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Benefits of living underground: the case of parasite release in the antlion *Myrmeleon timidus*

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Abstract

The omnipresence of pathogens makes them a strong selective pressure for most organisms, generating a variety of defensive responses to fight them. One mechanism by which organisms can release this pressure is avoidance of the pathogens in a spatial or temporal context. To date, only a few biological systems provide evidence that habitat selection can favour an escape from pathogens. An abiotic factor that varies across habitats and influences the evolution of host life history is humidity, which correlates positively with pathogen levels in the environment. Entomopathogenic fungi are abundant in soils and are obligate killers that require humidity for host infection. Antlions (Neuroptera: Myrmeleontidae) are typical soil-dwelling insects that can live several years as larvae, buried in dry and fine substrates where they build their traps. This lifestyle may release them from selective pressure by entomopathogenic fungi. Here, we investigated whether living underground provides protection against the generalist entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. in the antlion *Myrmeleon timidus* Gerstaecker under low or high humidity in the environment. Individuals that were inoculated with the fungus showed greater survival rates when buried in dry sand compared to individuals that were kept half-buried or without sand, with similar outcomes across humidity levels. Given that the diversity of antlions is higher in arid environments, this benefit obtained from pit-building behaviour seems to be a coincidental by-product of this foraging strategy, which would be particularly beneficial in environments where fungal pathogens are common.

Introduction

The omnipresence of pathogens and parasites has made them a strong selective force for most organisms, driving genetic diversity (Haldane, 1949), leading to arms race coevolution between host and pathogen (Pedrini et al., 2015), influencing population dynamics (Hudson et al., 1992), or even affecting community structure and ecosystem functioning (Hudson et al., 2006). In the case of insects, hosts have evolved a number of mechanisms to resist pathogen pressures, including genetic, physiological, and behavioural responses; or even acquiring symbionts that provide protection (Currie et al., 1999; Rolff &

Reynolds, 2009; de Roode & Lefèvre, 2012; Ye et al., 2013). Another proposed evolutionary mechanism to cope with natural enemies is simply moving away from them. For instance, arboreality in vertebrates and invertebrates seems to reduce the pressure by predators, pathogens, or other hazards found in the ground, providing an adaptive significance and reducing the investment in defences (Shattuck & Williams, 2010; Walker & Hughes, 2011; Saeki et al., 2017).

To date, only a few biological systems provide evidence that habitat selection may produce an escape from pathogens or parasites in a spatial, temporal, or evolutionary context (Wcislo, 1996; Mendes et al., 2005; Horrocks et al., 2011). For instance, an abiotic factor that varies regionally and seems to influence host life-history evolution is humidity, which positively correlates with the levels of parasites and pathogens in the environment (Evans, 1974; Moyer et al., 2002). Besides, species living in the humid

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tropics are presumably exposed to more diverse parasites (Moyer *et al.*, 2002; Mendes *et al.*, 2005; Froeschke *et al.*, 2010).

Soils are an example of environments rich in pathogens in which humidity plays an important role for host infection, as is the case for entomopathogenic fungi (Meyling & Eilenberg, 2007; Reber & Chapuisat, 2012). A typical soil-dwelling group of insects are antlion larvae (Neuroptera: Myrmeleontidae). They are distributed worldwide and typically live several years in dry and sandy substrates where they spend a major part of their life buried, using funnel-shaped pits to trap arthropod prey (Topoff, 1977). This lifestyle suggests that building their traps in fine and dry substrates, besides being ideal for foraging (Devetak & Arnett, 2015), may release the antlions from the pressure of entomopathogenic fungi. Here, we investigated whether the pit-building behaviour of antlions provides protection against pathogenic fungi. For this, we studied the antlion *Myrmeleon timidus* Gestaecker and the generalist entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill., which is found in soils globally including urban, agricultural, and natural areas (Rehner, 2005). There is evidence that this fungus is able to kill other fossorial insects in dry soil that shares the same habitats as antlions (Fuxa & Richter, 2004). As successful host infection occurs through the cuticle and depends on humidity (Luz & Fargues, 1999), we measured infection and survival of antlions in soils with different air humidity levels to test whether the pit-building behaviour provides protection in environments that vary in humidity.

