)
S. aurea	Moderate	*38.0	48.5 (0.54)	26.4 (1.8)
F. pruinosus	Low	*40.4	52.9 (0.48)	44.4 (1.6)
N. pallidicornis	Herbivore	43.0	55.9	-

Herbivore frequency and abundance

In the field, herbivore frequency (P=0.049) and abundance (P=0.013) were both higher on plants that were not defended by ants (Fig. 2; ANOVA; $R^2 = 0.446$, $F_{8,71} = 1.570$, P=0.049). After accounting for the effect of the number of fruits per plant, herbivore frequency was not related to ant species identity (ANOVA; $R^2 = 0.446$, $F_{4,71} = 1.099$, P=0.366, log-transformed proportion of plants with herbivores). Overall, however, *Narnia* were rare, and the majority of plants had none present during any given observation. Frequency of *Narnia* (i.e., the percentage of plants with at least one individual) ranged from 0% in June to 9% in September. *Narnia* abundance (number per plant) ranged from 0-5. Daily high temperatures in the field ranged from 18.3 to 43.9°C; there was no relationship between temperature and either frequency (regression; $r^2 = 0.014$, $F_{1,71} = 1.011$, P=0.318) or abundance ($r^2 = 0.0001$, $F_{1,71} = 0.010$, P=0.922) of *Narnia*. Surprisingly, *Narnia* frequency (regression; $r^2 = 0.446$, $F_{1,71} = 71.789$, P<0.0001) and abundance ($r^2 = 0.437$,

$F_{1,71} = 56.301$, P<0.0001) were both positively correlated with the number of fruits produced per plant (Fig. 3).
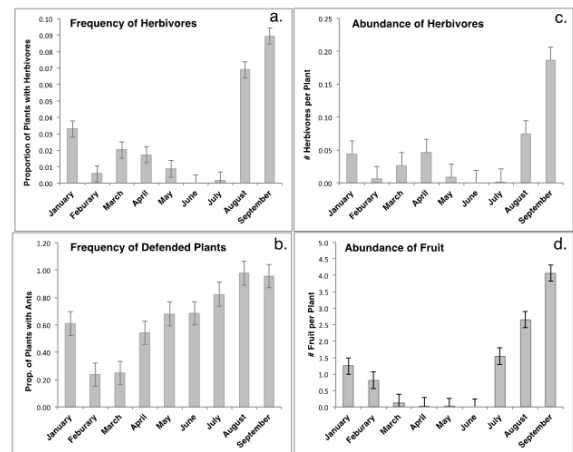
Fig 2. Change in observed frequency and abundance data over time (Jan-Sept 2011). a) Frequency of herbivores measured as the proportion of plants with at least one herbivore observed (ANOVA; $R^2 = 0.196$, $F_{8,71} = 1.920$, P=0.072). b) Frequency of defended plants measured as the proportion of plants on which at least one ant was observed ($R^2 = 0.963$, $F_{8,14} = 19.343$, P=0.001). c) Abundance of herbivores measured as the mean number of herbivores per plant ($R^2 = 0.219$, $F_{8,71} = 2.213$, P=0.038). d) Abundance of fruit measured as the mean number of fruit per plant ($R^2 = 0.218$, $F_{8,71} = 2.219$, P=0.039).

