Phenotypic plasticity in desiccation physiology of closely related, range restricted and broadly distributed fruit fly species

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ABSTRACT

1. Variation in geographical range size among closely related species may result from differences in physiological traits, such as desiccation tolerance, that enable these species to interact with their environment or adapt to new surroundings.
2. We tested the hypothesis that insect species with a broader geographical range have either a higher basal desiccation tolerance or mount a more plastic response than more narrowly distributed species by exposing four fruit fly species (*Ceratitis capitata*, *Ceratitis rosa*, *Ceratitis cosyra* and *Ceratitis podocarpi*) to one of three acclimation treatments (control: standard relative humidity (RH) and temperature; desiccation: standard temperature and low humidity; and temperature: low RH and high temperature) and measuring metabolic rate, activity, water loss rates and survival.
3. The targeted physiological responses differed between species and acclimation treatments. Survival of the widely distributed *C. capitata* improved by up to 43% after short-term exposure to high temperature and desiccation (35°C; 0% RH) treatment, while survival in the more narrowly distributed species only improved by 4%–30% after a desiccation treatment (25°C; 0% RH).
4. Less water was lost by broadly distributed *C. capitata* through excretion after both high temperature and desiccation treatments, but only activity and respiratory water loss (RWL) were reduced after the temperature treatment, and total water loss and cuticular water loss declined after the desiccation treatment. The narrowly distributed *C. rosa* also lost less water through excretion after both acclimation treatments but showed reduced cuticular and RWL only after desiccation. While basal tolerance in *C. cosyra* was high, acclimation responses in this species and *C. podocarpi* were insignificant in that they did not produce a measurable survival benefit.
5. Broadly distributed species successfully employed unique combinations of physiological strategies, with some having highly flexible responses to stressful environmental conditions, which ultimately results in beneficial acclimation to enhance survival during dry conditions. By contrast, range restricted species showed limited responses to desiccation stress. Flexible desiccation responses likely contribute to species geographical ranges in changing climate conditions.

Keywords : climate variability, drought, jack-of-all-trades, moisture availability, respirometry, thermal acclimation

1 INTRODUCTION

The geographical distribution of any species can be linked to the range of climatic variables it tolerates (Braschler et al., 2021; Halsch et al., 2021; Pearson & Dawson, 2003), with organisms thriving within a narrow range of climatic variables while tolerating a somewhat broader range (reviewed by Chown et al., 2010). The ability to tolerate a broad range of environmental conditions (basal tolerance) or to rapidly adjust physiological responses to shift optima or tolerance of extremes (phenotypic plasticity) are adaptive responses to enhance fitness in varying environmental conditions (Chown et al., 2010; Huey et al., 2012; Kellerman & van Heerwaarden, 2019; Maebe et al., 2021; Rohr et al., 2018). Plastic physiological responses could drive invasions of species into new areas where they encounter a novel range of environmental conditions (Davidson et al., 2011; Knop & Reusser, 2012; Richards et al., 2006), and at the same time help native species persist in their current environment as environmental conditions change globally (Chown, Marais, et al., 2007; Chown, Slabber, et al., 2007; Davidson et al., 2011; Knop & Reusser, 2012). Phenotypic plasticity occurs when a single genotype encodes for a range of phenotypes, and a specific set of environmental conditions stimulates selective gene expression that leads to the most favourable phenotype under those conditions (Bradshaw & Hardwick, 1989; Ghalambor et al., 2007; West-Eberhard, 2005). Such phenotypic variation can occur at a range of time-scales, within hours (e.g. Boardman et al., 2013), within a single generation or over multiple generations (Allen et al., 2012), in different life stages (Boardman et al., 2013; van Heerwaarden et al., 2016), and may either be reversible or irreversible (reviewed in Piersma & Drent, 2003; Sgro et al., 2016; Beaman et al., 2016; Kellerman & van Heerwaarden, 2019).

Insect phenotypic plasticity has been well documented in response to a broad range of stressful environmental conditions including water deficiency (e.g. Boardman et al., 2013), temperature variation (e.g. Bjørge et al., 2018; Chidawanyika & Terblanche, 2011) and food shortage (e.g. Mitchell et al., 2017; Renault et al., 2002). Insects can also respond in diverse ways towards the same stimulus and, conversely, diverse stressors can elicit a similar plastic physiological response (Bubliy et al., 2012; Gotcha et al., 2018). Furthermore, acclimation responses that enhance fitness in a predictable manner relative to the environmental cue (Allen et al., 2012; Chidawanyika & Terblanche, 2011) contribute to arguments for adaptive plasticity shaping evolutionary responses (Fox et al., 2019; Ghalambor et al., 2007; Leonard & Lancaster, 2020; Sgro et al., 2016). For example, exposure to low humidity conditions prompts a shift in epicuticular lipid expression, thus lowering water loss rates and enhancing survival in desiccating environments (Baumgart et al., 2022; Gibbs et al., 1997). The type and magnitude of response varies between species and is further affected by the timing, duration and intensity of acclimation conditions (Weldon et al., 2011). Plasticity can decrease as basal tolerance increases, potentially representing a trade-off between these strategies and indicating that expression of both responses is likely costly (Beaman et al., 2016; Kellerman & van Heerwaarden, 2019; Kellermann et al., 2018; Maebe et al., 2021; Sgro et al., 2016).

Insects must maintain an optimal water balance to persist in an environment with variable moisture availability (e.g. pulses in rainfall, humidity, or food and water resources). This is achieved by regulating both water intake from drinking, digestion of food and metabolic water gain, and water loss through respiratory, cuticular and excretory pathways (reviewed by Chown et al., 2011; Hadley, 1994). The small body size of most insects makes them

particularly vulnerable to cuticular water loss (CWL), as a high body surface area-to-volume ratio results in a large surface area where water passively diffuses through the cuticle to the outer environment. To combat this problem, insects possess an outer (epicuticular) layer comprising a waxy layer of (hydrophobic) long-chain unsaturated hydrocarbons (Blomquist & Jackson, 1979). Cuticular compounds differ between species and have multiple functions including facilitating chemical communication (Soares et al., 2017) while detectable differences in either the amount or combination of epicuticular hydrocarbons have been shown to affect water loss in ants and flies (Baumgart et al., 2022; Bazinet et al., 2010). Insect cuticles can be modified by natural selection to be less permeable to water over evolutionary timescales (Bazinet et al., 2010; Gibbs et al., 1997; Yoder et al., 1997), but they can also change within the lifetime of individuals to permit survival of anticipated dry conditions (Baumgart et al., 2022; Benoit & Denlinger, 2010; Chown et al., 2011).

Another avenue of insect water loss is through the respiratory pathway, when internal water, along with CO_2, passively diffuses through spiracles opened for gas exchange. This incurs a hygric penalty to respiratory metabolism in terrestrial environments (Kestler, 1984; Woods & Smith, 2010). Although respiratory water loss (RWL) rate can be as low as 2% (Quinlan & Hadley, 1993) and rarely makes up more than 30% of total water loss rates in many insects (Chown, 2002; Chown & Davis, 2003), even small increases in water loss rates can have large fitness consequences (Chown, 2002; Chown & Davis, 2003; Schimpf et al., 2012). Variation in metabolic rate can drive changes in RWL (e.g. Bazinet et al., 2010; Terblanche et al., 2010), although many intrinsic and extrinsic factors influence metabolic rate, including insect body size (Addo-Bediako et al., 2001; Chown, Marais, et al., 2007; Chown, Slabber, et al., 2007), activity levels (Halsey et al., 2015) and temperature, and these may interact with each other in complex ways (Bjørge et al., 2018; Irlich et al., 2009; Terblanche et al., 2010). Therefore, variation in metabolic rate, especially if modulated in the face of changing moisture availability (Terblanche et al., 2010), should be able to explain some variation in desiccation resistance.