Materials and methods

We collected all antlions in the campus of Ciudad del Saber, Panama City, Panama (9.0014°N, 79.5814°W), which is outside any protected area and did not require permission for collection. We identified the species following Heckman's (2017) key for adults and this species is abundant around Panama City. We kept them 1 week in the laboratory and fed them once with one worker of the ant *Atta sexdens* (L.) before the experiments. We cultured *B. bassiana* on a potato dextrose agar for 6 days at 26 °C and harvested the conidia into a water solvent (0.05% Tween 20). We challenged antlions by placing 2 µl of a LD₅₀ solution (8.5×10^7 conidia ml⁻¹), on the thorax and abdomen of each individual, a concentration determined in an unpublished pilot study. The control antlions consisted of individuals treated only with 2 µl of the solvent. To avoid loss of the inoculated solutions, we placed all antlions initially in containers without sand during at least 15 min.

To test whether living underground influences death caused by the pathogen, one third of the antlions were placed in a 460-ml (16 oz) lidded container with 149 g of sterilized sand (n = 51), one third of them with 10.5 g of sterilized sand (n = 50), and the other third in sandless containers (n = 50; hereafter buried, half-buried, and exposed, respectively). We used sieved sand that was on average 18 ± 0.03 µm in diameter. To test whether humidity influences the rate of infection, about half of the antlions from each sand treatment were placed under high-humidity conditions (ca. 88%), similar to conditions observed in Panama during the rainy season (ca. 87–93%). To achieve this, we placed 6 ml of water on a piece of cotton wool, hung it from the lid with a 5-cm wire, and re-humidified it every 2 days with 2 ml of water. The other half of each sand treatment was placed under low-humidity conditions (ca. 55%), with the wire but no wet cotton, and the experiments ran for 21 days. We maintained all the containers at 26 °C under a L12:D12 photoperiod regime. We assured similar distribution of body sizes for all treatments (0.71 ± 0.13 cm) and humidity conditions. In order to monitor survival, we disturbed all individuals by touching them with a spatula to detect responsiveness. We surface-sterilized dead antlions by following Lacey (2012); the method consists of placing and rinsing corpses in 70% alcohol, NaClO, and distilled water. After the surface sterilization, we placed them in tubes with wet cotton wool to confirm death by the fungus (Table S1). Only one of all the challenged individuals did not sporulate (one from the buried treatment); perhaps due to unfavourable environmental conditions for sporulation in that particular container and it was not removed from the analysis as it did not change the output of the analysis.

Statistical analysis

We carried out all the analyses with the statistical software R v.3.6.1 (R Core Team, 2016). We assessed differences in survival rates between treatments using Cox proportional hazard models, as implemented in the survival package. We performed separate analyses for each humidity condition (high or low). We performed pairwise comparisons with the function 'subset' in R and included: (1) challenges as compared to their respective controls, (2) comparisons between challenges, and (3) comparisons between controls. Moreover, we performed pairwise comparisons of the same challenge treatments in the two humidity levels (e.g., exposed – challenge: low vs. high humidity).

Results

In the exposed treatment, the fungal challenge reduced the survival of antlions (control vs. challenge, low humidity: 0

vs. 37.5% mortality, logrank = 10.9, $P < 0.001$; high humidity: 3.8 vs. 65.4% mortality, logrank = 21.5, $P < 0.0001$; Figure 1). The fungal challenge reduced the survival of antlions only at high humidity for the half-buried (control vs. challenge: 3.8 vs. 46.1% mortality, logrank = 12.2, $P < 0.001$; Figure 1B) and buried treatments (control vs. challenge: 0 vs. 19.2% mortality, logrank = 5.4, $P = 0.02$; Figure 1B), but not at low humidity (control vs. challenge, half-buried: 4.1 vs. 4.2% mortality, logrank = 0, $P > 0.05$; buried: 0 vs. 12% mortality, logrank = 3.0, $P = 0.08$; Figure 1A).

We found evidence that being buried provided protection against the fungal challenge because antlions that were buried showed higher survival after challenge with the pathogen compared to the exposed antlions (buried vs. exposed, low humidity: 12 vs. 37.5% mortality, logrank = 4.3, $P = 0.04$; high humidity: 19.2 vs. 65.4% mortality, logrank = 12.8, $P < 0.001$; Figure 1). Similarly, buried antlions showed higher survival than half-buried antlions with the pathogen at high humidity (19.2 vs. 46.1% mortality, logrank = 4.3, $P = 0.04$; Figure 1B) but not at low humidity (12 vs. 4.2% mortality, logrank = 0.96, $P = 0.3$; Figure 1A).

