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EXPOSURE TO DESICCATING CONDITIONS AND CROSS-TOLERANCE WITH THERMAL STRESS IN THE LESSER MEALWORM *ALPHITOBIUS DIAPERINUS* (COLEOPTERA: TENEBRIONIDAE)

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RÉSUMÉ.— *Exposition à des conditions desséchantes et tolérance croisée à des stress thermiques chez le petit ténébrion* Alphitobius diaperinus (Coleoptera : Tenebrionidae).— Le développement et la prolifération rapides des espèces exotiques dans de nouvelles régions peuvent être liés à leur importante plasticité phénotypique. Par ailleurs, les phénomènes de tolérance croisée, autrement dit les réponses mises en place afin de mieux tolérer un stress environnemental amélioreraient également la tolérance à un autre stress, pourrait contribuer au succès invasif de certains insectes exotiques. Par exemple, de bonnes capacités de résistance au froid ont été documentées chez plusieurs insectes d'origine tropicale, bien que ces espèces ne soient pas soumises à de basses températures dans leurs milieux naturels. Des observations similaires ont été signalées chez le petit ténébrion *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), un coléoptère invasif d'origine tropicale, qui se développe dans les denrées stockées. Cette espèce présente un niveau élevé de tolérance à la dessiccation, et l'existence d'une tolérance croisée entre la tolérance à la dessiccation et au froid pourrait ainsi faciliter établissement et son expansion dans les milieux naturels des régions tempérées. Dans cette étude, nous avons examiné si les adultes d'*A. diaperinus* préalablement exposés à la dessiccation présentaient des capacités de survie accrues lorsqu'ils étaient réexposés cette condition de dessiccation (7 % d'humidité relative), ou bien à des températures basses (5°C), ou élevées (38°C). Nos résultats indiquent que la durée de la survie est similaire entre les insectes pré-exposés à la dessiccation et les témoins lorsque ceux-ci sont ensuite maintenus à 7 % HR ou 5°C. Cependant, nous avons constaté que les adultes d'*A. diaperinus* préalablement exposés à 7 % HR présentaient une durée de survie accrue au chaud (temps nécessaire pour obtenir une mortalité de 50 % de la population allongé de 4 jours). Dans une seconde expérience, nous avons inclus une période de récupération suite à l'exposition préalable à la dessiccation (7 % HR). Les insectes ont été maintenus à humidité modérée (50% HR) ou bien à forte humidité (100% HR) pendant 12h avant que leur survie à la dessiccation, la tolérance au froid et au chaud ne soient testées. Nous avons alors constaté que la réhydratation générait des réponses différentes en termes de tolérance croisée par rapport à la première expérience. En effet, la survie au froid (5°C) a dans ce cas été augmentée (temps nécessaire pour obtenir une mortalité de 50% de la population allongé de 3 jours), tandis qu'aucun effet n'a été obtenu sur la tolérance au chaud. Par ailleurs, nous n'avons trouvé aucun effet de l'exposition préalable à la dessiccation sur la capacité de survie ultérieure à cette condition expérimentale. En résumé, nous avons obtenu des preuves de tolérance croisée entre les conditions expérimentales desséchantes (7 % HR) et chaudes (38°C), et entre la dessiccation et les conditions froides (5°C). Ces effets sont hautement dépendants des conditions expérimentales, plus particulièrement de la mise en place d'une période de récupération avant l'exposition à un autre stress. Ces résultats suggèrent l'existence de mécanismes de tolérance croisée complexes.

