


Tethered-flight performance of thermally-acclimated pest fruit flies (Diptera: Tephritidae) suggests that heat waves may promote the spread of *Bactrocera* species

Kevin Malod,^a  Eleftheria-Maria D. Bali,^b Corentin Gledel,^c Laura Moquet,^c Anandi Bierman,^a Evmorfia Bataka,^b Christopher W. Weldon,^d Minette Karsten,^a H el ene Delatte,^e Nikos T. Papadopoulos^b and John S. Terblanche^{a*}



Abstract

Background: Thermal history may induce phenotypic plasticity in traits that affect performance and fitness. One type of plastic response triggered by thermal history is acclimation. Because flight is linked to movement in the landscape, trapping and detection rates, and underpins the success of pest management tactics, it is particularly important to understand how thermal history may affect pest insect flight performance. We investigated the tethered-flight performance of *Ceratitis capitata*, *Bactrocera dorsalis* and *Bactrocera zonata* (Diptera: Tephritidae), acclimated for 48 h at 20, 25 or 30 °C and tested at 25 °C. We recorded the total distance, average speed, number of flight events and time spent flying during 2-h tests. We also characterized morphometric traits (body mass, wing shape and wing loading) that can affect flight performance.

Results: The main factor affecting most flight traits was body mass. The heaviest species, *B. dorsalis*, flew further, was faster and stopped less often in comparison with the two other species. *Bactrocera* species exhibited faster and longer flight when compared with *C. capitata*, which may be associated with the shape of their wings. Moreover, thermal acclimation had sex- and species-specific effects on flight performance. Flies acclimated at 20 °C stopped more often, spent less time flying and, ultimately, covered shorter distances.

Conclusion: Flight performance of *B. dorsalis* is greater than that of *B. zonata* and *C. capitata*. The effects of thermal acclimation are species-specific. Warmer acclimation temperatures may allow pest fruit flies to disperse further and faster.

  2023 The Authors. *Pest Management Science* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Supporting information may be found in the online version of this article.

Keywords: Tephritidae; thermal history; flight performance; phenotypic plasticity

1 INTRODUCTION

Thermal history may induce phenotypic plasticity so that a past thermal environment positively or negatively affects fitness.^{1,2} One type of phenotypic plasticity induced by a change in the thermal environment is acclimation. Perhaps due to their importance in understanding the invasive potential of pest insects in various environments, the first traits that are usually investigated in response to thermal acclimation are cold and heat tolerance. However, understanding the flight performance of a pest insect is also crucial, whether it is to improve success of methods used in pest management (e.g., sterile insect technique), or to predict movements (e.g., trapping and detection) and risk of invasion as well as quarantine areas.^{3–6}

Several fruit fly species (Diptera: Tephritidae) are among the most destructive horticultural pests, leading to large economic

* Correspondence to: John S. Terblanche, Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Stellenbosch, South Africa, E-mail: jst@sun.ac.za

a Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Stellenbosch, South Africa

b Laboratory of Entomology and Agricultural Zoology, Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Volos, Greece

c CIRAD, UMR PVBM, Saint-Pierre, France

d Department of Zoology and Entomology, University of Pretoria, Hatfield, South Africa

e CIRAD, UMR PVBMT, Antananarivo, Madagascar

losses to fruit industries worldwide, and necessitating implementation of pest management programs. Some of these fruit flies are particularly problematic as they expanded outside of their native range and became invasive in new regions of the world. Most species of major economic importance are found in the genera *Anastrepha* (Americas), *Bactrocera* and *Zeugodacus* (Asia, Africa), *Ceratitis* (Africa, Australia, Europe, Central and South America) and *Rhagoletis* (Europe and North America).^{7–9} In Africa and Europe, the most concerning species to food security are *Bactrocera dorsalis* (Hendel), *Bactrocera zonata* (Saunders), and *Ceratitis capitata* (Wiedemann); all three highly polyphagous and the latter notorious for its cosmopolitan distribution. These species can cause significant economic losses depending on the country and the type of cultivated fruit, estimated at between 1.9 and 116 million USD yearly⁸ for *B. dorsalis* and *C. capitata*, and 190 million EUR for *B. zonata*.¹⁰ Furthermore, the economic impact of these pests might be even greater in the near future, as suitable areas for *C. capitata*,¹¹ *B. dorsalis*^{12,13} and *B. zonata*¹⁴ are predicted to expand into higher latitudes with climate change. The literature documenting the biology of these species is extensive, yet little is known about their dispersal ability in relation to thermal history and how it could relate to their invasiveness.

Past thermal environment experienced by insects affects flight performance in various species.^{4,15–18} However, for tephritids this has so far only been investigated in *C. capitata*. Adult flight performance of *C. capitata* in the laboratory was enhanced when pupae were acclimated to cold temperatures, relative to those exposed to warmer temperatures.¹⁹ However, the opposite was not true, warm pupal acclimation did not confer any benefit to adults when tested in a warm environment. Furthermore, Steyn *et al.*²⁰ found that *C. capitata* adults acclimated at 20 °C dispersed better in the field than flies acclimated at 25 or 30 °C, especially in warmer environments. In *B. dorsalis*, although the impact of thermal history on flight performance has not been investigated to our knowledge, its flight performance as a function of sex, age, physiological status, environmental temperature and how far it can disperse has been documented.^{5,21–24} Despite *B. zonata* being listed as a strong flier,²⁵ and having a maximum recorded dispersal range of about 40 km,²⁶ information on its flight ability is scarce. Nevertheless, thermal history could affect flight performance in *B. zonata*, as acclimation at 30 °C leads to increased body mass and energy reserves.²⁷

To improve knowledge of the dispersal potential of three major tephritid pests, we investigated how thermal history affects traits associated with flight performance. We established whether cold or warm acclimation temperatures during the adult life-stage increases or decreases flight performance and if the same pattern is observed across all three species. Due to their invasiveness and significant geographic range expansion (that is predicted to expand further), we assessed if there are flight performance traits shared between these three species that explain their rapid spread outside of their native range. As the morphology of the wings may be linked to flight performance in tephritids,^{19,22} we also investigated how morphological traits (body mass, wing shape and size) characterize strong *versus* weak flying individuals. Based on observations from Steyn *et al.*,²⁰ we predicted that cold acclimated adult flies would exhibit better flight performance when tested at an intermediate flight temperature. Because *Bactrocera* species are widely regarded to be the most mobile tephritids of economic significance,²³ we anticipated that *B. dorsalis* and *B. zonata* would fly for longer periods of time and further than *C. capitata*, and that this would relate to morphological characteristics.