Species of true fruit flies (Diptera: Tephritidae) vary in their geographical distributions (Hill et al., 2016) and naturally occur under a wide range of climatic conditions. To some extent, the geographical range of tephritid species may result from measurable variation in several key traits that underlie environmental stress resistance (Weldon et al., 2016, 2018). This same plasticity perhaps contributes to their ability to colonise and invade areas outside of their natural range (Diamantidis et al., 2011) where they cause economic losses through crop damage to a wide range of fruits (Grové et al., 2016) and restricted access to export markets. Current changes in weather patterns are altering the range of temperature and humidity to which fruit flies are exposed in their native range while potentially creating more areas with a favourable environment for fruit fly invasion (Hill et al., 2016; Hill & Terblanche, 2014). Differences in desiccation resistance have been found between three *Ceratitis* species (Weldon et al., 2016), with the pathways for water loss (respiratory, cuticular, excretory or whole animal), differences in water loss rates and lipid catabolism suggested as possible physiological mechanisms that are yet to be comprehensively studied. Therefore, we determined the basal and plastic desiccation physiology of broadly distributed and narrowly distributed fruit fly species using high temporal resolution, flow-through respirometry to partition routes of water loss, and thus, identify the major mechanisms underlying water balance trait variation among treatment conditions and among closely related species. We

hypothesised that broadly distributed tephritid species such as *C. capitata* would display either broad tolerance or greater phenotypic plasticity in their desiccation physiology, but the potential costs associated with these responses would favour one over the other. Regarding plastic responses, we predicted that either a low relative humidity (RH) or high temperature treatment would reduce rates of CWL or RWL. A reduction in RWL could be achieved through suppression of metabolic rate with accompanying reduction in RWL—potentially coupled to activity reductions. We expect range restricted or more narrowly distributed species to have lower plasticity, or be less plastic in the face of diverse stressors, or lower trait means than more broadly distributed species.

2 MATERIALS AND METHODS

2.1 Rearing of wild caught species

Pupae of *Ceratitis capitata* (Wiedemann, 1824), *Ceratitis rosa* (Karsch, 1887), *Ceratitis cosyra* (Walker, 1849) and *Ceratitis podocarpi* (Bezzi, 1924) were seasonally collected from stung host fruit (Table 1) and reared using standard culture methods to the F2 generation for experiments (see online Supporting Information for details).

TABLE 1. Average mass (mg ± SD) of 15 females and males of the four tested *Ceratitis* species, and information about where each species was collected. Information given includes collection area, temperature range of this area during breeding season of the fly species, as well as host fruit from which flies were collected. Temperature data were obtained from South African Weather Services (SAWS) and calculted as the average daily minima and maximum for each area and season of collection

Species	Sex	Average mass (mg)	SD	Host fruit	Average monthly temperature during collection season
C. capitata	F	10.16	1.17	*Psidium guajava*	13°C
	M	8.73	1.05	(guava)	(Western Cape)
C. cosyra	F	10.18	1.01	*Sclerocarya birrea*	20–23°C
	M	6.84	1.32	(marula)	(Limpopo)
C. rosa	F	10.27	0.99	*Mangifera indica*	20–23°C
	M	6.93	1.11	(mango)	(Limpopo)
C. podocarpi	F	8.58	1.83	*Podocarpus gracilior*	13°C
	M	8.69	3.20	(African fern pine)	(Western Cape)

2.2 Acclimation treatments

The potential phenotypic plasticity of the desiccation physiology of adult flies was tested by rearing the flies at standard conditions and then exposing adults to one of two short-term acclimation treatments of 24 h to test if the four fly species had different rapid responses to variation in temperature and humidity relative to each other and a control. Acclimation treatments were chosen to test the effects of different combinations of temperature and RH on both respiration rate and cuticle composition. Temperature could affect both CWL and RWL, while RH might only affect CWL since the humidity gradient between the internal

environment and surrounds of an insect drives passive CWL, but active RWL should be unaffected. The selected treatments were as follows: (1) Control treatment, representing basal conditions (exposure to 25°C and 76% RH for 8 h), (2) 'heat+desiccation' or Temperature treatment (exposure to 35°C and 0% RH for 2 h), and (3) Desiccation treatment (exposure to 25°C and 0% for 8 h). The desired RH was achieved following the methods of Winston and Bates (1960) with a saturated solution of NaCl creating a RH of 76%, while airtight desiccators filled with silica gel were used to achieve <5% RH (hereafter referred to as 0% RH). Age was controlled to measure only flies between 5 and 7 days old (Weldon et al., 2013; Weldon & Taylor, 2010). The acclimation treatments were achieved by placing females and males of known age in individual microcentrifuge tubes containing a small (~1 mm) air hole in the lid. The tubes containing the flies were then transferred to airtight containers in which RH was controlled, and these were put into incubators set to the desired temperature. Temperature and RH during acclimation were verified with data loggers (DS1923, hygrochron-iButton, Maxim) placed within each airtight container. Flies allocated to the control and desiccation treatments were acclimated for 8 h while flies allocated to the temperature treatment were acclimated for 2 h as pilot trails determined that longer acclimation treatments led to mortality. For the duration of the acclimation treatments, food and water were withheld but provided again during a 12-h resting period (at 25°C and 75% RH) before measurements of either mortality or partitioning of routes of water loss were undertaken in separate groups of flies. The effects of the combined temperature and humidity treatments on longevity (desiccation resistance), water loss at death, metabolic rate, activity and water loss rates (partitioned into RWL and CWL) were recorded.

2.3 Desiccation resistance assay

Equal numbers of female and male flies (n_{total} = 180) were weighed to the nearest 0.1 mg (Mettler Toledo, MS104S/01), acclimated to each of the three treatments ($n_{treatment}$ = 60) and then given a 12 h rest period, under the control conditions described above. Following this rest period, flies were placed in individual, marked micro-centrifuge tubes, weighed, then transferred to a container set up as for the desiccation treatment and checked every 2 h. Flies that were found dead at each of the observations were removed and immediately re-weighed, after which they were placed in a drying oven at 60°C for 72 h. The flies were removed from the drying oven and weighed a final time to determine dry mass. Body water was estimated as the difference between initial wet mass at the start of the assay and final dry mass. While this estimate of body water does not account for weight lost due to catabolism of stored nutrients, the difference is likely to be within the range of measurement error for the balance that we used (e.g. see Weldon et al., 2016).