SUMMARY.— The ability of invasive species to rapidly expand into new regions may be related to their greater phenotypic plasticity. In addition, cross-tolerance, *i.e.* a response by which tolerance to one stress can enhance tolerance to another stress, may contribute to the invasive success of some alien insects. For instance, a certain level of cold hardiness has been documented in several tropical insects, although these species never experience cool or freezing temperatures in their natural environments. Similar observations were reported in the lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), an invasive beetle of tropical origin that thrives in stored products. This species shows a high basal desiccation tolerance, and cross-tolerance between desiccation and cold could have facilitated its successful establishment in temperate areas. In this study, we examined if dry pre-exposed beetles have increased ability to survive desiccation (7 % RH), cold (5°C), or heat stress (38°C). Survival duration remained similar between dry pre-exposed and control beetles that were subjected to desiccation or cold stress. However, we found that dry pre-exposed beetles had increased heat tolerance (Lt_{50} postponed by 4 days). In a second experiment, dry pre-exposed insects were allowed to recover either with water supply or with moderate humidity (50 % RH) before being assessed for desiccation, cold and heat tolerance. We found that rehydration changed cross-tolerance patterns: cold survival was promoted (Lt_{50} postponed by 3 days), while heat tolerance was not affected anymore. We found no effect of dry pre-exposure on the subsequent ability to survive dry condition. In summary, we found some evidence of cross-tolerance between desiccating and heat

conditions, and between desiccating and cold conditions, but these effects depended on whether the beetles were allowed to recover or rehydrate before being exposed to another stress. These findings suggest complex and differential cross-tolerance mechanisms.

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In many natural habitats, insects have to cope with varying environmental conditions, with the magnitude and frequency of these fluctuations varying geographically (Colinet *et al.,* 2015). As a result, environmental variability can impose range limits on natural populations, depending on a population's ability to cope with environmental perturbation (Addo-Bediako *et al.*, 2000). As a group, insects have been successful in colonizing large geographical areas and a diverse range of habitats. The worldwide distribution of insects results from a diverse set of responses for dealing with environmental constraints. These responses may act on different timescales, from long-term evolutionary adaptation to rapid phenotypic adjustment (Fischer & Karl, 2010). At short timescales, insects have the capacity to adjust their behaviour, morphology or physiology through plastic responses that often promote fitness (Angilletta, 2012; Colinet & Hoffman, 2012).

The ability of aliens to rapidly colonize new regions has long been thought to be related to a higher degree of phenotypic plasticity (Baker, 1965; Richards *et al.*, 2006; Davidson *et al.*, 2011) compared to native populations. Interestingly, this assumption has often been examined by investigating the responses of alien insects to a single environmental condition, whereas in nature individuals may have to cope with several environmental stresses occurring concurrently, or to successive environmental stresses whose effects could be related or distinct. The possible occurrence of common responses for dealing with different stressors has thus emerged in the literature (Sulmon *et al.*, 2015), starting from the idea of exaptation (Ring & Danks, 1994), and now developed within the concept of cross-tolerance (Sinclair *et al*., 2013). Cross-tolerance refers to a phenomenon by which tolerance to one environmental stressor results in increased tolerance to another stress (Sinclair *et al.*, 2013). This concept could result from shared pathways ('cross talk') and common mechanisms underlying tolerance to different types of stress. For instance, in responses to thermal and water stresses, organisms commonly exhibit increased hemolymph osmolality, which is achieved by the synthesis and accumulation of compatible solutes (polyols, amino acids, sugars) (Yancey, 2001). These so-called low molecular weight compounds are referred to as cryoprotectants or osmoprotectants, depending on the environmental stressor, even though these metabolites can be identical. Tolerance to multiple stressors has been reported following selection on a single stress resistance trait (Kellermann *et al.*, 2009; MacMillan *et al.*, 2009; Bubliy *et al.*, 2012); hence, it is also conceivable that pre-exposure or acclimation to one stress could promote tolerance to other stresses. Such cross-tolerance may be adaptive in organisms living in multiple-stress environments. For instance, episodes of heat stress often coincide with water restriction, and thus responses to either heat or desiccation may promote resistance to both stresses. Along the same line, cold acclimation has also been reported to confer increased ability to survive desiccating conditions and *vice versa* (Ring & Danks, 1994; Bayley *et al.*, 2001; Worland & Block, 2003; Michaud *et al.*, 2008), and this can most probably be explained by the similar effects generated from cold and desiccation at a cellular level (Sinclair *et al.*, 2013).