2 MATERIALS AND METHODS

2.1 Fly husbandry

Cultures of *Bactrocera dorsalis* (South Africa), *Bactrocera zonata* (La Réunion) and *Ceratitis capitata* (Greece) were maintained in climate rooms in different countries under controlled laboratory conditions at 25 °C (see Supplementary Information for details).

2.2 Acclimation

Flies were acclimated in incubators for 48 h at either 20, 25 or 30 °C. Flies were only acclimated after reaching sexual maturity (i.e., 14–16 days for *B. dorsalis*, 12–50 days for *B. zonata*, and 9–11 days for *C. capitata*). Less than 48 h of thermal acclimation is sufficient to induce changes in physiological traits in *C. capitata*.²⁸

2.3 Tethered flight procedure

The flight mill assays were conducted in a temperature-controlled room at 25 °C (see Supplementary Information for details). We used flight mills connected to a multi-channel flight mill data acquisition (DAQ) system controlled from a laptop computer. A maximum of nine flies were tested per session (i.e., group of flies tested at the same time). The body mass of each fly was determined using analytical balances before being placed on the flight mill (see Supplementary Information for details). Each session included at least two flies from each temperature group. Each session started when the last fly was attached to the flight mill and was recorded for exactly 2 h. We ran the assays until having between 14 and 24 flying individuals of each sex and temperature group. Individuals completing fewer than one revolution (two data points) were considered as non-flying. To determine whether data from different laboratories using different flight mills were comparable, *B. dorsalis* of both sexes from La Réunion and acclimated at 25 °C were tested (see Table S1 and Fig. S3).

2.4 Wing morphometry

For each species, from the 15 flying individuals of each sex and temperature group, we identified the five flies that flew the longest and shortest distances (total = 60 per species). Both wings of each fly were removed and imaged for measurements. For both wings (one if damaged), measurements were taken for wing length, wing width and seven landmarks used to calculate wing area (Figs. S1 and S2). These measurements were used to calculate the aspect ratio (wing length²/wing area) and the wing loading (body mass/wing area). A low aspect ratio indicates short and broad wings that might be better for maneuverability, whereas a high aspect ratio indicates long and thin wings that might be better for speed and long distance.²⁹ Low wing loading indicates large wings relative to the mass of the individual (more lift), in contrast, high wing loading indicates small wings relative to the mass (less lift).

2.5 Statistical analyses

All statistical analyses were performed in R software (v 4.2.1, The R Foundation for Statistical Computing) (see Supplementary Information for detailed procedure).

2.5.1 Intraspecific comparisons of flight performance

Data were analyzed using generalized mixed effects models. Acclimation temperature, sex and their interactions were entered as fixed effects, body mass as a covariate, and flight mill channel as a random effect. Model reduction was performed by removing the interaction term if it was not significant, and models (full and reduced) were compared using Akaike information criterion (AIC)

to determine the best one based on the lowest AIC. If a significant main effect or interaction was detected, *post hoc* pairwise comparison tests were performed. To assess if the characteristics of the wings affect flight performance, we replaced body mass in the generalized linear models with either the aspect ratio, wing loading or wing area as a covariate. No *post-hoc* analyses were performed after running the generalized linear models using wing characteristics as we were only interested in the effect of the covariate.

2.5.2 Interspecific comparisons of flight performance

For each flight performance trait, performance across species was compared using the models from the intraspecific comparisons with the addition of species as a fixed factor. If a significant main effect or interaction was detected, *post hoc* pairwise comparison tests were performed. Because of the size difference between species, we first compared the species without including body mass as a covariate in the models. We used the outputs of these models to interpret the differences in flight performance between species. However, we also checked differences between species when body mass was added to the models as a covariate.

2.5.3 Intraspecific comparison of wing and body morphometry

We used generalized linear models for all traits (aspect ratio, wing loading and wing area). Sex, acclimation temperature and their interactions were added as fixed effects, and body mass as a covariate (except for wing loading). No random effect was added as morphometric measurements are independent of the flight mill apparatus used. If a significant main effect or interaction was detected, *post hoc* pairwise comparison tests were performed.

2.5.4 Interspecific comparison of wing and body morphometry

We compared species to one another for all traits (aspect ratio, wing loading and wing area) using generalized linear models. Sex, acclimation temperature and their interactions were added as fixed effects, and body mass as a covariate (except for wing loading). If a significant main effect or interaction was detected, *post hoc* pairwise comparison tests were performed.

3 RESULTS

3.1 Flight performance

3.1.1 Intraspecific comparisons of flight performance

Body mass was a strong predictor of flight performance as it affected almost all the traits within each species (Table 1, Fig. S5) (see Supplementary Information for more details).

In *C. capitata*, the distance flown was only affected by body mass (Table 1). The distance increased with body mass (coefficient = 0.14, $P = 0.012$). In *B. dorsalis*, heavier flies also covered more distance (coefficient = 271.54, $P < 0.001$), but it was also affected by acclimation (Table 1). Regardless of the sex, *B. dorsalis* acclimated at 20 °C flew shorter distances than those acclimated at 25 or 30 °C (respectively: estimate = -1142, $P = 0.02$; estimate = -1246, $P = 0.01$) and there was no difference between 25 and 30 °C (Fig. 1(a)). In *B. zonata*, acclimation and sex together affected flight distance. When acclimated at 25 °C females covered shorter distances than males (estimate = -1997, $P < 0.001$), and in males, flies acclimated at 20 °C flew shorter distances than those acclimated at 25 °C (estimate = -1240.8, $P = 0.002$).

In all species, average speed was positively correlated with body mass (Table 1) (*C. capitata*: coefficient = 0.07, $P = 0.002$; *B. dorsalis*: coefficient = 0.02, $P = 0.004$; *B. zonata*: coefficient = 0.01, $P = 0.007$). The average speed for *C. capitata*, *B. dorsalis* and *B. zonata* were 0.39 ± 0.01 , 0.63 ± 0.02 and 0.44 ± 0.01 m/s respectively (Fig. 1(b)). Similarly, body mass was the only factor affecting maximum speed in all species (Table 1). Maximum speed increased as fly body mass increased (*C. capitata*: coefficient = 0.05, $P < 0.001$; *B. dorsalis*: coefficient = 0.04, $P < 0.001$; *B. zonata*: coefficient = 0.01, $P = 0.033$). The average top speed recorded was 0.55 ± 0.01 for *C. capitata*, 0.98 ± 0.03 for *B. dorsalis* and 0.69 ± 0.02 m/s for *B. zonata* (Fig. 1(c)).