2.4 Metabolic rate and water loss

Gravimetric total water loss does not identify the avenue of water loss (e.g. water loss through the cuticle, from respiration or by excretion). To partition routes of water loss and determine if CWL, RWL or excretory water loss rates differed between the four *Ceratitis* species, CO_2 (as an indirect measure of metabolic rate) and H_2O outputs were measured with flow-through respirometry using methods like those in Terblanche et al. (2010). All measurements were taken under desiccating conditions (0% RH and 25°C). Flies aged between 5 and 7 days were selected (n = 20 per treatment) and weighed to the nearest 0.1 mg after being placed in

individual respirometry chambers constructed from 5 ml sterile syringes. These respirometry chambers were then connected to a LiCor 7000 CO_2/H_2O gas analyser that was calibrated using a CO_2 standard and a LiCor 610 dew point generator following methods in Terblanche et al. (2010). The gas analyser was connected to a desktop computer running Sable Systems LiCor software (LI-7000-500 Windows® Software, v2.0.0), which recorded CO_2 and H_2O output. The test temperature of 25°C was achieved by placing the respirometry chamber containing the individual fly inside a programmable water bath (CC410wl; Huber). A type T thermocouple connected to a temperature data logger (TC-08; Pico Technologies) was also inserted into the syringe via a small opening to measure the temperature inside the respirometry chamber for the duration of the measurement and the opening sealed to prevent air loss. Each individual was measured for approximately 120 min at an airflow of 100 ml min^{-1} with air that was first passed through soda lime, silica gel and Drierite columns, to ensure that the air that entered the system was free of water vapour and CO_2. A Sable Systems AD-2 electronic activity detector was connected to the respirometry chamber, recording a pronounced Voltage change every time the fly interrupted a light beam, and used to qualitatively identify resting periods, although activity and rest periods were also readily visible in the $\dot{V}CO_2$ traces.

2.5 Statistical analysis

2.5.1 Desiccation resistance

A set of model selection analyses were run in R (R software v. 4.1.3; R Core Team, 2022) (Cranley package; Leoncio, 2022) using the 'dredge' function in the MuMin library (Burnham & Anderson, 2002) to find the simplest model that best explained the variation in survival time between acclimation treatments in each species. Body water and body mass were positively correlated (Spearman, $r = 0.944$) and therefore only one of these variables was included in the test models to avoid autocorrelation. For each species, two models were tested with the dredge function, namely model 1 = Treatment × Sex × Body mass and model 2 = Treatment × Sex × Body water. From these two models, the combination of variables that produced the lowest Akaike's information criterion (AIC) score was selected as the best model. Where AIC values were similar between models, the model with the fewest predictor variables was selected. Cox proportional hazards models were run with the determined variables and the differences between groups summarised.

2.6 Metabolic rate and water loss

All metabolic data were extracted using Expedata (v 1.9.1 Sable Systems). The CO_2 and H_2O output traces for each individual were corrected for baseline drift, which was typically non-existent. All measurements were standard temperature and pressure corrected by the internal calibration of the infrared gas analyser and mass flow control valve. After each CO_2 and H_2O trace was drift corrected, they were converted from mMol/m and ppm to ml CO_2 h^{-1} and mg H_2O h^{-1}, respectively (standard gas equations; Supporting Information). Thereafter, a range of metabolic rate and water loss variables were extracted. Resting metabolic rate and average water loss rates were determined as the most level 120 s where there was no corresponding activity recorded by the activity detector, and the mean value and standard deviation were extracted for each individual. As the flies in this study were not observed to

exhibit any obvious cyclic or discontinuous gas exchange pattern (where the spiracles are periodically closed), we could estimate CWL for each individual. To do so, we used ordinary least squares regression to relate H_2O (mg h^{-1}) against CO_2 (ml h^{-1}) output, and determined CWL as the H_2O output when CO_2 output was zero (i.e. the *y*-intercept of the regression equation) (Gibbs & Johnson, 2004). Excretion events during the course of the full 2-h measurement were clearly visible as spikes in the H_2O trace that did not correspond with a spike in the CO_2 trace and for each individual the number of excretion events was tallied. The area under the trace of each excretion event was measured and taken as the volume of H_2O excreted and corrected by subtracting the estimated CWL from the peaks. The cumulative volume of these excretion events was also recorded for each individual. Activity was determined as the percentage time each individual spent active during the 2-h measurement period. The duration of activity spikes was determined in each activity trace and totalled before being divided by the total duration of the respirometry recording.

Before testing our hypotheses, we checked our data for the assumptions of ANOVA for each dependent variable using Levene's tests (homogeneity of variance), Shapiro–Wilk tests (normal distribution) and likelihood ratio tests (over dispersion). None of the data were normally distributed, even following log transformation. Therefore, generalised linear models (GLZ's) with Gaussian error distribution and log link function were used to test our hypotheses. A Gaussian distribution offered the best fit for our data based on comparison of AIC relative to other investigated distributions. These were followed by post-hoc Tukey multiple comparisons tests to identify groups that differed from each other. For metabolic rate, activity, water loss rate, and CWL and RWL, the best model predictors were species, sex and body mass, as determined through model selection. For convenience, all mass-adjusted metabolic rate data are presented as µl h^{-1} mg^{-1} and water loss rates presented as µg h^{-1} mg^{-1}. Statistical analyses were performed using R (R software v. 4.1.3; R Core Team, 2022), packages used included 'car' (Fox & Weisberg, 2019), and 'lsmeans' (Russell, 2016).

3 RESULTS

3.1 Desiccation resistance

The best model to compare the basal response (as represented by the control treatment) between the four species included sex but excluded body mass and body water (Table S1). Cox proportional hazards analyses showed that the basal mortality risk under desiccating conditions was similar for *C. capitata* and *C. cosyra* ($z = -0.971$, $p = 0.765$), and that of *C. rosa* and *C. podocarpi* was also similar ($z = -0.731$, $p = 0.884$). The risk of mortality for *C. capitata* and *C. cosyra* when experiencing desiccation was significantly lower relative to *C. podocarpi* (*C. capitata*: $z = 3.031$, $p = 0.013$; *C. cosyra*: $z = 3.674$, $p = 0.001$) and *C. rosa* (*C. capitata*: $z = 2.574$, $p = 0.049$; *C. cosyra*: $z = 3.290$, $p = 0.005$) (Figure 1).