There are several examples of tropical insects exhibiting significant cold hardiness (Ring & Danks, 1994; Nedved, 1999), even though they are never exposed to cool or freezing temperatures in their natural environments (but see Cloudsley-Thompson, 1973 for a study of the cold tolerance of arachnids from desert environments). Similar observations were reported from the lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), an invasive beetle of tropical origin that was first observed from poultry houses in Brittany (France) in 1977 (Le Torc'h, 1979). Despite the sub-Saharan origin of this beetle (Vuattoux, 1968), this species has recently been detected outside buildings in Europe, in cultivated areas (Klejdysz & Nawrot, 2010) and in forests

(Borges & Mériguet, 2005). Hence, this pest has the potential to permanently populate wild habitats of temperate colonized areas, and this invasion success would require the development of certain level of cold tolerance.

In its natural habitat, the lesser mealworm is most often found in stored products, which are usually hot and dry micro-environments. Adult *A. diaperinus* are thus characterized by a great ability to survive desiccating (Renault & Coray, 2004) and heat (Salin *et al.* 2006), and cross tolerance between these two environmental variables is thus expected. Most importantly, it has been suspected that these responses could contribute to enhancing its survival to prolonged cold exposures (Renault *et al.*, 1999, 2004). For instance, cross-tolerance between desiccation and low temperature could provide increased ability to survive cool conditions in temperate ecosystems. So far, such cross-tolerance response has not been explored in this insect model. In this study, we examined cross-tolerance among different environmental stressors: desiccation, cold and heat stresses in adult *A. diaperinus*. We hypothesized that (1) pre-exposure to dry conditions would increase the subsequent desiccation tolerance of the beetles, (2) the beneficial effects of dry pre-exposure would persist even after a rehydration recovery (*i.e.* carry over effect), and (3) dry pre-exposure would promote cold and heat tolerance (*i.e.* cross-tolerance).

MATERIAL AND METHODS

INSECTS AND REARING CONDITIONS

Adults of the lesser mealworm, *A. diaperinus* were hand-collected from the litter of a poultry house in Taupon (Brittany, France, 47°57'27"N, 2°26'21"W) in April 2014. Larvae and adults were then transported to the laboratory and reared under controlled conditions in aerated plastic boxes (28.5 x 27.5 x 9.0 cm) containing a 3 cm layer of the litter from the poultry house, at 25°C and 50 % relative humidity (RH). The specimens were supplied with water and food (in the form of dry dog food) *ad libitum*. Adult beetles were 1-3 months old when they were used for the experiments.

PRE-TREATMENT

The effect of dry pre-exposure on the desiccation tolerance of *A. diaperinus* adults was assessed either without (experiment 1) or with (experiment 2) rehydration recovery phase just before the subsequent stress exposure. In the first experiment, the beetles were not allowed to recover after they were pre-exposed to dry conditions. Adults were pre-exposed at 7 % RH and 20°C for 4.5 days, and were directly assessed for desiccation, cold and heat tolerance. Control insects were pre-exposed at 100 % RH and 20°C for 4.5 days. Four hundred and twenty individuals were used per experimental condition to ensure sufficient number of individuals and replicates at each time point during the stress tolerance assays. In the second experiment, two dry pre-exposure durations were used: (i) insects exposed at 7 % RH and 20°C for 0.5 day (short exposure), and (ii) insects exposed at 7 % RH and 20°C for 4.5 days (long exposure). Control insects were pre-exposed at 100 % RH and 20°C for 4.5 days. These three groups were then divided into two sub-treatments: (i) insects that were allowed to recover for 0.5 day at 23 ± 3°C (laboratory temperature) with water *ad libitum* and 100 % RH (N = 420 individuals) (*i.e.* recovery with rehydration), thereafter referred to as 'with rehydration' or (ii) insects that were returned to moderate humidity (50-55 % RH; laboratory conditions) at 23 ± 3°C (laboratory temperature) for 0.5 day (N = 420 individuals), thereafter referred to as 'without rehydration'. During dry exposure treatments, all insects were individually held into opened 2 mL microtubes and then placed inside sealed plastic box filled with *ca.* 3 cm of silicagel. All beetles were food- and water-deprived during the experiment. RH was monitored inside the boxes using Hobo data logger (model U12-012, Onset Computer Corporation, Bourne, USA). The same procedure was applied to control insects, except that silicagel was replaced by water soaked filter paper.