The benefit in surviving the fungal challenge when buried is likely due to reduction of humidity in the sand and not merely due to stress when infected in the exposed condition (challenge, buried vs. exposed), as exposed antlions showed higher mortality against the fungal challenge at high humidity than at low humidity (65 vs. 37.5% mortality, logrank = 4.7, $P = 0.03$). The same trend was observed for half-buried antlions at low and high humidity (4.2 vs. 46.1% mortality, logrank = 10.08, $P = 0.001$).

Discussion

We found evidence that the underground pit-building behaviour of antlions provides protection against a common soil-dwelling pathogenic fungus and this adaptation seems to be protective in environments with varying levels of air humidity, an important factor for fungal infection (Evans, 1974; Luz & Fargues, 1999). Our results also suggest that pit rebuilding by the buried individuals, due to monitoring with the spatula during the experiments, was not metabolically expensive, similar to other studies (Eltz, 1997; Meißner et al., 2012) and did not influence their survival. Moreover, the similar survival rates of controls

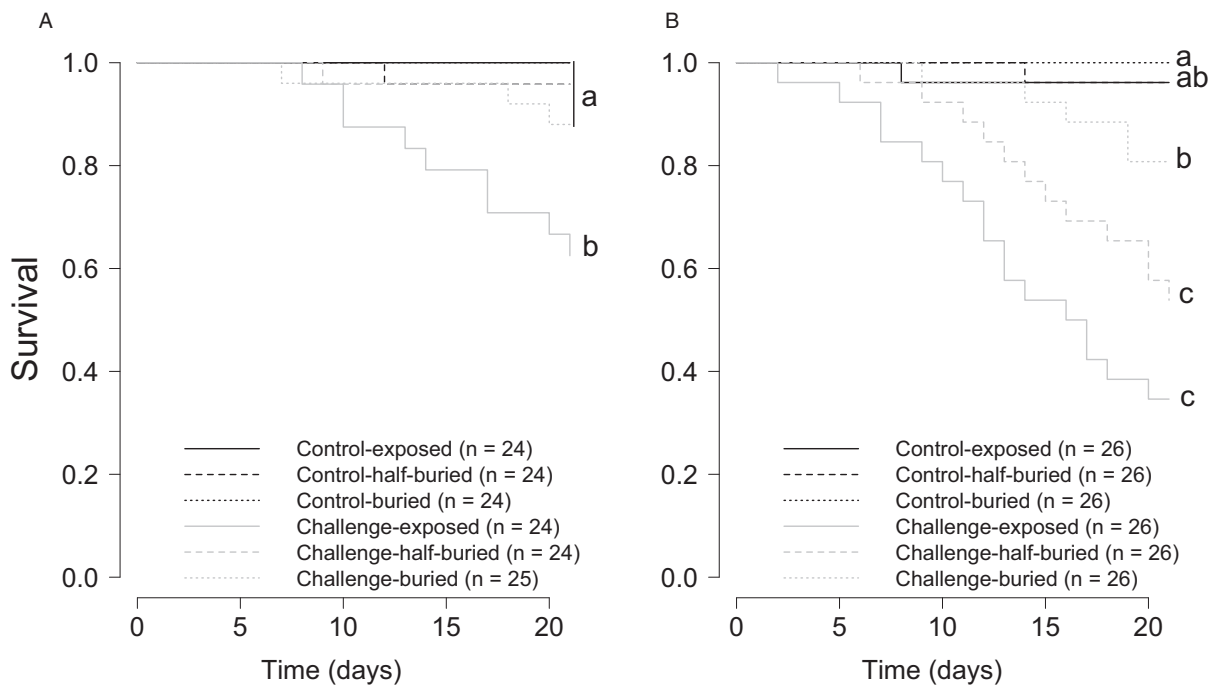


Figure 1 Survival curves of *Myrmeleon timidus* under various sand and inoculation treatments, under (A) low- and (B) high-humidity conditions. Control antlions were inoculated only with the solvent (control), in containers without sand (exposed) or with 10.5 (half-buried) or 149 g of sand (buried). In the challenge, antlions were inoculated with a high dose of *Beauveria bassiana* in containers with the same amounts of sand. Curves within a panel marked with different letters indicate treatments that differed significantly (Cox proportional hazard models: $P < 0.05$). In (A), the line control-exposed overlaps the line control-buried.

indicate that the exposed condition per se was not a significant stress factor influencing the results. Besides, the higher mortality of exposed and half-buried antlions when challenged at high humidity corroborates that the cause was not stress.