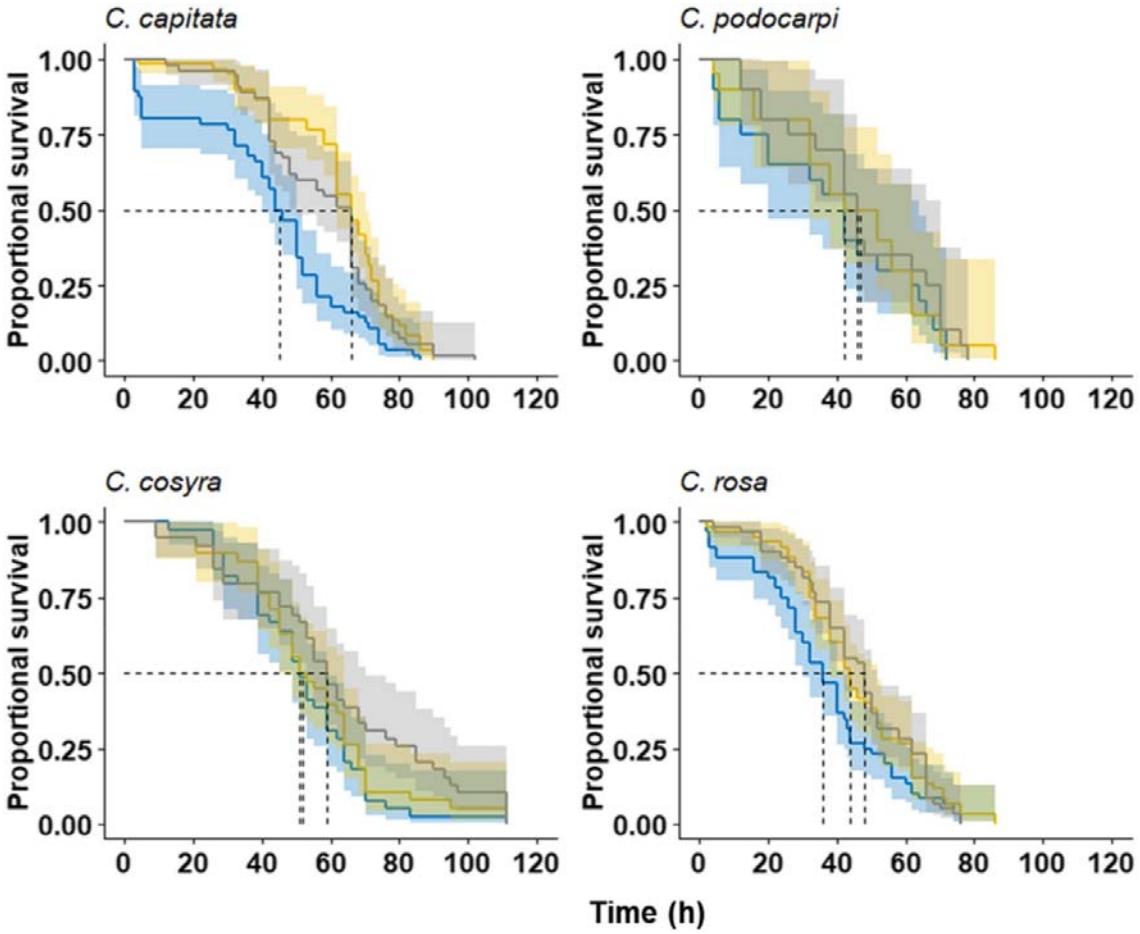

FIGURE 1. Survival curves for *Ceratitis capitata*, *Ceratitis rosa*, *Ceratitis cosyra* and *Ceratitis podocarpi* (n = 60 per treatment, males and females pooled). Fly mortality was recorded every 2 h under desiccating conditions (0% RH and 25°C) after one of three acclimation treatments, namely: Desiccation (0% RH and 25°C; yellow line), temperature (0% RH and 35°C; grey line) or control (76% RH and 25°C; blue line). Lethal times (LT) for each treatment group is represented by the dashed line on each graph. Basal survival for each *C. capitata* and *C. cosyra* was higher than both *C. podocarpi* (*C. capitata*: p = 0.013; *C. cosyra*: p = 0.001) and *C. rosa* (*C. capitata*: p = 0.049; *C. cosyra*: p = 0.005). Survival of *C. capitata* was increased by both treatments (desiccation: p = 0.008; temperature: p = 0.023) while survival in the other three species were increased only in the desiccation treatment (*C. rosa*: p = 0.001, *C. cosyra*: p = 0.022, *C. podocarpi*: p = 0.002) and not in the temperature treatment.

The plastic responses associated with the three acclimation treatments were tested for each species separately. The best model for both *C. capitata* and *C. rosa* contained body mass and treatment, and excluded sex and body water as predictors (Tables S2 and S3). For *C. cosyra* and *C. podocarpi*, the best models included body water and treatment as predictor variables but excluded sex and body mass (Tables S4 and S5). Cox proportional hazards analyses based on the optimal models indicated that both temperature and desiccation treatments significantly reduced the risk of mortality (relative to the control) in subsequent desiccating events for *C. capitata* (desiccation: z = −2.973, p = 0.008; temperature: z = −2.639, p = 0.023, Figure 1) but the proportional hazard did not differ between the desiccation and temperature treatments for this species (z = 0.116, p = 0.992, Figure 1). *Ceratitis capitata* from the control

treatment reached 50% mortality (LT50) within 45 h but after desiccation and temperature treatments this period increased to 66 h, indicating a 43% improvement in longevity with acclimation.

In *C. rosa*, *C. cosyra* and *C. podocarpi*, Cox proportional hazards analyses run with body mass and treatment showed that only a desiccation treatment significantly reduced the risk of mortality in subsequent desiccating conditions (Cox proportional hazards *C. rosa*: $z = -3.212$; $p = 0.001$, Figure 1; *C. cosyra*: $z = 2.642$, $p = 0.022$, Figure 1, *C. podocarpi*: $z = -3.169$; $p = 0.002$, Figure 1). The increase in longevity for each species (relative to the control) was 30% for *C. rosa* (LT50 control = 36 h; desiccation = 48 h), 4% for *C. cosyra* (LT50 control = 50 h; desiccation = 52 h), and 25% for *C. podocarpi* (LT50 control = 40 h; desiccation = 50 h). The LT50 values of 46, 60 and 50 h recorded for *C. rosa*, *C. cosyra* and *C. podocarpi* (Figure 1) suggest that temperature treatment has an initial effect but this effect ultimately evens out so that there is no significant difference in longevity at LT100 (Cox proportional hazards, *C. rosa*: $z = -1.625$; $p = 0.104$; *C. cosyra*: $z = -0.545$, $p = 0.847$; *C. podocarpi*: $z = 0.292$; $p = 0.771$). The temperature and desiccation treatments differed from each other in these species (Cox proportional hazards, *C. rosa*: $z = -1.898$; $p = 0.058$; *Ceratitis cosyra*: $z = -2.710$, $p = 0.018$; *C. podocarpi:* $z = -3.110$, $p = 0.026$) (Figure 1).

3.2 Metabolic rate and activity patterns

For all species tested, $\dot{V}CO_2$ correlated positively with body mass so that heavier individuals had higher metabolic rates (Table S6). To control for this effect of body mass, metabolic rate measurements were mass-adjusted and converted from mg h^{-1} to μg h mg^{-1} and all further analyses were conducted on mass-adjusted values. The resting metabolic rate under standard conditions was similar for *Ceratitis capitata* and *C. podocarpi* (GLZ, $z = 1.602$, $p = 0.377$), as well as for *C. cosyra* and *C. rosa* (GLZ, $z = -0.033$, $p > 0.05$, $p = 1.000$). Metabolic rates of *C. capitata* and *C. podocarpi* were significantly higher than for the other two species (Table 2, Figure 2).

TABLE 2. Comparison of basal responses of four *Ceratitis* species under standard conditions (25°C and 76% RH) for a range of metabolic and water loss measurements

Basal responses		***C. cosyra***		***C. podocarpi***		***C. rosa***	
		***z*-score**	***p***	***z*-score**	***p***	***z*-score**	***p***
Metabolic rate (µl h^{-1} mg^{-1})	*C. capitata*	−5.606	**<0.001**	1.693	0.327	−5.366	**<0.001**
	C. cosyra			6.958	**<0.001**	−0.101	1.000
	C. podocarpi					−6.677	**<0.001**
Percentage active (%)	*C. capitata*	1.022	0.736	−0.197	0.997	1.251	0.594
	C. cosyra			−1.158	0.653	0.291	0.991
	C. podocarpi					1.370	0.518
Total water loss (µl h^{-1} mg^{-1})	*C. capitata*	−2.179	0.129	0.363	0.984	−1.941	0.211
	C. cosyra			2.410	0.075	0.106	1.000
	C. podocarpi					−2.179	0.129
Cuticular water loss (µl h^{-1} mg^{-1})	*C. capitata*	−1.701	0.323	0.463	0.967	−2.174	0.130
	C. cosyra			2.061	0.166	−0.576	0.939
	C. podocarpi					−2.494	0.060
Respiratory water loss (µl h^{-1} mg^{-1})	*C. capitata*	−0.565	0.942	0.074	1.000	−0.257	0.994
	C. cosyra			0.605	0.931	0.273	0.993
	C. podocarpi					0.314	0.989
Excretion events	*C. capitata*	−0.876	0.817	−0.754	0.875	0.809	0.850
	C. cosyra			0.069	1.000	1.632	0.360
	C. podocarpi					1.479	0.450
Excretion volume (µl)	*C. capitata*	−0.018	1.000	−0.125	0.999	1.663	0.343
	C. cosyra			−0.108	1.000	1.680	0.334
	C. podocarpi					1.691	0.328