The number of flight events was affected by body mass in all species (Table 1). Heavier flies stopped less often than lighter ones (*C. capitata*: coefficient = -0.16, $P = 0.005$; *B. dorsalis*: coefficient = -0.10, $P < 0.001$; *B. zonata*: coefficient = -0.15, $P < 0.001$). In all species, the number of flight events was affected by the interaction between acclimation and sex (Fig. 1(d)). In female *C. capitata*, flies stopped more when acclimated at 20 than 25 °C (estimate = 0.60, $P = 0.002$), and less at 25 than 30 °C (estimate = -0.63, $P = 0.001$). In males, flies stopped more when acclimated at 20 than 30 °C (estimate = 0.45, $P = 0.015$). For groups acclimated at 30 °C, only female and male *C. capitata* differed, with females stopping more often than males (estimate = 0.74, $P < 0.001$). In *B. dorsalis*, differences between acclimation groups only occurred in females, with both the 20 and 25 °C groups resting more often than the 30 °C group (respectively, estimate = 1.51, $P < 0.001$; estimate = 1.08, $P < 0.001$). In this species, females rested more often than males when acclimated at 20 °C (estimate = 0.66, $P < 0.001$), but less often than those acclimated at 30 °C (estimate = -0.97, $P < 0.001$). For *B. zonata*, flight interruptions occurred more often as the acclimation temperature increased. Females from the 20 °C group stopped less often than those acclimated at 25 or 30 °C (respectively, estimate = -0.42, $P = 0.026$; estimate = -1.32, $P < 0.001$), and females acclimated at 25 °C rested less often than if they were acclimated at 30 °C (estimate = -0.89, $P < 0.001$). Males acclimated at 20 or 25 °C had less interruptions than those acclimated at 30 °C (respectively, estimate = -1.05, $P < 0.001$; estimate = -1.33, $P < 0.001$). Females stopped more often than males if acclimated at 25 or 30 °C (respectively, estimate = 0.85, $P < 0.001$; estimate = 0.42, $P = 0.002$).

The time spent flying was affected by body mass in *C. capitata* and *B. dorsalis* but not in *B. zonata* (Table 1). The flight duration increased with body mass (*C. capitata*: coefficient = 693.3, $P = 0.034$; *B. dorsalis*: coefficient = 0.05, $P = 0.026$). Furthermore, the total duration of the flight in *B. dorsalis* was also affected by acclimation (Table 1). Flies acclimated at 20 °C spent less time flying than those acclimated at 30 °C (estimate = -0.43, $P = 0.016$) (Fig. 1(e)). None of the tested parameters affected the total flight duration in *B. zonata*.

3.1.2 Interspecific comparisons of flight performance

Body mass was affected by an interaction between species, acclimation and sex (Table S4). This is because in *B. zonata* females acclimated at 20 °C were lighter than the ones acclimated at 25 °C (estimate = -4.95, $P < 0.001$) or 30 °C (estimate = -3.38, $P < 0.001$) (Fig. S5). Overall, *C. capitata* was lighter than *B. dorsalis* (estimate = -10.91, $P < 0.001$) and *B. zonata* (estimate = -1.79, $P < 0.001$), and *B. dorsalis* was heavier than *B. zonata* (estimate = 9.12, $P < 0.001$).

Table 1. Within species effects of acclimation temperature (20, 25 and 30 °C) and sex on the flight performance at 25 °C of three tephritid species

		χ^2	df	P
Body mass				
<i>C. capitata</i>	Acclimation	3.91	2	0.141
	Sex	28.15	1	<0.001
	Acclimation × Sex	2.27	2	0.321
<i>B. dorsalis</i>	Acclimation	4.38	2	0.112
	Sex	25.55	1	<0.001
	Acclimation × Sex	3.24	2	0.198
<i>B. zonata</i>	Acclimation	57.38	2	<0.001
	Sex	14.24	1	<0.001
	Acclimation × Sex	19.84	2	<0.001
Distance				
<i>C. capitata</i>	Acclimation	1.26	2	0.533
	Sex	0.33	1	0.563
	Body mass	6.31	1	0.012
	Acclimation × Sex	1.03	2	0.598
<i>B. dorsalis</i>	Acclimation	15.19	2	<0.001
	Sex	3.63	1	0.056
	Body mass	16.38	1	<0.001
	Acclimation × Sex	5.35	2	0.069
<i>B. zonata</i>	Acclimation	2.03	2	0.362
	Sex	0.86	1	0.353
	Body mass	1.88	1	0.169
	Acclimation × Sex	13.2	2	0.001
Average speed				
<i>C. capitata</i>	Acclimation	0.19	2	0.908
	Sex	0.81	1	0.367
	Body mass	9.07	1	0.002
	Acclimation × Sex	0.07	2	0.966
<i>B. dorsalis</i>	Acclimation	0.37	2	0.831
	Sex	0.49	1	0.481
	Body mass	8.38	1	0.004
	Acclimation × Sex	1	2	0.606
<i>B. zonata</i>	Acclimation	3.75	2	0.153
	Sex	0.02	1	0.873
	Body mass	7.26	1	0.007
	Acclimation × Sex	0.74	2	0.691
Maximum speed				
<i>C. capitata</i>	Acclimation	0.56	2	0.753
	Sex	0.27	1	0.598
	Body mass	12.94	1	<0.001
	Acclimation × Sex	1.25	2	0.535
<i>B. dorsalis</i>	Acclimation	0.86	2	0.648
	Sex	0.28	1	0.595
	Body mass	12.24	1	<0.001
	Acclimation × Sex	0.41	2	0.812
<i>B. zonata</i>	Acclimation	4.85	2	0.088
	Sex	3.73	1	0.053
	Body mass	4.52	1	0.033
	Acclimation × Sex	3.16	2	0.206
Flight events				
<i>C. capitata</i>	Acclimation	15.08	2	<0.001
	Sex	2.54	1	0.111
	Body mass	7.61	1	0.006
	Acclimation × Sex	15.63	2	<0.001
<i>B. dorsalis</i>	Acclimation	35.95	2	<0.001
	Sex	9.61	1	0.002
	Body mass	17.77	1	<0.001
	Acclimation × Sex	30.55	2	<0.001