Other soil-dwelling insects such as ants and termites show behavioural and physiological adaptations to counteract hazards from pathogenic fungi in the ground, both at the individual and collective levels, such as grooming, spreading of antiseptic substances, and trophallaxis, among others (Rosengaus et al., 1999; Hughes et al., 2004; Fernández-Marín et al., 2006; Hamilton et al., 2011). Less is known about how solitary sedentary organisms such as trap-building predators deal with pathogens, with few studies finding that gregarine parasites (Marques & Ormières, 1978; Devetak & Klokočovník, 2011) and symbiotic bacteria could perhaps influence pathogen resistance (Dunn & Stabb, 2005; Nishiwaki et al., 2007). Because the rate of pit relocation may be low due to predation risk and metabolic cost as seen in other *Myrmeleon* species (Crowley & Linton, 1999), selecting dry substrates for their traps may have coincidentally provided an evolutionary way of escape, as theoretically predicted for host–pathogen coevolution (Gilman et al., 2012). This lower pathogen pressure in dry substrates perhaps contributed to the higher diversity and success of antlions to colonize arid habitats as compared to other myrmeleontiforms (Badano et al., 2018).

However, a phylogenetic approach across species of Myrmeleontiformia could reveal whether there are alternative defences against fungal pathogens. For instance, a prediction could be that species that hunt just beneath the surface without a trap while changing their ambush site frequently (sit-and-pursue strategy) or camouflagers (Loria et al., 2008; Klokočovník & Devetak, 2014; Badano et al., 2018) are under higher pathogen pressures and possess a higher investment in immune defences than trap-building species (sit-and-wait). In fact, the two strategies may show differences in their metabolic rates (higher in sit-and-pursue; van Zyl et al., 1997) and respond differently to other biotic factors. For instance, in the sit-and-wait antlion *Myrmeleon hyalinus* Olivier, individuals were less active in the presence of an active predatory beetle than a sit-and-wait spider. On the contrary, the sit-and-pursue antlion *Lopezus fedtschenkoi* McLachlan showed the reversed pattern, indicating that responses of the two antlion strategies are associated with their distinct foraging modes and to the foraging mode of their predators (Loria et al., 2008).

Our results suggest a multifunctional role of the fossorial trap-building behaviour in antlions, not only for hunting and predator evasion (Badano et al., 2018) but also for

avoidance of pathogenic fungi in environments where they might be common. We could exclude that the passive removal of the conidia by the digging behaviour of the antlions act as a mechanism to avoid the cuticle infection, as we recorded an increase in the mortality of buried challenged individuals compared to controls, at high humidity. Finally, whether specialist pathogenic fungi of antlion larvae exist is unknown, nor is it known whether the relationship antlion – fungus is influenced by other factors such as type of habitat or season. However, we used a generalist pathogenic fungus which is likely to represent the response of other pathogenic fungi to abiotic conditions such as humidity, in line with our hypothesis (Lacey, 2012). Moreover, it is possible that this benefit is observed in other insects with convergent pit-building behaviours (wormlions; Dor et al., 2014).