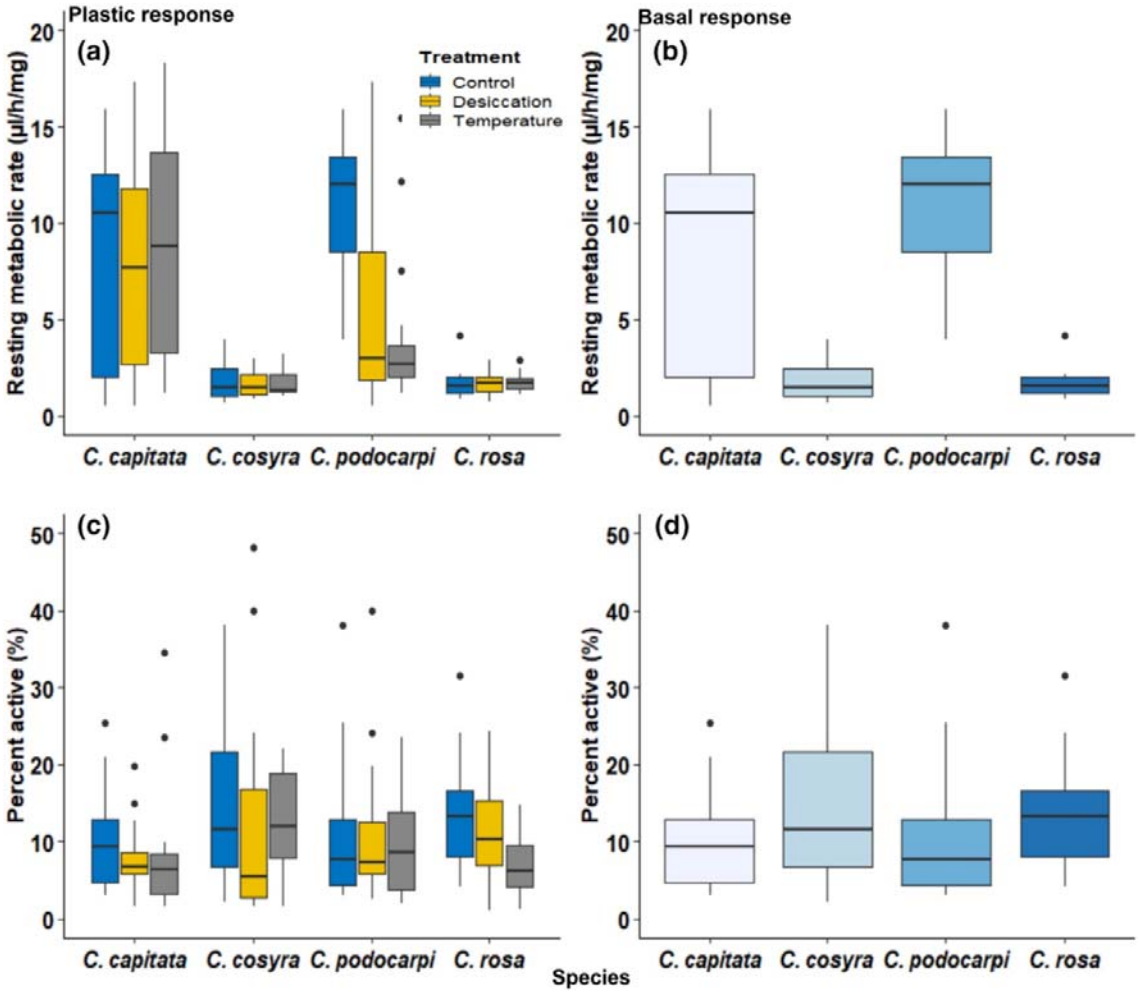

FIGURE 2. Resting metabolic rate and the percentage time flies were active for four *Ceratitis* species. Plastic (a) and basal responses (b) are shown for metabolic rate, and for activity (c and d, respectively). Before measurement, 60 flies from each species (n = 20 per treatment, females and males pooled) were acclimated to one of three acclimation treatments: Desiccation (0% RH and 25°C), temperature (0% RH and 35°C) or control (76% RH and 25°C). Respirometry measurements were taken over 2 h under standard conditions (0% RH and 25°C) and resting metabolic rate was taken as CO_2 emission during a 120-s inactive period. Basal responses are presented per species and plastic responses per species and treatment. Basal metabolic rates of *C. capitata* and *C. podocarpi* were significantly higher than for the other two species (Table 1). *C. podocarpi* metabolic rates were significantly reduced in desiccation (p = 0.002) and temperature (p < 0.001) treatments. No significant differences in basal activity between the four species. Both *C. capitata* and *C. rosa* were less active after a temperature treatment (*C. capitata*: p = 0.077; *C. rosa*: p = 0.035) but not after a desiccation treatment.

For *C. capitata*, *C. rosa* and *C. cosyra*, the mass-adjusted resting metabolic rates of flies that were hardened did not differ from the flies in the control group (desiccation treatment (*C. capitata*: z = −1.401; p = 0.166; *C. rosa*: z = 0.101; p = 0.994; *C. cosyra*: z = −0.800; p = 0.427); temperature treatment: (*C. capitata*: z = 1.198; p = 0.236; *C. rosa*: z = 0.217; p = 0.974; *C. cosyra*: 0.372; p = 0.711)) (Figure 2a). Furthermore, for these three species, metabolic rates did not differ between the two acclimation treatments (*C. capitata*: z = 0.903; p = 0.638, *C. rosa*: z = 0.119; p = 0.992; *C. cosyra*: z = 1.238; p = 0.431, Figure 2a). Only *C. podocarpi*

exhibited significantly reduced metabolic rates after being exposed to both acclimation events (desiccation: $z = -3.324$; $p = 0.002$; temperature: $z = -4.271$; $p < 0.001$, Figure 2a), and there was no difference in response between temperature and desiccation treatments ($z = -0.912$; $p = 0.633$, Figure 2a).

The percentage of time that flies were active did not differ between the species under control conditions, indicating no difference in basal response between the species (Table 2, Figure 2). Both *C. capitata* and *C. rosa* were less active after a temperature treatment (*C. capitata*: $z = -1.797$; $p = 0.077$; *C. rosa*: $z = -2.485$; $p = 0.035$) but not after a desiccation treatment (*C. capitata*: $z = -0.818$; $p = 0.417$; *C. rosa*: $z = -0.769$; $p = 0.722$, Figure 2b). The activity of other tested species did not change in response to temperature (*C. cosyra*: $z = -0.580$; $p = 0.565$; *C. podocarpi*: $z = -0.526$; $p = 0.602$) or desiccation treatment (*C. cosyra*: $z = -0.123$; $p = 0.992$; *C. podocarpi*: $z = -0.060$; $p = 0.952$) (Figure 2b). There was no difference within any species in activity of flies from desiccation and temperature treatments (*C. capitata*: −0.973; $p = 0.594$; *C. rosa*: $z = -1.755$; $p = 0.185$; *C. cosyra*: −0.123; $p = 0.992$; *C. podocarpi*: $z = -0.473$; $p = 0.884$) (Figure 2b).