Table 1. Continued

		χ^2	df	P
<i>B. zonata</i>	Acclimation	124.28	2	<0.001
	Sex	1.54	1	0.214
	Body mass	89.41	1	<0.001
	Acclimation × Sex	10.51	2	0.005
Flight duration				
<i>C. capitata</i>	Acclimation	1.54	2	0.462
	Sex	1.07	1	0.299
	Body mass	4.65	1	0.031
	Acclimation × Sex	1.11	2	0.575
<i>B. dorsalis</i>	Acclimation	14.08	2	<0.001
	Sex	2.09	1	0.148
	Body mass	5.16	1	0.023
	Acclimation × Sex	4.57	2	0.101
<i>B. zonata</i>	Acclimation	3.99	2	0.136
	Sex	2.04	1	0.153
	Body mass	1.67	1	0.196
	Acclimation × Sex	5.99	2	0.051

Note: Bold values indicate significant terms.

Flight distance was affected by an interaction between species, acclimation and sex (Table S4) (Fig. 1(a)). At 25 °C, *B. dorsalis* females covered more distance than *B. zonata* females (estimate = 1860.42, $P < 0.001$), and *C. capitata* males flew shorter distances than those of *B. dorsalis* (estimate = -1713.54, $P < 0.001$) or *B. zonata* (estimate = -1383.90, $P < 0.001$). When acclimated at 30 °C, *C. capitata* females and males covered less distance than their *B. dorsalis* counterparts (Female: estimate = -2337.33, $P < 0.001$; Male: estimate = -1196.04, $P = 0.034$), and *B. dorsalis* females reached greater distances than *B. zonata* ones (estimate = 2646.51, $P < 0.001$).

The average and maximum speed differed between species (Table S4) (Fig. 1(b)). Average speed was lower in *C. capitata* than in *B. dorsalis* (estimate = -0.15, $P < 0.001$) or *B. zonata* (estimate = -0.04, $P = 0.043$), and higher in *B. dorsalis* in comparison to *B. zonata* (estimate = 0.11, $P < 0.001$). The maximum speed of *C. capitata* was lower than that of *B. dorsalis* (estimate = -0.42, $P < 0.001$) and *B. zonata* (estimate = -0.14, $P < 0.001$), and *B. dorsalis* was faster than *B. zonata* (estimate = 0.28, $P < 0.001$).

The number of flight events was affected by an interaction between species and acclimation (Table S4) (Fig. 1(c)). At 20 °C, *B. dorsalis* stopped less often than *C. capitata* (estimate = -0.59, $P = 0.035$). At 30 °C, *C. capitata* (estimate = -0.73, $P = 0.006$) and *B. dorsalis* (estimate = -1.57, $P < 0.001$) stopped less than *B. zonata*, and *C. capitata* stopped more regularly than *B. dorsalis* (estimate = 0.84, $P = 0.006$). There was no difference between species at 25 °C.

No main effects or interactions were detected for the total flight duration (Table S4).

3.2 Wing and body morphometry

3.2.1 Intraspecific comparison of wing and body morphometry

In *C. capitata*, wing aspect ratio was affected by sex and body mass (Table 2) (Fig. 2(a)). Aspect ratio was lower in males than females (coefficient = -0.01, $P = 0.006$), and the aspect ratio