Discussion

Herbivores threaten the survival and reproduction of plants by consuming and damaging plant tissue. In this study, we documented that the herbivore *Narnia pallidicornis*, a common phytophagous insect that specializes on cacti, had a higher thermal tolerance than any of the four ant species that defend its host, the fishhook barrel cactus *Ferocactus wislizeni* (Table 1). As we also found that undefended plants had more herbivores, these results suggest that herbivory may become an increasing threat for *F. wislizeni* as temperatures rise. However, at least at the low frequencies of *Narnia* present at our study site, there was no evidence that the presence of *Narnia* inflicted a fitness cost on *F. wislizeni*. Indeed, fruits were more numerous on plants with *Narnia*. It is possible that initially, cacti with more flowers, buds, and fruit attract more herbivores, producing a positive correlation between fruit number and *Narnia* abundance. On the tree cholla cactus (*Opuntia imbricata*), Miller (2008) found that population dynamics of *Narnia pallidicornis* are determined largely by host plant quality, and that increased allocation to reproduction resulted in more attack by *Narnia* and a higher rate of flower abortion. Our study terminated in September, a month when fruit are in the early stages of formation. If the study had persisted through January, it is possible that the correlation between *Narnia* abundance and fruit number would have reversed, as damage from herbivory might cause a reduction
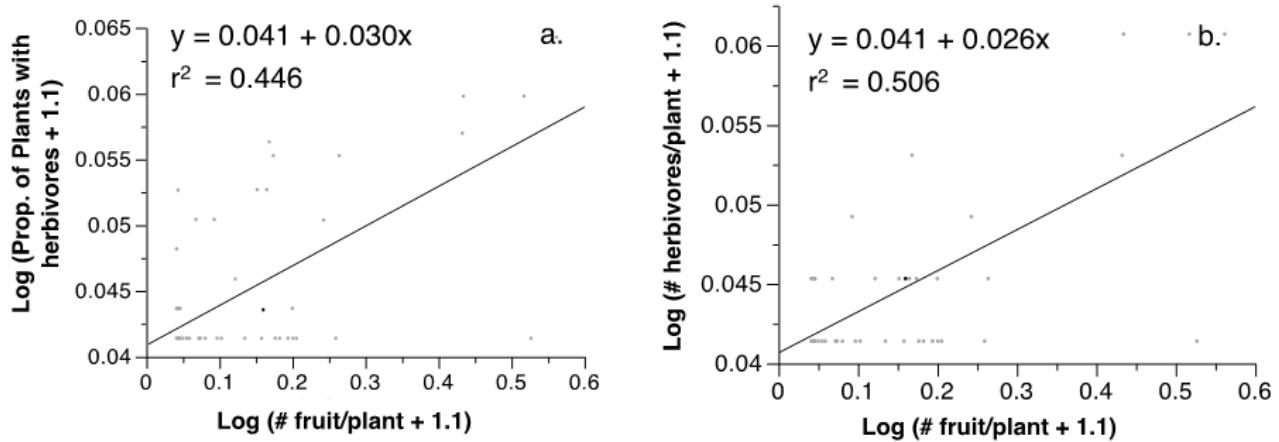
Fig 3. Linear log-log relationship between number of fruit per plant and a) frequency of herbivores ($F_{1,71} = 71.789$, $P < 0.0001$) and b) abundance of herbivores ($F_{1,71} = 56.301$, $P < 0.0001$).

in fruit maturation and an increase in fruit abortion. Also, Miller (2008) observed much higher numbers of *Narnia* on cacti than in this study, which may account for the difference in findings. Future studies that measure seed number and recruitment in relation to herbivory are necessary to accurately estimate the consequences of *Narnia* on cacti reproduction, especially at high temperatures when plants are undefended by ants.

No study to our knowledge has investigated herbivore activity and damage at high temperatures in relation to the presence and identity of ant defenders. In general, warmer temperatures tend to increase insect development rate, which could result in a higher frequency and abundance of herbivores (Ayres & Lombardero, 2000) and increased interaction with the plants on which they feed (O'Connor 2009), as well as with the ants that defend the plants; however, interaction with ant defenders would be lower at high temperatures beyond the tolerance of the ants. Barton (2011) found no direct effect of temperature on grasshoppers, but at high temperatures their predators were forced to seek thermal refuge, which reduced spider predation risk. Barton (2011) also found that the predators from warmer study sites tolerated higher temperatures. In our study system, the least effective mutualistic ant species is a heat specialist that occupies plants that are on average hotter than those occupied by ant species that are more effective at removing and deterring herbivores (Fitzpatrick et al. in review). Thus, plants that are hotter will be defended by a less effective ant defender and consequently be vulnerable to increased abundance of herbivores.

During this study, *Narnia* frequency (the proportion of plants that had at least one individual present) and abundance did not vary predictably with daily high temperatures, although no herbivores were present on plants in the hottest month of June, and there was very low frequency in July. We note that we did not measure *Narnia*'s activity or feeding rate as a function of temperature; it is possible that it behaves differently on hotter days. Also, we studied thermal tolerance in the laboratory, and field studies are needed to confirm *Narnia*'s response to temperature in nature.

If temperatures continue to rise in southwestern North America, as projected by the IPCC (2007), temperatures in the Sonoran Desert could come to exceed *Narnia*'s thermal tolerance. Such high temperatures would also cause mortality in the ant and plant species. However, evidence suggests that in general, species will not be directly threatened by higher temperatures, but will instead adapt or disperse (Berg et al. 2010). This suggests that species interactions are likely to change in unforeseen ways. For this reason, understanding the thermal ecology of species interactions is essential. Given that the consequences of warming at the community level will likely be determined by the complicated interplay of species' life history traits, biotic interactions, and abiotic responses (Berg et al. 2010), more studies that investigate the direct and indirect effects of temperature on interaction networks such as the one that links plants, herbivores, and mutualistic ant defenders are necessary to predict changes in response to rising temperatures.

Acknowledgements

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