3.3 Total, CWL and RWL rates

Total, CWL and RWL rates measured under standard conditions, which is indicative of basal response, did not differ between the species (Table 2, Figure 3).

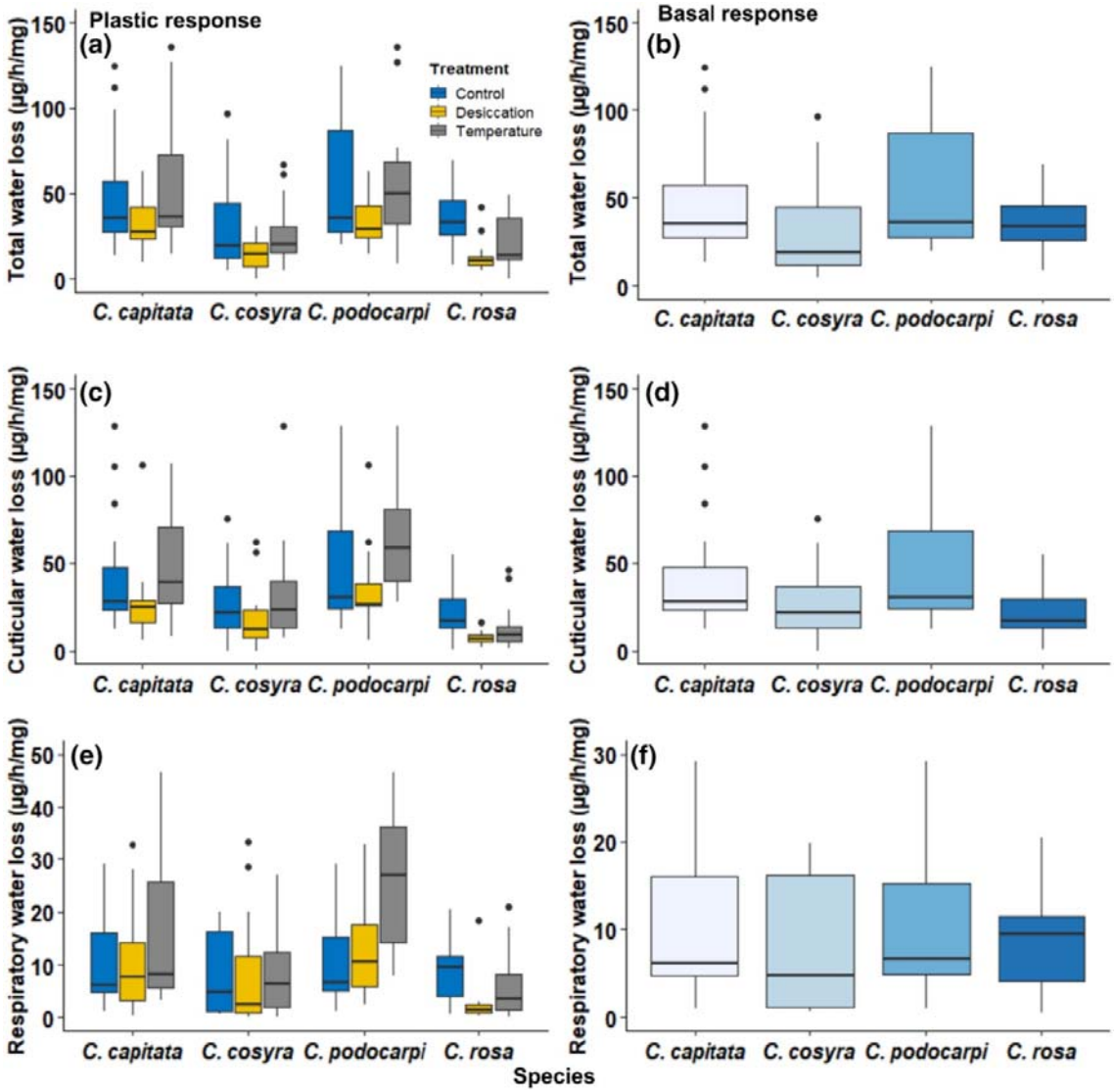

FIGURE 3. The total (gravimetric) water loss, cuticular water loss (CWL) and respiratory water loss (RWL) for four *Ceratitis* species. Plastic and basal responses are shown for total water loss rate (a and b, respectively), cuticular water loss (c and d, respectively) and respiratory water loss (e and f, respectively). Prior to measurement, 60 flies from each species (n = 20 per treatment, females and males pooled) were acclimated to one of three acclimation treatments: Desiccation (0% RH and 25°C), temperature (0% RH and 35°C) or control treatment (76% RH and 25°C). The basal total, CWL and RWL rates did not differ between the species. The total water loss rate was significantly lower in the desiccation treatment for *C. capitata* (p = 0.007), *C. rosa* (p < 0.001) and *C. cosyra* (p = 0.040). Temperature treatment reduced water loss in *C. rosa* (p = 0.012). CWL rate was significantly reduced in desiccation treatment in *C. capitata* (p = 0.008) and *C. rosa* (p = 0.002). RWL in desiccation treatment significantly lower in *C. rosa* (p = 0.002). Temperature treatments resulted in lower RWL rates in *C. capitata* (p = 0.041) and *C. podocarpi* (p < 0.001).

The total water loss rate lost during survival assays was significantly lower than the controls after a desiccation treatment for *C. capitata* (z = −1.816; p = 0.007), *C. rosa* (z = −4.823; p < 0.001) and *C. cosyra* (z = −2.112; p = 0.040) but not in *C. podocarpi* (z = −1.828; p = 0.075)

(Figure 3a). After a temperature treatment, total water loss was lower than from controls for *C. rosa* ($z = -2.847$; $p = 0.012$, Figure 3a). In contrast, none of the other species had total water loss affected by a temperature treatment (*C. capitata*: $z = 0.226$; $p = 0.822$; *C. cosyra*: $z = 0.076$; $p = 0.940$; *C. podocarpi*: $z = 2.274$; $p = 0.060$) (Figure 3a).

Average water loss rate of inactive flies (determined from respiration traces) was significantly lower after a desiccation treatment in *C. capitata* ($z = -3.269$; $p = 0.002$) and *C. rosa* (GLZ, $z = -4.368$; $p < 0.001$) but not *C. cosyra* ($z = -1.805$; $p = 0.078$) or *C. podocarpi* ($z = -0.788$; $p = 0.435$). Temperature treatment affected water loss rates in *C. capitata* (GLZ, $z = -4.167$; $p = 0.015$), *C. rosa* ($z = -2.458$; $p = 0.037$) and *C. podocarpi* ($z = 2.442$; $p = 0.019$) but not *C. cosyra* ($z = -0.027$; $p = 0.979$). Water loss rates of inactive *C. capitata* ($z = -3.269$; $p = 0.002$) and *C. podocarpi* ($z = 3.301$; $p = 0.003$) given desiccation and temperature treatments differed from each other, but this was not the case in *C. rosa* ($z = 1.871$; $p = 0.147$) or *C. cosyra* (GLZ, $z = 1.716$; $p = 0.199$).