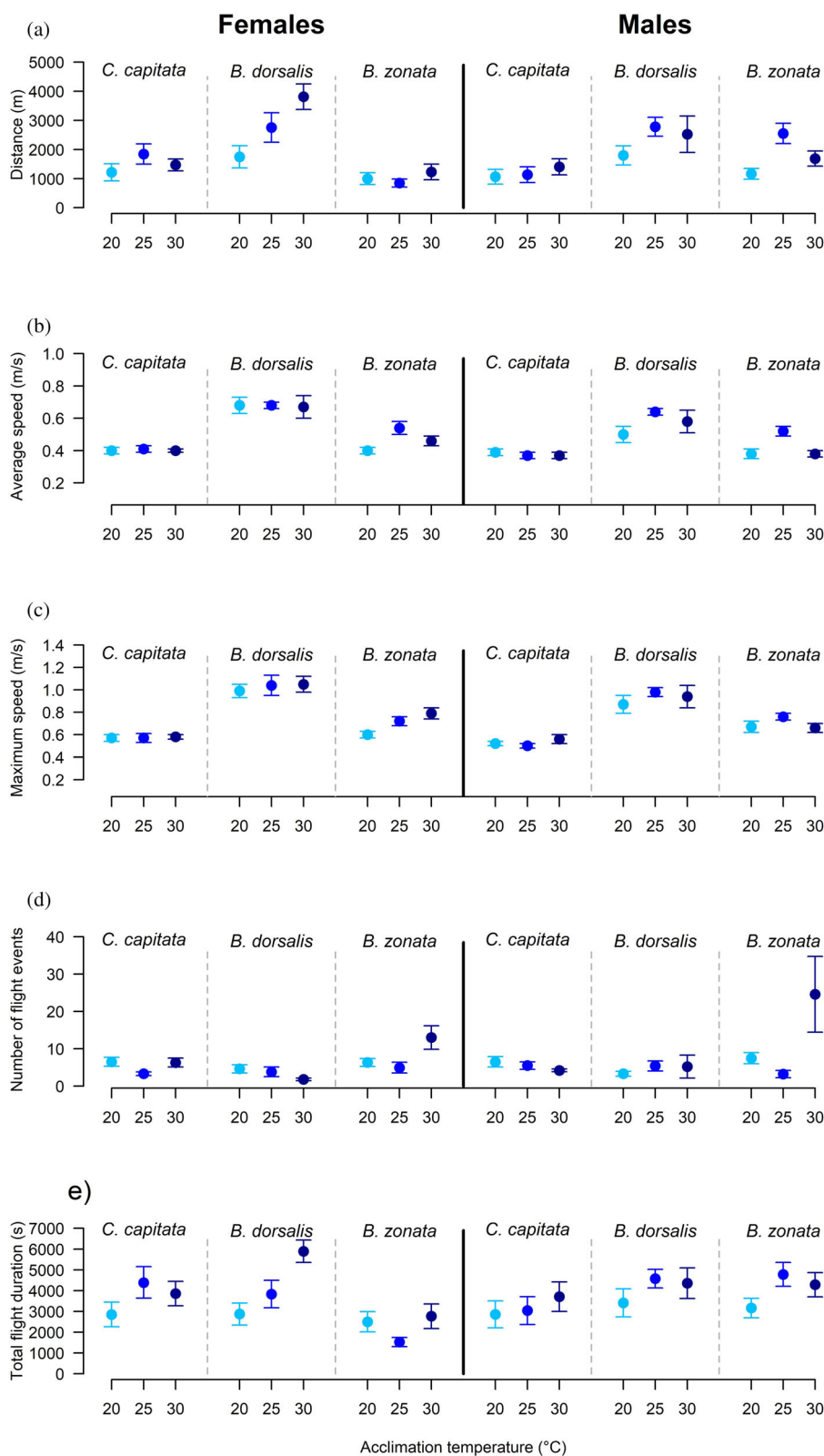


Figure 1. Flight performance traits of females and males from three tephritid species acclimated at either 20, 25 or 30 °C and tested at 25 °C. Total distance flown (a), average speed (b), maximum speed (c), number of flight events (d) and the total time spent flying (e). Each circle represents the means from 14 to 24 individuals and error bars represent the standard error of the mean.

slightly increased with body mass (coefficient = 0.01, $P = 0.001$). In *B. dorsalis* and *B. zonata*, none of the tested factors affected wing aspect ratio.

Wing loading in *C. capitata* was affected by sex, acclimation temperature and their interaction (Table 2) (Fig. 2(b)). This was because wing loading between acclimation groups differed in

Table 2. Within species effects of acclimation temperature (20, 25 and 30 °C) and sex on the morphometry of three tephritid species

		χ^2	df	P
Aspect ratio				
<i>C. capitata</i>	Sex	8.32	1	0.003
	Body mass	11.79	1	<0.001
<i>B. dorsalis</i>	Sex	2.76	1	0.096
<i>B. zonata</i>	Acclimation	2.69	2	0.259
	Sex	1.52	1	0.218
	Body mass	0.67	1	0.413
	Acclimation × Sex	2.57	2	0.277
Wing loading				
<i>C. capitata</i>	Acclimation	10.94	2	0.004
	Sex	9.07	1	0.002
	Acclimation × Sex	8.15	2	0.017
<i>B. dorsalis</i>	Acclimation	11.3	2	0.003
	Sex	3.23	1	0.072
	Acclimation × Sex	19.39	2	<0.001
<i>B. zonata</i>	Acclimation	21.99	2	<0.001
	Sex	4.17	1	0.041
	Acclimation × Sex	9.27	2	0.009
Wing area				
<i>C. capitata</i>	Sex	10.33	1	0.001
	Body mass	8.75	1	0.003
<i>B. dorsalis</i>	Sex	3.52	1	0.061
<i>B. zonata</i>	Acclimation	1.05	2	0.592
	Sex	1.96	1	0.161
	Body mass	47.53	1	<0.001
	Acclimation × Sex	14.83	2	<0.001

Note: Bold values indicate significant terms.

females only, with females acclimated at 20 °C having a lower wing loading than those acclimated at 25 °C (estimate = -0.12, $P = 0.011$) or 30 °C (estimate = -0.11, $P = 0.021$). In *B. dorsalis*, wing loading was also affected by the acclimation temperature and its interaction with sex (Table 2). Females had a higher wing loading than males when acclimated at 25 °C (estimate = 0.23, $P = 0.039$) but lower when acclimated at 30 °C (estimate = -0.38, $P = 0.001$). For *B. zonata*, wing loading was affected by sex, acclimation temperature and their interaction (Table 2). Wing loading between acclimation groups only differed in females, with females acclimated at 20 °C having a higher wing loading than those acclimated at 25 °C (estimate = 0.29, $P < 0.001$) or 30 °C (estimate = 0.27, $P < 0.001$).

Wing area in *C. capitata* was affected by sex and body mass (Table 2) (Fig. 2(c)). Wing area was higher in males (coefficient = 0.22, $P = 0.001$) and the area increased as body mass increased (coefficient = 0.07, $P = 0.004$). None of the factors affected wing area in *B. dorsalis*. The wing area of *B. zonata* was affected by an interaction between acclimation temperature and sex, as well as body mass (Table 2). In males only, wing area was higher when flies were acclimated at 25 °C instead of 30 °C (estimate = 0.75, $P < 0.001$). Wing area in *B. zonata* increased as body mass increased (coefficient = 0.17, $P < 0.001$).

3.2.2 Interspecific comparison of wing and body morphometry

Wing aspect ratio was affected by an interaction between species and sex and an interaction between sex and acclimation

(Table S6). Female *B. dorsalis* had a lower aspect ratio than female *B. zonata* (estimate = -0.02, $P = 0.006$) but this was not the case in males (estimate = 0.01, $P = 0.985$) (Fig. 2(a)). In females only, flies acclimated at 25 °C had a lower aspect ratio than those acclimated at 30 °C (estimate = -0.02, $P = 0.034$). Overall *C. capitata* had a lower aspect ratio than *B. dorsalis* (estimate = -0.53, $P < 0.001$) and *B. zonata* (estimate = -0.54, $P < 0.001$), and there was no difference between *B. dorsalis* and *B. zonata* (estimate = -0.01, $P = 0.092$). This indicates that *C. capitata* had shorter and wider wings than the two *Bactrocera* species. In all species, males had a lower aspect ratio than females (coefficient = -0.12, $P < 0.001$).

There was an interaction effect of species, sex and acclimation on wing loading (Table S6). Wing loading was overall the lowest in *C. capitata* in comparison with *B. dorsalis* (estimate = -0.24, $P < 0.001$) and *B. zonata* (estimate = -0.08, $P < 0.001$), and *B. dorsalis* had a higher wing loading than *B. zonata* (estimate = 0.16, $P < 0.001$), this was not the case at all temperatures and was sex dependent (Fig. 2(b)). There was no difference between *C. capitata* and *B. zonata* in both sexes for flies acclimated at 20 °C. At 25 °C, there was no difference between *B. dorsalis* and *B. zonata* females. In males acclimated at 30 °C, there was no difference between *C. capitata* and *B. zonata*. Wing loading was lower in males than in females in all species (coefficient = -0.08, $P = 0.011$).

