Journal of Insect Physiology 82 (2015) 114–121

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Accumulation of free amino acids during exposure to drought in three springtail species

Martin Holmstrup [a,*], Stine Slotsbo [a], Jan Rozsypal [b], Per G. Henriksen [c], Mark Bayley [c]

[a] Aarhus University, Department of Bioscience, Vejlsøvej 25, PO Box 314, 8600 Silkeborg, Denmark
[b] Institute of Entomology, Biology Centre of The Czech Academy of Sciences, Branišovská, 370 05 České Budějovice, Czech Republic
[c] Aarhus University, Department of Bioscience, C.F. Møllers Allé, Universitetsparken, 8000 Aarhus C, Denmark

ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 23 September 2015
Accepted 25 September 2015
Available online 30 September 2015

Keywords:
Collembola
Desiccation
Osmoregulation
Soil water content
Soil fauna

ABSTRACT

Springtails are closely related to insects, but they differ from these with respect to water balance, in particular because springtails are small and have high integumental permeability to water. Here we report a series of experiments addressing the dynamics of osmoregulation, water content and accumulation of free amino acids (FAAs) in three springtail species during exposure to a gradually increasing environmental desiccation simulating conditions in drought exposed soil. *Folsomia candida* and *Protaphorura fimata* (both living in the deeper soil layers; euedaphic species) were active throughout the 3 week exposure, with the developing drought regime ending at −3.56 MPa (the soil water activity at the permanent wilting point of plants is −1.5 MPa) and remained hyperosmotic (having an body fluid osmolality higher than the corresponding environment) to their surrounding air. *Sinella curviseta* (living in upper soil/litter layers; hemiedaphic species) also survived this exposure, but remained hypoosmotic throughout (i.e. with lower osmolality than the environment). The body content of most FAAs increased in response to drought in all three species. Alanine, proline and arginine were the most significantly upregulated FAAs. By combining our results with data in the literature, we could account for 82% of the observed osmolality at −3.56 MPa in *F. candida* and 92% in *P. fimata*. The osmolality of *S. curviseta* was only slightly increased under drought, but here FAAs were considerably more important as osmolytes than in the two other species. We propose that FAAs probably have general importance in drought tolerance of springtails.

1. Introduction

Springtails (Collembola) are a species rich group of invertebrates inhabiting soil, litter or vegetation of most terrestrial ecosystems (Hopkin, 1997). Springtails can be very abundant in soil decomposer communities where they are grazers of microorganisms, thus stimulating nutrient cycling (Faber, 1991; Seastedt, 1984; Verhoef and Brussard, 1990). Soil-dwelling springtails are closely related to insects, but have several unique characteristics, in particular with respect to water balance. These characteristics include small size (body length usually 1–3 mm and rarely up to 5 mm), epidermal respiration (no spiracles), and high integumental permeability.

Early studies on drought tolerance of springtails employed methodologies adapted from insect studies often with focus on evaporative water loss at very low humidities or even in dry air (e.g. Harrison et al., 1991). At such low humidities, springtail survival can be measured in minutes to hours and dry air is obviously not relevant for soils, where RH typically does not fall below 90% RH even during severe drought episodes (Hillel, 1998; Maraldo and Holmstrup, 2009). Moreover, soil drying is a relatively slow process, allowing physiological acclimation responses of springtails to be effective. Studies of drought tolerance of springtails (and other soil invertebrates) should therefore strive to mimic the conditions of drying soil as closely as possible. When mimicking soil conditions and exposing the springtails to high relative humidity (e.g. >98% RH) we have previously shown that soil dwelling springtails are able to absorb water vapour from the soil pore air by decreasing the osmotic pressure of their fluids below the equivalent soil water potential (Bayley and Holmstrup, 1999; Holmstrup et al., 2001; Kærsgaard et al., 2004). Body fluid osmotic pressure of drought-exposed springtails is decreased (i.e. osmolality increases) by production of compatible osmolytes such as sugars (e.g. glucose) and sugar alcohols (e.g. myo-inositol) (Bayley and Holmstrup, 1999; Holmstrup and Bayley, 2013; Holmstrup et al., 2001). Compatible osmolytes are small organic molecules that can occur in high concentrations without detrimen-

* Corresponding author.
E-mail address: martin.holmstrup@bios.au.dk (M. Holmstrup).

http://dx.doi.org/10.1016/j.jinsphys.2015.09.005
0022-1910/

tal effects on cellular processes (Hochachka and Somero, 2002). Our previous studies have shown that soil-dwelling springtails can reduce or completely replenish water loss and survive during exposure to drought conditions equivalent to 98.2% RH or a soil water potential of ca. −2.5 MPa (the permanent wilting point of plants is ca. −1.5 MPa).

Drought tolerance of springtails can be considerably enhanced if the animals are allowed to acclimate to mild drought conditions before exposure to severe drought (Holmstrup et al., 2002). Sjursen et al. (2001) showed that the RH causing 50% mortality of *Folsomia candida* acutely exposed to drought conditions was 97% RH, but dropped to 94.7% RH if animals were pre-acclimated at 98.2% RH for a week before exposure to more severe drought conditions. Thus, slowly increasing the drought intensity (simulating natural progression of drought in the field) can give more realistic indications of the drought tolerance potential of a particular springtail species than acute exposure in simpler laboratory experiments. Such ramping experiments have shown that *F. candida* and *Protaphorura tricampata*, both species that inhabit the deeper soil layers, can survive and remain active for at least a month at conditions equivalent to soil water potentials down to −3.5 MPa (Holmstrup and Bayley, 2013; Waagner et al., 2011, 2012).