DESICCATION, COLD AND HEAT TOLERANCE ASSAYS

After dry pre-exposure, the ability of *A. diaperinus* to survive desiccation, cold and heat stress was assessed by continuously maintaining adults in the following conditions: (i) dry: 7 % RH and 20°C, (ii) cold: 100 % RH and 5°C and or (iii) heat: 100 % RH and 38°C. The control consisted of individuals maintained at 100 % RH at 23°C [This condition induces *ca.* 3 % mortality after 40 d]. For each experimental condition, beetles were individually placed into open 2 mL microtubes transferred to sealed plastic box that contained either silicagel (for desiccation stress) or filter paper saturated with water (cold and heat tolerance assays). The boxes were then maintained into thermoregulated incubators (Sanyo™, MIR-153) set at the desired temperatures. Fourteen sets of 10 individuals were used for each survival assay. One set of ten individuals was removed from the stressing conditions at regular intervals (every two or three days) for each experimental assay, starting after 6 days of exposure. Two days after the beetles were removed from the experimental conditions and

allowed to recover at 23°C (100 % RH), the survival was scored. The relative humidity was constantly monitored over the duration of the experiment, using Hobo data loggers (model U12-012, accuracy; Onset Computer Corporation, Bourne, USA). In the desiccating condition, results showed that RH varied between 4 and 11 % RH in boxes containing silicagel, with a mean RH of *ca.* 7 %.

STATISTICAL ANALYSES

Survival data were analysed using a generalized linear model with logistic link function for binary outcome. The survival was dependent either on stress duration and dry pre-exposure (experiment 1), or on stress duration, dry pre-exposure, recovery treatment and dry pre-exposure*recovery treatment interaction (experiment 2). Lethal times for 50 % of the individuals (Lt_{50}) were retrieved from binary logistic regressions, and overlap of 95 % fiducial limits was used for assessing differences among the values (Payton *et al.*, 2003). R version 3.0.2 and MiniTab 12.2 were used for the statistical analysis.

RESULTS

The stress tolerance of dry pre-exposed beetles (without recovery after dry exposure) is presented in Figure 1; the associated statistical results are presented in Table I. Dry pre-exposure had no effect on desiccation (X^2-value = 76.42, $P = 0.68$) or cold tolerance (X^2-value = 0, $P = 1$), with mortality curves completely overlapping in this latter condition (Fig 1B). The most striking difference appeared at 38°C, with mortality prediction of dry pre-exposed insects being significantly longer than control insects (Fig 1C). In this first experiment, for beetles that were submitted to dry pre-exposure, the longest duration of survival was observed in the beetles exposed to desiccation stress, whereas the shortest duration of survival was measured under cold conditions, and this result was confirmed by the Lt_{50} values (Tab. II).
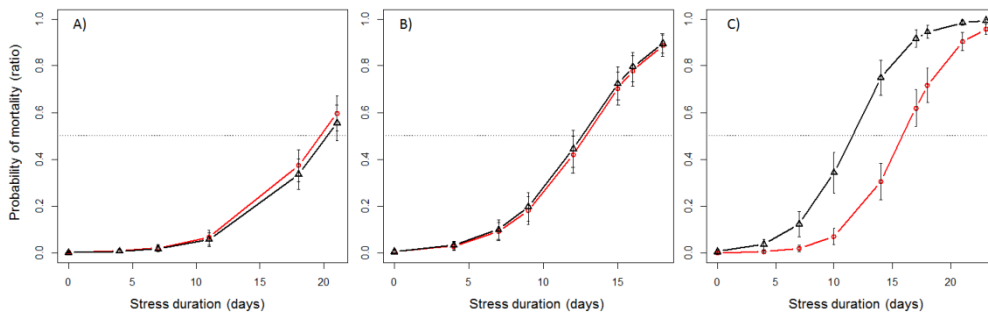

Figure 1.— Probability of mortality (± SE) (Y-axis) as function of time (days) (x-axis) of adult beetles that were continuously exposed to the following stress conditions : desiccation at 7 % RH (A), cold at 5 °C (B) or heat at 38 °C (C). Red lines: individuals pre-exposed at 7 % RH for 4.5 days before stress exposure; Black lines: control individuals exposed at 100 % RH for 4.5 days. For each survival assay, 140 beetles were used. Probability lines and estimates SE were obtained from fitted generalized linear model with binomial logit link function.