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References

- Badano D, Engel MS, Basso A, Wang B & Cerretti P (2018) Diverse Cretaceous larvae reveal the evolutionary and behavioural history of antlions and lacewings. *Nature Communications* 9: 3257.
- Crowley PH & Linton MC (1999) Antlion foraging: tracking prey across space and time. *Ecology* 80: 2271–2282.
- Currie CR, Scott JA, Summerbell RC & Malloch D (1999) Fungus-growing ants use antibiotic producing bacteria to control garden parasites. *Nature* 398: 701–704.
- Devetak D & Arnett AE (2015) Preference of antlion and wormlion larvae (Neuroptera: Myrmeleontidae; Diptera: Vermileonidae) for substrates according to substrate particle sizes. *European Journal of Entomology* 112: 500–509.
- Devetak Du & Klokočovník V (2011) Eugregarinorida in *Myrmeleon* and *Euroleon*: first evidence of the gregarines (Protozoa: Apicomplexa) in adult antlions (Insecta: Neuroptera: Myrmeleontidae). *Annales Series Historia Naturalis* 21: 119–124.
- Dor R, Rosenstein S & Scharf I (2014) Foraging behaviour of a neglected pit-building predator: the wormlion. *Animal Behaviour* 93: 69–76.
- Dunn AK & Stabb EV (2005) Culture-independent characterization of the microbiota of the ant lion *Myrmeleon mobilis* (Neuroptera: Myrmeleontidae). *Applied and Environmental Microbiology* 71: 8784–8794.
- Eltz T (1997) Foraging in the ant-lion *Myrmeleon mobilis* Hagen 1888 (Neuroptera: Myrmeleontidae): behavioral flexibility of a sit-and-wait predator. *Journal of Insect Behavior* 10: 1–11.