Wing area was affected by an interaction between species, sex and acclimation (Table S6). This was due to differences between acclimation groups in *B. zonata* and because in some treatments and sex there was no difference between *C. capitata* and *B. zonata* (Fig. 2(c)). In *B. zonata* males, the wing area was larger for flies acclimated at 20 or 25 °C than 30 °C (respectively, estimate = 0.08, $P = 0.003$; estimate = 0.16, $P < 0.001$), and smaller in flies acclimated at 20 than 25 °C (estimate = -0.08, $P = 0.002$). In comparison with *B. dorsalis* (estimate = -0.14, $P < 0.001$) and *B. zonata* (estimate = -0.02, $P < 0.001$), *C. capitata* was the species with the smallest wing area, and *B. dorsalis* had a larger wing area than *B. zonata* (estimate = 0.16, $P < 0.001$). Body mass significantly affected wing area (Table 2), which increased in heavier flies (coefficient = 0.02, $P < 0.001$).

4 DISCUSSION

In this study, we investigated how adult acclimation at temperatures of 20, 25 or 30 °C affects the flight performance of three tephritid species at an intermediate flight temperature. We found that acclimation at low temperature was deleterious, and the effect of thermal acclimation on flight performance traits was species specific. In addition, we confirmed that *Bactrocera* species are stronger flyers than *C. capitata*. This is because body mass was a main predictor of flight performance, and *Bactrocera* species are also equipped with wings that are better adapted for speed and long-distance flight.

Body mass was the main factor that defined the flight performance of individuals and species (with some exceptions in *B. zonata*). Heavier flies flew further, faster, stopped less often, and spent more time flying. This was also verified at the intraspecific level between populations of *B. dorsalis* of different geographical origins (i.e., South Africa and La Réunion) (see Table S1 and Fig. S3). Flight capacity is usually positively correlated with body mass in insects as it may translate into larger thorax and flight muscles.^{30,31} In *Drosophila* species, muscles' efficiency increases in larger flies, which contributes to decreasing energy

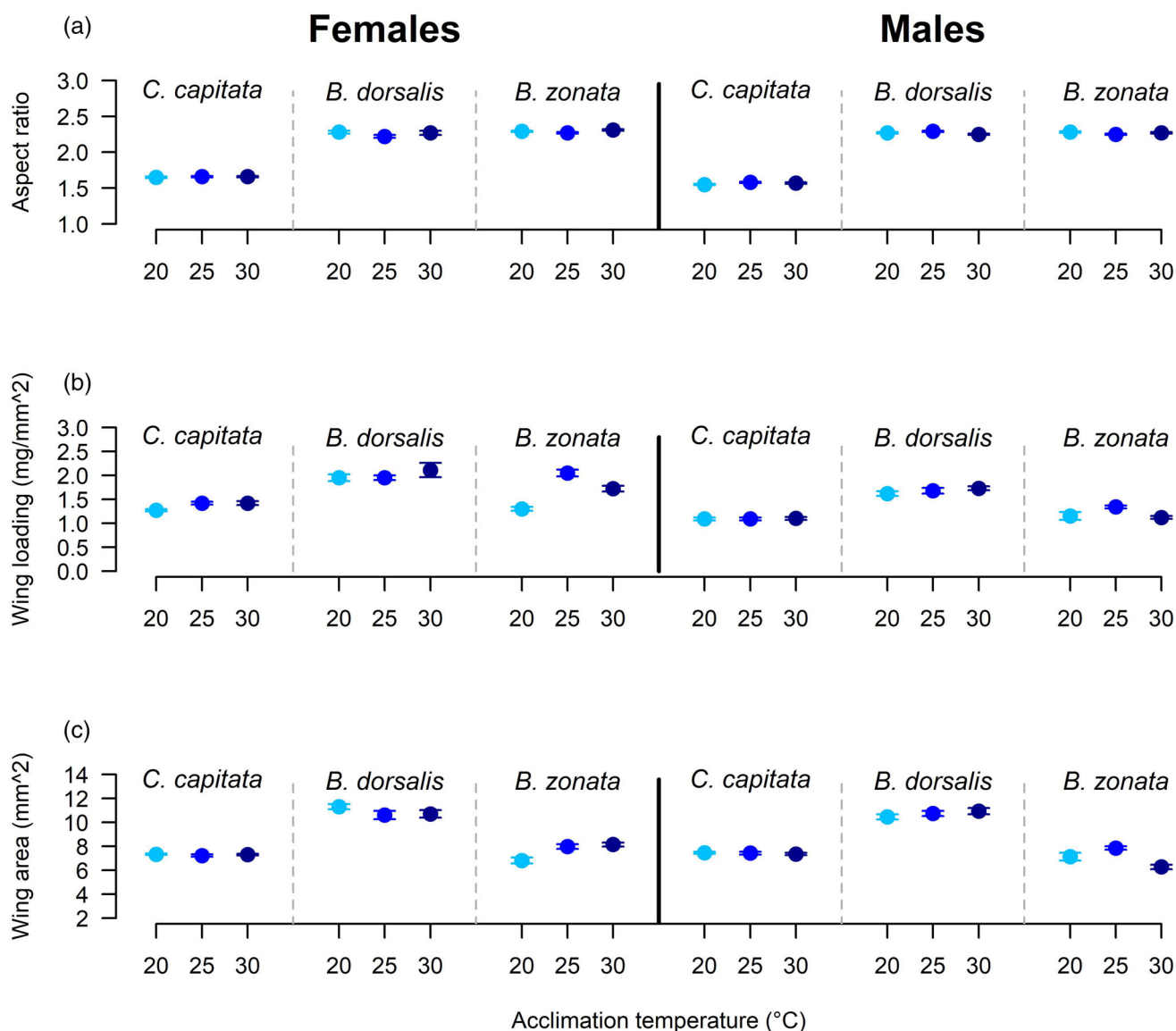


Figure 2. Wing morphometry of females and males from three tephritid species acclimated at either 20, 25 or 30 °C. Aspect ratio (a), wing loading (b) and wing area (c). Each circle represents the means from 7 to 10 individuals and error bars represent the standard error of the mean.

expenditure.³² This suggests that populations originating from environments that support the development of larger individuals may be better at dispersing. Our finding that a *B. dorsalis* population with larger individuals exhibited better tethered-flight performance aligns with the prediction that larger flies may disperse better. Consequently, because of the size variation between populations, the flight performance of an invasive tephritid species should be measured on a population that has already invaded locally (and reared from local host-plants), or that is likely to invade due to its geographical proximity or available pathways of invasion facilitated by human activities.