Water loss measurements were further partitioned into CWL, RWL and excretion events (both number of events as well as total volume of excretions) to pinpoint how much water was lost through each of these avenues in each species and for each treatment. As with the total water loss, neither CWL nor RWL differed between the species when measured under standard conditions (Table 2, Figure 3). CWL rate was significantly reduced after a desiccation treatment in *C. capitata* ($z = -1.789$; $p = 0.008$) and *C. rosa* ($z = -3.349$; $p = 0.002$) but unaffected in *C. cosyra* ($z = -1.636$; $p = 0.109$) and *C. podocarpi* ($z = -1.124$; $p = 0.268$) (Figure 3b). Meanwhile, temperature treatments had no effect on the CWL in any of the species tested: *C. capitata* ($z = 0.586$; $p = 0.559$), *C. rosa* ($z = -1.626$; $p = 0.234$), *C. cosyra* ($z = -0.085$; $p = 0.932$) and *C. podocarpi* ($z = 1.384$; $p = 0.174$), (Figure 3b). The desiccation and temperature treatments differed from each other in *C. capitata* ($z = 2.517$; $p = 0.032$) and *C. podocarpi* ($z = 2.572$; $p = 0.027$) but not in *C. rosa* ($z = -1.626$; $p = 0.235$) or *C. cosyra* ($z = 1.516$; $p = 0.283$) (Figure 3b).

RWL rates after a desiccation treatment were significantly lower than the control in *C. rosa* ($z = -3.432$; $p = 0.002$, Figure 3c). However, it was unaffected in *C. capitata* ($z = 0.097$; $p = 0.923$), *C. cosyra* ($z = -0.008$; $p = 0.994$) and *C. podocarpi* ($z = 0.678$; $p = 0.501$, Figure 3c). Temperature treatments resulted in lower RWL rates in *C. capitata* ($z = 2.092$; $p = 0.041$) and *C. podocarpi* ($z = 3.861$; $p < 0.001$) but not in *C. rosa* ($z = -2.255$; $p = 0.062$) or *C. cosyra* ($z = 0.062$; $p = 0.951$, Figure 3c). The desiccation and temperature treatments differed from each other in *C. podocarpi* ($z = 3.230$; $p = 0.004$) but not in *C. capitata* ($z = 2.048$; $p = 0.101$), *C. cosyra* ($z = 0.066$; $p = 0.998$, Figure 3c) or *C. rosa* ($z = -1.898$; $p = 0.058$).

3.4 Excretion

Neither the number of excretion events nor the total volume excreted under controlled conditions differed significantly between the four species (Table 2, Figure 4). The frequency of excretion events was reduced after desiccation treatment only in *C. capitata* (excretion events: $z = -2.226$; $p = 0.030$, Figure 4a) but not in *C. rosa* ($z = 0.210$; $p = 0.976$, Figure 4a), *C. cosyra* ($z = 1.455$; $p = 0.152$, Figure 4a) or *C. podocarpi* ($z = 0.000$; $p = 0.9997$, Figure 4a). The number of excretion events was unaffected by a temperature treatment in all the species

tested: *C. capitata* (z = −1.488; p = 0.142, Figure 4a), *C. rosa* (z = −0.698; p = 0.765, Figure 4a) and *C. cosyra* (z = 0.982; p = 0.331, Figure 4a).

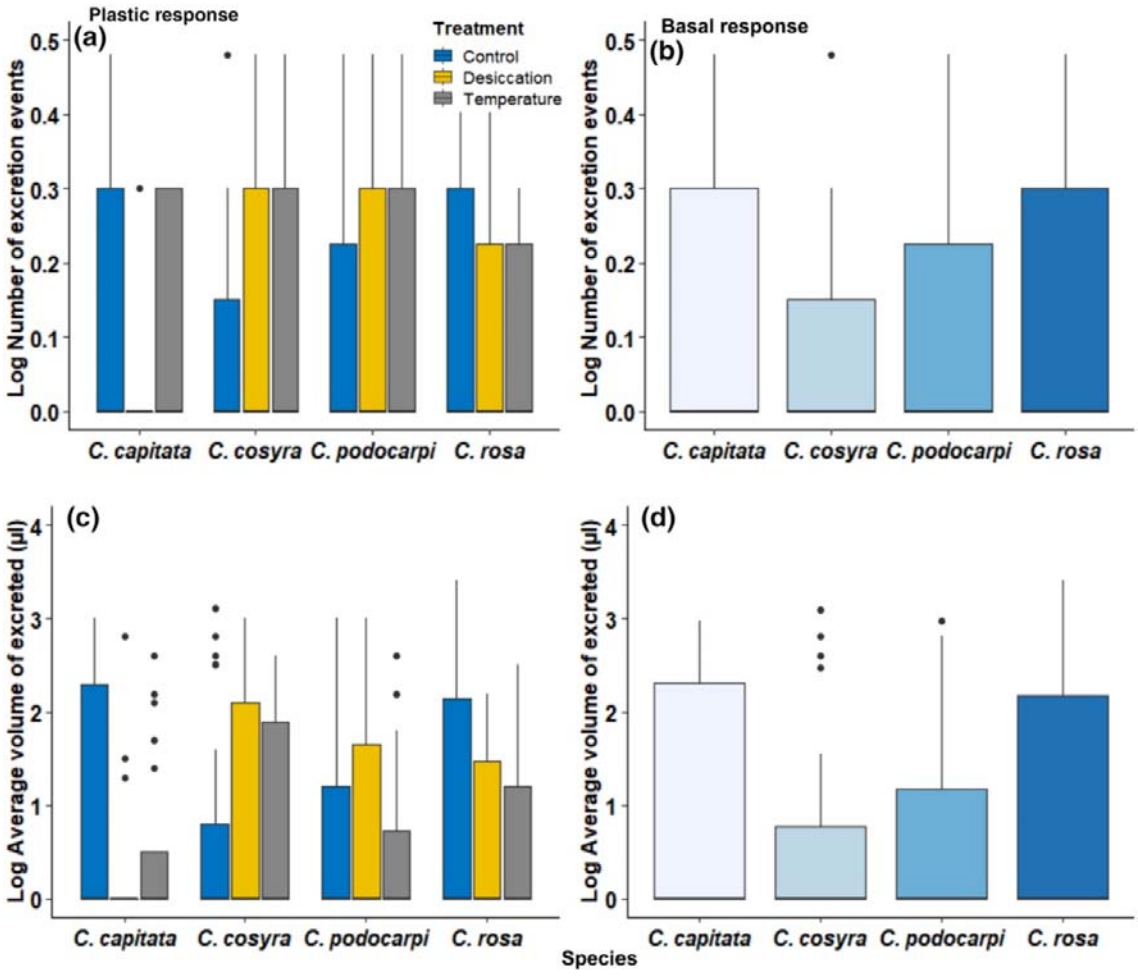

FIGURE 4. The number of excretion events and the cumulative volume of water lost for pooled excretion events recorded for four *Ceratitis* species, during a 2-h respirometry measurement for flies that experienced one of three acclimation treatments, namely: Desiccation (0% RH and 25°C), temperature (0% RH and 35°C) or control treatment (76% RH and 25°C). Plastic (a) and basal responses (b) are shown for number of excretion events, and for volume excreted (c and d, respectively). Basal excretion events (number) and total volume excreted did not differ between four species. The frequency of excretion events was reduced only after a desiccation treatment in *C. capitata* (p = 0.030). Total volume water loss was reduced after both a desiccation treatment in the species *C. capitata* (p = 0.046) and *C. rosa* (p = 0.026). Total water loss volume was also reduced by a temperature treatment *C. capitata* (p = 0.071) and *C. rosa* (p = 0.035).