TABLE I

Results of the generalized linear model with logistic link function for binary outcome, showing the effect of dry pre-exposure (4.5 days at 7 % or 100 % RH) on the survival at 7 % RH, 5 °C or 38 °C (experiment 1)

Stress	Explanatory variable	d.f.	X^2-value	P
Desiccation	Pre-exposure	1	76.422	0.68
Cold	Pre-exposure	1	0	1
Heat	Pre-exposure	1	13.382	< 0.001

The temporal mortality patterns of the beetles resulting from the second experiment are presented in Figure 2. Under desiccating conditions (Fig 2A), the factors dry pre-exposure,

recovery treatment and their interaction had a significant effect on the survival of adult *A. diaperinus* (Table III).

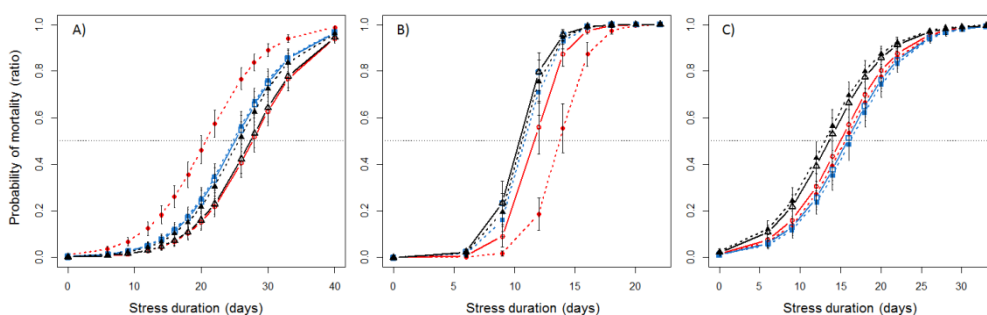| Stress	Pre-treatment	Lt_{50}	SE	CI 95 %	
Desiccation	Dry pre-exposure	19.70	0.97	17.81	21.87
	Control	20.25	0.98	18.36	22.51
Cold	Dry pre-exposure	12.81	0.75	11.26	14.34
	Control	12.81	0.75	11.26	14.34
Heat	Dry pre-exposure	15.88	0.78	14.24	17.44
	Control	11.49	0.86	9.69	13.21

Figure 2.— Probability of mortality (± SE) (Y-axis) as function of time (days) (x-axis) of adult beetles that were continuously exposed to the following stress conditions : desiccation at 7 % RH (A), cold at 5°C (B) or heat at 38°C (C). Blue lines: individuals pre-exposed at 7 % RH for 0.5 days; red lines: individuals pre-exposed at 7 % RH for 4.5 days; black lines: individuals exposed at 100 % RH for 4.5 days (control). Dotted lines: recovery at 50 % RH before stress exposure (without rehydration); full lines: recovery at 100 % RH and water supplies before stress exposure (with rehydration). For each survival assay, 140 beetles were used.

TABLE III

Results of the generalized linear model with logistic link function for binary outcome, showing the effect of pre-exposure (0.5 or 4.5 days at 7 %, or 4.5 days at 100 % RH), recovery (0.5 day at 100 % RH with water supply or at 50 % RH without water supply), and their interaction, on the survival at 7 % RH, 5°C or 38°C (experiment 2)

Stress	Explanatory variables	d.f.	X^2-value	P-Value
Desiccation	Pre-exposure	2	12.21	0.002
	Recovery	1	20.04	< 0.001
	Pre-exposure*Recovery	2	10.24	0.006
Cold	Pre-exposure	2	20.05	< 0.001
	Recovery	1	7.56	0.006
	Pre-exposure*Recovery	2	3.03	0.22
Heat	Pre-exposure	2	5.81	0.05
	Recovery	1	0.16	0.68
	Pre-exposure*Recovery	2	0.36	0.84

The mortality of *A. diaperinus* submitted to dry pre-exposure for 4.5 d and further exposed to 50 % RH for 0.5 day before the desiccation tolerance assay was significantly increased as compared with the other experimental conditions (Fig 2A, Tab. IV). Under cold stress (Fig 2B), the survival of the insects was significantly affected by dry pre-exposure (X^2-value = 20.05, P <