Flight performance traits of *Bactrocera* species appeared to be more sensitive to acclimation temperature than *C. capitata*, in which only the number of flight events was affected. Overall, we observed a deleterious effect of an acclimation at 20 °C, which might be the result of behavioral rather than physiological changes. Previous studies suggest no change in metabolic rate after thermal acclimation in insects,^{19,33} and changes in the

willingness to fly may rather explain differences in flight performance in *C. capitata*.^{19,34} Our result contrasts with the colder or hotter is better hypotheses, which predict better performance at all test temperatures after warm or cold acclimation.^{16,35} Although our experimental procedure was not set up to test the various acclimation hypotheses (i.e., with acclimated flies tested at various flight temperatures), our observations on flies acclimated at the lowest temperature provide some support for the detrimental acclimation hypothesis.³⁶ The findings of this study differ from what has been previously observed in *C. capitata* in the laboratory,¹⁹ and in the field,²⁰ where cold acclimation was beneficial in both studies under some environments. The difference in the outcome of cold acclimation between Esterhuizen *et al.*¹⁹ and our study might be explained by the type of assay used to measure flight performance and the life stage at which individuals were acclimated. We measured traits of flight performance that directly relate to dispersal (i.e., distance and speed), whereas the traits used by Esterhuizen *et al.*¹⁹ were an estimate of the ability

to initiate flight. More importantly, in Esterhuizen *et al.*,¹⁹ *C. capitata* was acclimated during the pupal stage, whereas we acclimated adults. Although flight performance was not assessed directly, Steyn *et al.*²⁰ found a beneficial effect of acclimation at 20 °C in *C. capitata* under field conditions. Nevertheless, they also noted that the effect of the acclimation treatments differed between field and semi-field conditions. Thus, if cold acclimation has a negative or no effect, as in our study when tested in controlled laboratory conditions at 25 °C, it might be that cold acclimation can be beneficial in a fluctuating environment. Although field temperatures fluctuate, results by Hoskins and colleagues³⁷ suggest that at equal means, fluctuating and constant acclimation temperatures have similar effects on the phenotype (i.e., thermal tolerance). Therefore, our results may help to predict future dispersal based on the recent field temperatures.

In the species investigated here, there was little evidence for an effect of thermal history on wing morphometry. This is probably because the variation in the thermal environment occurred during the adult stage and for a short period of time, which is unlikely to trigger changes in wing shape. Previous observations have reported very minor variation in wing length and width between *B. dorsalis* from different age groups,²² meaning that the wings do not undergo significant changes in the days following adult emergence. When comparing the wings between species, we observed that *Bactrocera* species had a higher aspect ratio in comparison with *C. capitata*, indicating longer and narrower wings, with less drag (i.e., also lower energy consumption) and better suited for distance and speed. In contrast, the lower aspect ratio and wing loading of *C. capitata* provides better maneuverability. These results align with a review on tephritid dispersal showing that *Bactrocera* species are more mobile and disperse further than *Ceratitidis* species in mark-release-recapture studies.²³ Our results suggest that this higher mobility of *Bactrocera* species may be aided by their wing morphology.

In conclusion, we showed that thermal history can affect traits related to flight performance in three tephritid species of major economic importance. Overall, acclimation at the lowest temperature had negative effects on flight performance. Nevertheless, under the specific acclimation conditions used in this study, *C. capitata* was less affected by adult thermal history than the two *Bactrocera* species. We suggest that this may relate to the evolutionary history of *C. capitata*, a species that evolved in temperate environments with higher temperature fluctuations. In laboratory conditions at 25 °C, we observed that *Bactrocera* species are better flyers than *C. capitata*. This is because *Bactrocera*, and especially *B. dorsalis*, are larger, which may allow for larger and more efficient flight muscles, and that the morphology of their wings is more suitable for higher speed and long-distance flight. Altogether, our results are also informative for pest management of those species, as they suggest that the dispersal and spread of *Bactrocera* species are more likely to be affected by climate change, which might be one of the factors explaining the recent detections of *B. dorsalis* in southern Europe.³⁸ Furthermore, these observations may also be informative to determine the size of new quarantine areas based on the thermal environment. Finally, the data presented may also be of interest for biological control programs (e.g., sterile insect technique) where insects are mass reared, and their dispersal could potentially be manipulated through adult thermal acclimation. Further studies should consider a larger number of species from these genera, as well as the effect of thermal history during the developmental stage. There is also a need to investigate the effect of acclimation at

different flight temperatures and in fluctuating environments (i.e., field studies), as some acclimation hypotheses predict that a given temperature may be detrimental or beneficial only in certain thermal environments.

ACKNOWLEDGEMENTS

The European Union's Horizon 2020 research and innovation program under agreement No 818184 (FF-IPM) funded this research. We thank Aruna Manrakhan for providing the pupae to establish cultures of *B. dorsalis*. We also thank Pertunia Moropa for her assistance with the flight mill procedure. The authors acknowledge the Plant Protection Platform (3P, IBISA), where some experiments were conducted. Valuable reviews from an anonymous reviewer and Nicholas Manoukis greatly improved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data is available on Figshare repository at [10.6084/m9.figshare.23300726](https://doi.org/10.6084/m9.figshare.23300726).