- Evans HC (1974) Natural control of arthropods, with special reference to ants (Formicidae), by fungi in the tropical high forest of Ghana. *Journal of Applied Ecology* 11: 37–49.
- Fernández-Marín H, Zimmerman JK, Rehner SA & Wcislo WT (2006) Active use of the metapleural glands by ants in controlling fungal infection. *Proceedings of the Royal Society of London B* 273: 1689–1695.
- Froeschke G, Harf R, Sommer S & Matthee S (2010) Effects of precipitation on parasite burden along a natural climatic gradient in southern Africa – implications for possible shifts in infestation patterns due to global changes. *Oikos* 119: 1029–1039.
- Fuxa JR & Richter AR (2004) Effects of soil moisture and composition and fungal isolate on prevalence of *Beauveria bassiana* in laboratory colonies of the red imported fire ant (Hymenoptera: Formicidae). *Environmental Entomology* 33: 975–981.
- Gilman RT, Nuismer SL & Jhwueng DC (2012) Coevolution in multidimensional trait space favours escape from parasites and pathogens. *Nature* 483: 328–330.
- Haldane J (1949) Disease and evolution. *Ricerca Scientifica* 19: 168–174.
- Hamilton C, Lejeune BT & Rosengaus RB (2011) Trophallaxis and prophylaxis: social immunity in the carpenter ant *Camponotus pennsylvanicus*. *Biology Letters* 7: 89–92.
- Heckman CW (2017) Neuroptera (Including Megaloptera). Springer, Cham, Switzerland.
- Horrocks NPC, Matson KD & Tieleman BI (2011) Pathogen pressure puts immune defense into perspective. *Integrative and Comparative Biology* 51: 563–576.
- Hudson PJ, Dobson AP & Newborn D (1992) Do parasites make prey vulnerable to predation? Red grouse and parasites. *Journal of Animal Ecology* 61: 681–692.
- Hudson PJ, Dobson AP & Lafferty KD (2006) Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology and Evolution* 21: 381–385.
- Hughes WOH, Thomsen L, Eilenberg J & Boomsma JJ (2004) Diversity of entomopathogenic fungi near leaf-cutting ant nests in a neotropical forest, with particular reference to *Metarhizium anisopliae* var. *anisopliae*. *Journal of Invertebrate Pathology* 85: 46–53.
- Klokočovník V & Devetak D (2014) Pit-builder vs. non-pit-builder: advantage of trap building strategy in antlion larvae does not mean. *Behaviour* 151: 653–668.
- Lacey LA (2012) *Manual of Techniques in Invertebrate Pathology*, 2nd edn. Academic Press, London, UK.
- Loria R, Scharf I, Subach A & Ovidia O (2008) The interplay between foraging mode, habitat structure, and predator presence in antlions. *Behavioral Ecology and Sociobiology* 62: 1185–1192.
- Luz C & Fargues J (1999) Dependence of the entomopathogenic fungus, *Beauveria bassiana*, on high humidity for infection of *Rhodnius prolixus*. *Mycopathologia* 146: 33–41.
- Marques A & Ormières R (1978) *Actinocephalus acanthaclisis* n. sp., eugregarine parasite des larves d'*Acanthaclisis* (Névropt. Myrmeleontidae). *Zeitschrift für Parasitenkunde* 165: 159–165.
- Meißner K, Feike M, Pires C & Stetzkowski J (2012) Überlebensstrategien und Kosten-Nutzen-Bilanzen einer räuberischen Insektenlarve (*Euroleon nostras* Fourcroy, Myrmeleontidae). *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie* 18: 591–599.
- Mendes L, Piersma T, Lecoq M, Spaans B & Ricklefs RE (2005) Disease-limited distributions? Contrasts in the prevalence of avian malaria in shorebirds species using marine and freshwater habitats. *Oikos* 109: 396–404.
- Meyling NV & Eilenberg J (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. *Biological Control* 43: 145–155.
- Moyer BR, Drown DM & Clayton DH (2002) Low humidity reduces ectoparasite pressure: implications for host life history evolution. *Oikos* 97: 223–338.
- Nishiwaki H, Ito K, Shimomura M, Nakashima K & Matsuda K (2007) Insecticidal bacteria isolated from predatory larvae of the antlion species *Myrmeleon bore* (Neuroptera: Myrmeleontidae). *Journal of Invertebrate Pathology* 96: 80–88.
- Pedrini N, Ortiz-Urquiza A, Huarte-Bonnet C, Fan Y, Juárez MP & Keyhani NO (2015) Tenebrionid secretions and a fungal benzoquinone oxidoreductase form competing components of an arms race between a host and pathogen. *Proceedings of the National Academy of Sciences of the USA* 112: E3651–E3660.
- R Core Team (2016) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Reber A & Chapuisat M (2012) Diversity, prevalence and virulence of fungal entomopathogens in colonies of the ant *Formica selysi*. *Insectes Sociaux* 59: 231–239.
- Rehner SA (2005) Phylogenetics of the insect pathogenic genus *Beauveria*. *Insect-Fungal Associations: Ecology and Evolution* (ed. by FE Vega & M Blackwell), pp. 3–27. Oxford University Press, Oxford, UK.
- Rolf J & Reynolds SE (2009) *Insect Infection and Immunity: Evolution, Ecology and Mechanisms*. Oxford University Press, Oxford, UK.
- de Roode JC & Lefèvre T (2012) Behavioral immunity in insects. *Insects* 3: 789–820.
- Rosengaus RB, Traniello JFA, Chen T, Brown JJ & Karp RD (1999) Immunity in a social insect. *Naturwissenschaften* 86: 588–591.
- Saeki I, Niwa S, Osada N, Hyodo F, Ohta T et al. (2017) Adaptive significance of arboreality: field evidence from a tree-climbing land snail. *Animal Behaviour* 127: 53–66.
- Shattuck MR & Williams SA (2010) Arboreality has allowed for the evolution of increased longevity in mammals. *Proceedings of the National Academy of Sciences of the USA* 107: 4635–4639.
- Topoff H (1977) The pit and the antlion. *Natural History* 86: 65–71.
- Walker TN & Hughes WOH (2011) Arboreality and the evolution of disease resistance in ants. *Ecological Entomology* 36: 588–595.
- Wcislo W (1996) Parasitism rates in relation to nest site in bees and wasps (Hymenoptera: Apoidea). *Journal of Insect Behavior* 9: 643–656.

Ye YH, Woolfit M, Rancès E, O'Neill SL & McGraw EA (2013) *Wolbachia*-associated bacterial protection in the mosquito *Aedes aegypti*. PLoS Neglected Tropical Diseases 7: e2362.

van Zyl A, van der Linde TCDK & Grimbeek RJ (1997) Metabolic rates of pitbuilding and non-pitbuilding antlion larvae (Neuroptera: Myrmeleontidae) from southern Africa. Journal of Arid Environments 37: 355–365.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Total number of antlion corpses that showed sporulation of *Beauveria bassiana* after surface sterilization and isolation in a humid container, from the experiments with low- and high-humidity conditions. Almost all the corpses that were inoculated with the fungal solution (Challenge) showed evidence of fungal infection.

Figure S1. Example of the containers used for the experiments, showing buried, half-buried, and exposed treatments.

Figure S2. Example of exposed individuals in which the fungus has sporulated after death of the antlion.