0.001), recovery treatment (X^2-value = 7.56, P < 0.01), but the interaction dry pre-exposure x recovery treatment was not significant (X^2-value = 3.03, P = 0.22). The temporal mortality patterns were similar among the experimental conditions, except for the beetles exposed at 7 % RH for 4.5 days and further exposed to 50 % RH for 0.5 days (Fig 2B, Tab. III). For this group, Lt_{50} value was significantly increased (Lt_{50} =13.73 ± 0.52 days), by approximately 3 days as compared with the other experimental conditions (Tab. IV). Under heat stress (Fig 2C), survival patterns remained very comparable and all Lt_{50} values were similar (Tab. IV); however, a marginally significant dry pre-exposure effect was detected (Tab. III). Finally, no mortality was observed in the control specimens maintained at 23°C and 100 % RH (data not shown).

TABLE IV

Lethal times (± SE) for 50 % of the individuals (Lt_{50}) retrieved from binary logistic regressions. Adult Alphitobius diaperinus *were pre-exposed at 7 % for 0.5 day (short dry pre-exposure), 4.5 days (long dry pre-exposure) or at 100 % RH for 4.5 days (control), and further allowed to recover for 0.5 day at 100 % RH with water supply (with rehydration) or 50% RH without water supply (without rehydration) prior to being exposed at 7 % RH (desiccation), 5°C (cold) or 38°C (heat) (experiment 2). The 95 % confidence intervals (CI) were used for assessing differences among the values*

Stress	Dry exposure	Recovery	Lt_{50}	SE	CI 95%	
Desiccation	Long	With rehydration	27.95	1.32	25.52	31.1
	Long	Without rehydration	20.47	0.86	18.75	22.32
	Short	With rehydration	24.89	1.01	22.92	27.13
	Short	Without rehydration	25.08	1.25	22.71	27.92
	Control	With rehydration	28.19	1.53	25.45	31.93
	Control	Without rehydration	25.57	1.09	23.46	28.02
Cold	Long	With rehydration	11.77	0.51	10.41	12.82
	Long	Without rehydration	13.73	0.52	12.5	14.82
	Short	With rehydration	10.48	0.5	9.23	11.67
	Short	Without rehydration	10.9	0.6	9.43	12.12
	Control	With rehydration	10.37	0.6	8.92	11.62
	Control	Without rehydration	10.59	0.63	9.05	11.87
Heat	Long	With rehydration	14.97	0.94	12.92	16.85
	Long	Without rehydration	15.53	0.85	13.7	17.34
	Short	With rehydration	15.86	0.75	14.25	17.44
	Short	Without rehydration	16.14	1.17	13.63	18.54
	Control	With rehydration	13.51	1.05	11.11	15.57
	Control	Without rehydration	12.97	1.06	10.5	15

DISCUSSION

In the present study, we examined if dry pre-exposure would affect the subsequent desiccation tolerance of *A. diaperinus* adults. We also tested whether the effect of dry pre-exposure would persist after a recovery phase post desiccation. Finally, we tested whether dry pre-exposure would promote cold and heat tolerance (*i.e.* cross-tolerance). Pre-exposure of the organisms to sublethal abiotic conditions can confer increased tolerance when individuals are subjected to a similar stress (Renault *et al.*, 2012; Colinet *et al.,* 2013). The pre-exposure of *A. diaperinus* adults to low RH did not promote subsequent survival capacities under desiccating conditions. This result suggests that the pre-treatments used here were not prone to induce a detectable physiological conditioning. The basal desiccation tolerance is already very high in control specimens (Lt_{50} of *ca.* 20 days), and consequently, it is conceivable that the level of plasticity of this biological trait could be low. The ability to thrive in dry conditions is common in Tenebrionidae, with several species from this taxa being able to lose up to > 50 % of their body water before changes in osmoregulation strategies take place (Gehrken & Sømme, 1994). All beetles had died after a 40-day exposure at 7 % RH, corresponding to *ca.* 1/10 of the mean adult life span in this species (Preiss & Davidson, 1971), whereas it does not exceed *ca.* 30h for instance in *Drosophila* flies, which corresponds to about 1/50 of the adult life span in this species (Aggarwal *et al.*, 2013). In addition, acclimation