Total water loss volume was less after desiccation treatment in *C. capitata* (z = −2.041; p = 0.046, Figure 4b) and *C. rosa* (z = −2.309; p = 0.026, Figure 4b) but not in *C. cosyra* (z = −0.281; p = 0.780, Figure 4b) or *C. podocarpi* (z = −1.034; p = 0.307, Figure 4b). Similarly, the volume excreted was reduced after a temperature treatment in only *C. capitata* (z = −1.831; p = 0.071, Figure 4b) and *C. rosa* (z = −2.179; p = 0.035, Figure 4b) while *C. cosyra*

(z = −0.699; p = 0.488, Figure 4b) and *C. podocarpi* (z = −1.053; p = 0.543, Figure 4b) were unaffected.

4 DISCUSSION

Here, we compared basal and plastic desiccation physiology of broadly distributed and narrowly distributed fruit fly species using high temporal resolution, flow-through respirometry to partition routes of evaporative water loss, and subsequently identified major mechanisms underlying water balance trait variation. By examining the water loss physiology of closely related species, we show that both basal and plastic physiological responses can play a role in desiccation physiology, and that each species employs a specific combination of water loss mechanisms to combat desiccation stress.

Basal tolerance to desiccation stress was observed in the broadly-distributed *C. capitata* as well as the narrowly-distributed *C. cosyra*, but not in the other two narrowly-distributed species. The basal response was driven in part by resting metabolic rate and activity patterns, where *Ceratitis capitata* had significantly higher and *C. podocarpi* had significantly lower metabolic rates than the other two species, and *C. capitata* the lowest activity levels of all species tested. However, none of the water loss traits in non-acclimated individuals differed between the species under desiccating conditions. Therefore, further exploration of other metabolic traits, such as differential nutrient oxidation to supplement metabolic water, could potentially shed light on the innate desiccation tolerance of these species. For example, Weldon et al. (2016) presented evidence for broadly-distributed *C. capitata* catabolising lipids during water stress while narrowly-distributed *C. cosyra* and *C. rosa* did not.

While phenotypic plasticity was observed in both broadly distributed and narrowly distributed species, the metabolic and water balance traits were uniquely expressed in each species and temperature and humidity treatment. The widely distributed species reduced water loss rate after a desiccation treatment by reducing both excretion and CWL, meanwhile the narrowly distributed species had reduced CWL and RWL (*C. rosa*), as well as reduced metabolic rate (*C. podocarpi*). Meanwhile, temperature treatment reduced activity, RWL and excretory water loss in the widely distributed species (*C. capitata*) and reduced metabolic rate in the narrowly distributed species (*C. podocarpi*) which also led to a corresponding reduction in RWL rate.

As predicted, a low RH or high temperature treatment induced a particular plastic physiological response by reducing either CWL or RWL rates. CWL is related to insect epicuticular hydrocarbon composition which change during the adult life stage of some insects to decrease water loss (Benoit & Denlinger, 2010; Botella-Cruz et al., 2021; Chown et al., 2011), and is here shown to be affected by desiccation treatment but not temperature treatment. Lipid phase changes reported at high temperatures in other insects (Gibbs, 2002; Lighton & Feener Jr., 1989; Rourke & Gibbs, 1999) are largely absent in this study, suggesting this is not likely a universal response, or perhaps more sustained thermal variation is required to trigger such a response in our focal species. A deeper exploration into cuticular hydrocarbon composition, gene expression and lipidomic variation before, during and after recovery from desiccation stress will help shed light on plastic variation of the epicuticle in response to desiccation stress in tephritids (see e.g. Baumgart et al., 2022).

We have shown through measurement of various desiccation-related traits that species with a high basal desiccation tolerance (*C. cosyra*) have low phenotypic plasticity in desiccation responses while, by contrast, the widely distributed species (*C. capitata*) derived a significant survival benefit from its cumulative plastic responses but had lower basal tolerance than the more geographically range-restricted species. Therefore, our hypothesis that both high basal tolerance and phenotypic plasticity are costly responses and that only one approach will be attempted by each species, is supported by our results.

Host specialisation could prevent the need for phenotypic plasticity as is seen in the specialist *Ceratitis podocarpi* that feeds only on the fruits of *Podocarpus* trees (De Meyer et al., 2002) found in remnant Afromontane forests with substantial annual rainfall (>525 mm pa; Low & Rebelo, 1998), which along with the shade created by the canopy should allow for a cooler and more humid environment. Thus, *C. podocarpi* might not rely on rapid or pronounced phenotypic plasticity responses as it is found in a relatively stable environment. While *C. cosyra* may not have exhibited much plasticity, it did have high basal tolerance to desiccation. The main hosts of *C. cosyra* are tropical marulas *Sclerocarya birrea* and mangoes, with the former growing in highly seasonal savannah environments with cold dry winters and hot wet summers (Jinga et al., 2022; Mansourian et al., 2018; Theron et al., 2017). Therefore, *C. cosyra* is also not expected to regularly encounter desiccation or temperature stress, which would require a pronounced plastic response. It may be useful to further explore how longer-term acclimation or selection can affect these species and their ability to adapt, especially as there may also be considerable costs associated with phenotypic plasticity in desiccation resistance that are currently poorly understood in tephritids (Sgro et al., 2016; but see e.g. Weldon et al., 2018).

Ultimately, the fitness benefits of phenotypic plasticity are best described by the cumulative effects of all the plastic responses that result in survival benefits under desiccating conditions of different magnitude for each species. Here we show that most broadly distributed species, *C. capitata*, ultimately derives the largest survival benefit from its cumulative desiccation resistance mechanisms in both hot and dry conditions, while the beneficial acclimation effect on water loss physiology in the narrowly distributed species was usually limited to one of the treatments. Therefore, broadly distributed species that can respond flexibly to a wide range of environmental conditions have the potential to shift their distribution to areas with novel climatic conditions (Davidson et al., 2011; Knop & Reusser, 2012; Richards et al., 2006). This plastic desiccation physiology of *C. capitata* should significantly reduce the challenge xeric conditions pose to geographical range expansion; indeed, *C. capitata* is predicted to shift its range into more temperate regions following predictions of climate change (Hill et al., 2016). Meanwhile, the weaker plasticity observed in narrowly distributed species (*C. podocarpi* and *C. rosa*) indicates that while they have limited potential to adapt to changing climatic conditions, a combination of environmental stresses (e.g. both hotter and drier) could overwhelm their capacity to mount a phenotypic response that will enable them to expand their geographical range. In some cases it is thought that plastic responses of desiccation resistance in insects might not contribute much to their overall climate change responses (Kellerman et al., 2020), yet our research suggests it might not be the case in other taxa; further work is thus needed to reveal any generalities. This research has broad implications for understanding the mechanistic basis of species responses to climate change in the face of increasing variability and concurrent stressors.

AUTHOR CONTRIBUTIONS

Henrika J. Bosua, Christopher W. Weldon and John. S. Terblanche conceived the ideas and designed the methodology; Henrika J. Bosua collected the data and processed the samples; Henrika J. Bosua analysed the data; Henrika J. Bosua, Chris W. Weldon and John. S. Terblanche led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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