These studies on body fluid osmoregulation and water vapour absorption in springtails have focused on the sugars and polyols that are synthesized and accumulated during drought. However, previous studies have indicated that free amino acids (FAAs) also play an important role in increasing the body fluid osmolality. Witteveen et al. (1987) showed that FAAs are strongly upregulated in *Anurida maritima* during saline inundation of its littoral habitat. Further, *P. tricampata* subjected to drought accumulated alanine and proline during increasing drought stress, and these compatible osmolytes increased from about 20 to more than 300 μmol g^{-1} dry weight (combined) when the drought intensity was highest (Holmstrup and Bayley, 2013). In addition, Bayley et al. (2010), suggested that FAAs might play a dual role during dehydration by functioning as both compatible osmolytes and as a sink for ammonium in situations where urine production might be reduced. In order to explore whether FAAs are also of importance in other springtail species, and hence a more general phenomenon, the aim of the present study was to investigate the dynamics of osmoregulation, water content and FAA accumulation during exposure to gradually increasing drought stress.

2. Materials and methods

2.1. Springtails

We have included species representing a variety of vertically distributed habitats in the soil profile. Three species of springtails were studied in detail: *Protaphorura fimata* and *F. candida*, which are euedaphic species, and *Sinella curviseta* which has a more hemiedaphic life-history (Kærsgaard et al., 2004). Euedaphic species live in deeper soil layers whereas hemiedaphic species are more confined to upper litter layers and soil surface. All studied species were cultured on moistened gypsum/charcoal and fed dried baker's yeast in our laboratory for several years. In all experiments presented here, we used a mixture of adult specimens of both sexes selected at random from stock cultures. Thus, no age synchronization was performed before experiments.

2.2. Drought experiment

Laboratory experiments were designed to simulate the soil water potential experienced by the springtails in the field during an extreme yet natural drought event (Holmstrup and Bayley, 2013). NaCl solutions were used to create controlled water potentials (relative air humidities) in small vials where the springtails were kept (Holmstrup, 1997). Briefly, the springtails were placed in open-top (but covered with 100 μm nylon net preventing escape of the springtails) plastic sample vials (3 cm high, 1.6 cm diameter) which were glued to the floor of 160-ml plastic cups (4.2 cm high, 7 cm diameter) containing 25 ml aqueous NaCl solution, sealed with Parafilm™ and tightly fitting plastic lids. The air in this small closed system rapidly equilibrates with the salt solution (following Raoult's law). By replacing and increasing the NaCl concentration of the beakers during a 22-d period between 0 and 45.3 g L^{-1}, water potential of this system was decreased gradually from 0 (i.e. control conditions) to −3.56 MPa, after which the water potential was quickly brought back to starting conditions (100% RH) allowing the springtails to recover after the drought exposure (i.e. recovery for 24 h at control conditions). Vials with springtails were sampled at intervals to estimate survival, total water content, osmotically active water, osmotic pressure of body fluids and accumulation of free amino acids. Only survivors were used for these measurements. In addition, the osmotic pressure of the NaCl solution used to control humidity in the drought beakers was measured to verify exposure.

2.3. Survival and behaviour

The survival of representative vials was scored on the basis of animals that showed locomotor activity either spontaneously or after gentle tactile stimulus. Animals that failed to respond were scored as dead. Observations of behaviour (agility) were recorded. Survival of springtails in a control situation (i.e. at water saturated conditions) was not assessed over the experimental period in the present study since lethality of the tested species over a 25-day period under control conditions is negligible (M. Holmstrup, unpublished).

2.4. Water content and osmolality

Total water content of springtails and osmolality of their body fluids were measured during the drought experiments to follow the development of these parameters during increasing drought stress and after recovery. Total water content was determined gravimetrically using samples of 10–20 animals (determination of fresh mass, then drying at 60 °C until constant mass was achieved (24 h) followed by dry mass determination) and expressed as mg water mg^{-1} dry weight (dw). The weighing was carried out using a Sartorius Micro SC 2 balance accurate to ± 1 μg (Sartorius AG, Goettingen, Germany). The osmotic pressure of body fluids was determined using Wescor C-52 sample chambers connected to a Wescor HR 33T Dew Point Microvoltmeter operated in the dew point mode. The whole sample of surviving animals from each replicate was transferred to a sample holder of the C-52 chambers and crushed using a cylindrical aluminium rod fitting the well of the sample holder (Bayley and Holmstrup, 1999). Osmotic pressure of NaCl solutions used for drought beakers was measured by soaking filter paper discs in the solution and placing them in the sample holder. The accuracy of this equipment is within ±0.02 MPa.

2.5. Estimation of osmotically active water by differential scanning calorimetry

In order to estimate the osmotic contributions of compatible osmolytes it was necessary to measure how much of the total water content was osmotically active and thus functioning as a solvent. The fresh mass of samples of 10–15 springtails was determined to the nearest 0.01 mg (Mettler Toledo, Greifensee,