generally involves pre-exposures to moderately stressful conditions. Here, insects were pre-exposed to 7 % RH, a condition that was too stressful for the beetles, and which did not allow dry acclimation response. The results obtained from the *A. diaperinus* beetles exposed at 7 % RH for 4.5 days are consistent with this idea, and, without rehydration, this pre-exposure should be considered as an extension of the exposure time to stressful condition. This is consistent with the 7 days delay in the Lt_{50} between these insects with and without rehydration. Finally, even if the duration of the assays was rather long, even under desiccating conditions, it is unlikely that starvation confounded our conclusion on desiccation tolerance. Indeed, only 3 % of the starved beetles died after a 40 days exposure at 100 % RH at 23°C.

Cross-tolerance between desiccation and thermal stresses was analysed. We assumed that such effect could contribute to enhancing the invasion success of this species in natural habitats in temperate regions, as proposed by Block (1996). Indeed, this author stated that species from arid environments would be pre-adapted to cold conditions. Many Tenebrionids are adapted to arid/semi-arid conditions found in desert environments (Kergoat *et al.*, 2014) that are renowned for reaching extreme temperatures: cold overnight and hot during the day. A basal level of cold tolerance may be an evolutionary trait inherited from shared ancestry. Cross-tolerance may represent another facet of the arsenal supporting the invasion success of exotic species, and it is important to thoroughly consider the multifactorial responses evolved by the species that thrive in variable environments. Cold mortality was unaffected by dry pre-exposure when this pre-treatment was not followed by a recovery phase (Fig 1B). However, cold mortality was decreased in adults exposed long dry pre-exposure when this pre-treatment was followed by a recovery period of 0.5 d at 50 % RH (Fig. 2B), thus suggesting the existence of cross-tolerance in this case. It is also interesting to point out that the longest dry pre-exposure conferred the highest benefit on the subsequent cold survival, a finding that differs from the results obtained for desiccation tolerance. Similar results were obtained by Hayward *et al.* (2007), who observed that dry acclimated *Belgica antarctica* larvae had increased cold tolerance. The exposure to desiccating conditions prior to the transfer to 5°C may have contributed decreasing the body water content of the beetles, and have likely favoured the synthesis of protective compatible solutes (for instance free amino acids, trehalose, glycerol, see Coutchie & Crowe, 1979; Naidu, 2006). During cold exposure, these accumulated metabolites may play a significant role in maintaining membrane fluidity, by preventing protein unfolding or damages to DNA (Yancey, 2001).

The survival to heat stress was enhanced when insects were not allowed to recover after the dry pre-exposure (Fig 1C). However, this cross-tolerance response between desiccation and heat stresses disappeared when beetles recovered either with water supply or with 50 % RH after the dry pre-treatment (Fig 2C). This finding is consistent with the available literature, as Holmstrup *et al.* (2002) reported in the collembolan *Folsomia candida* that membrane adjustments to drought fit to cold adaptation but not heat adaptation.

To conclude, we found some evidence of a cross-tolerance between desiccating and cold conditions, and between desiccating and heat conditions, but the occurrence of these responses depended on whether the beetles were allowed to recover before being submitted to the other stress. A recovery phase just following dry pre-exposure revealed cross-tolerance response between desiccation and cold stress; but on the other hand, it suppressed cross-tolerance between desiccation and heat stress. This suggests complex and differential cross-tolerance mechanisms. As hypothesized, mechanisms that were evolved to enhance desiccation resistance in this insect also enhance, as least in part, its cold tolerance. This cross-tolerance could have served as an important starting point for the successful colonization of native habitats in temperate regions. We also suggest considering additional biological end proxies when assessing the cross-tolerance phenomenon, allowing a wider view of the effect on the general biological functioning of the organism. Other beneficial effects may be revealed on fecundity, longevity, or other fitness components. Finally, the concept of stress memory (or carry over effect) is extensively

investigated in plants (Ding *et al.*, 2013), and more efforts are to be done on insect models (but see Schiffer *et al.*, 2013) assess the possible existence of this phenomena.

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