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

- Kellermann V, van Heerwaarden B and Sgrò CM, How important is thermal history? Evidence for lasting effects of developmental temperature on upper thermal limits in *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences* **284**:20170447 (2017).
- Sgrò CM, Terblanche JS and Hoffmann AA, What can plasticity contribute to insect responses to climate change? *Annu Rev Entomol* **61**: 433–451 (2016).
- Chailleux A, Thiao DS, Diop S, Bouvery F, Ahmad S, Caceres-Barrios C *et al.*, Understanding *Bactrocera dorsalis* trapping to calibrate area-wide management. *Journal of Applied Entomology* **145**:831–840 (2021).
- Chidawanyika F and Terblanche JS, Costs and benefits of thermal acclimation for codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae): implications for pest control and the sterile insect release programme. *Evolutionary Applications* **4**:534–544 (2011).
- Froerer KM, Peck SL, McQuate GT, Vargas RI, Jang EB and McInnis DO, Long-distance movement of *Bactrocera dorsalis* (Diptera: Tephritidae) in Puna, Hawaii: how far can they go? *American Entomologist* **56**:88–95 (2010).
- Hendrichs J, Pereira R and Vreysen MJB, *Area-Wide Integrated Pest Management: Development and Field Application (1st Ed.)*. CRC Press, Boca Raton (2021).
- Health EPanel oP, Bragard C, Dehnen-Schmutz K, Di Serio F, Gonthier P, Jacques M-A *et al.*, Pest categorisation of non-EU Tephritidae. *EFSA Journal* **18**:e05931 (2020).
- Ekesi S, De Meyer M, Mohamed SA, Virgilio M and Borgemeister C, Taxonomy, ecology and management of native and exotic fruit fly species in Africa. *Annu Rev Entomol* **61**:219–238 (2016).
- Malacrida AR, Gomulski LM, Bonizzoni M, Bertin S, Gasperi G and Guglielmino CR, Globalization and fruitfly invasion and expansion: the medfly paradigm. *Genetica* **131**:1–9 (2007).
- EPPO, *Bactrocera zonata*. *EPPO Bulletin* **35**:371–373 (2005).
- Gilioli G, Sperandio G, Colturato M, Pasquali S, Gervasio P, Wilstermann A *et al.*, Non-linear physiological responses to climate change: the case of *Ceratitidis capitata* distribution and abundance in Europe. *Biol Invasions* **24**:261–279 (2022).
- Hill MP and Terblanche JS, Niche overlap of congeneric invaders supports a single-species hypothesis and provides insight into future

- invasion risk: implications for global management of the *Bactrocera dorsalis* complex. *PLOS ONE* **9**:e90121 (2014).
- 13 De Meyer M, Robertson MP, Mansell MW, Ekesi S, Tsuruta K, Mwaiko W *et al.*, Ecological niche and potential geographic distribution of the invasive fruit fly *Bactrocera invadens* (Diptera, Tephritidae). *Bull Entomol Res* **100**:35–48 (2010).
 - 14 Zingore KM, Sithole G, Abdel-Rahman EM, Mohamed SA, Ekesi S, Tanga CM *et al.*, Global risk of invasion by *Bactrocera zonata*: implications on horticultural crop production under changing climatic conditions. *PLOS ONE* **15**:e0243047 (2020).
 - 15 Boersma N, Boardman L, Gilbert M and Terblanche JS, Cold treatment enhances low-temperature flight performance in false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *Agricultural and Forest Entomology* **21**:243–251 (2019).
 - 16 Frazier MR, Harrison JF, Kirkton SD and Roberts SP, Cold rearing improves cold-flight performance in *Drosophila* via changes in wing morphology. *J Exp Biol* **211**:2116–2122 (2008).
 - 17 Ernst SA and Mutchmor JA, Dispersal of three species of grain beetles as a function of thermal acclimation, temperature, and larval size. *Journal of Stored Products Research* **5**:407–412 (1969).
 - 18 Arribas P, Velasco J, Abellán P, Sánchez-Fernández D, Andújar C, Calosi P *et al.*, Dispersal ability rather than ecological tolerance drives differences in range size between lentic and lotic water beetles (Coleoptera: Hydrophilidae). *J Biogeogr* **39**:984–994 (2012).
 - 19 Esterhuizen N, Clusella-Trullas S, van Daalen CE, Schoombie RE, Boardman L and Terblanche JS, Effects of within-generation thermal history on the flight performance of *Ceratitis capitata*: colder is better. *J Exp Biol* **217**:3545–3556 (2014).
 - 20 Steyn VM, Mitchell KA, Nyamakondiwa C and Terblanche JS, Understanding costs and benefits of thermal plasticity for pest management: insights from the integration of laboratory, semi-field and field assessments of *Ceratitis capitata* (Diptera: Tephritidae). *Bulletin of Entomological Research* **112**:1–11 (2022).
 - 21 Chen M, Chen P, Ye H, Yuan R, Wang X and Xu J, Flight capacity of *Bactrocera dorsalis* (Diptera: Tephritidae) adult females based on flight mill studies and flight muscle ultrastructure. *Journal of Insect Science* **15**:141 (2015).
 - 22 Makumbe LDM, Moropa TP, Manrakhan A and Weldon CW, Effect of sex, age and morphological traits on tethered flight of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) at different temperatures. *Physiological Entomology* **45**:110–119 (2020).
 - 23 Weldon CW, Schutze MK and Karsten M, Trapping to monitor Tephritid movement: results, best practice, and assessment of alternatives, in *Trapping and the Detection, Control, and Regulation of Tephritid Fruit Flies: Lures, Area-Wide Programs, and Trade Implications*, ed. by Shelly T, Epsky N, Jang EB, Reyes-Flores J and Vargas R. Springer Netherlands, Dordrecht, pp. 175–217 (2014).
 - 24 Manoukis NC and Carvalho LAFN, Flight burst duration as an indicator of flight ability and physical fitness in two species of tephritid fruit flies. *Journal of Insect Science* **20**:11 (2020).
 - 25 CABI, *Bactrocera zonata* (peach fruit fly) *Invasive Species Compendium* (2022).
 - 26 Qureshi ZA, Population fluctuation and dispersal studies of the fruit fly, *Dacus zonatus* Saunders, in *Proceedings of the Symposium on the Sterility Principle for Insect Control Jointly Organized by the IAEA and the FAO of the United Nations and Held in Innsbruck*. International Atomic Energy Agency, Vienna, Austria (1975).
 - 27 Ben-Yosef M, Verykoui E, Altman Y, Nemni-Lavi E, Papadopoulos NT and Nestel D, Effects of thermal acclimation on the tolerance of *Bactrocera zonata* (Diptera: Tephritidae) to hydric stress. *Frontiers in Physiology* **12**:686424 (2021).
 - 28 Nyamakondiwa C, Kleynhans E and Terblanche JS, Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitis capitata*). *Ecological Entomology* **35**:565–575 (2010).
 - 29 Schunk C, Swartz SM and Breuer KS, The influence of aspect ratio and stroke pattern on force generation of a bat-inspired membrane wing. *Interface Focus* **7**:20160083 (2017).
 - 30 Berwaerts K, Van Dyck H and Aerts P, Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. *Functional Ecology* **16**:484–491 (2002).
 - 31 Gula CC, Rinehart JP, Greenlee KJ and Bowsler JH, Body size allometry impacts flight-related morphology and metabolic rates in the solitary bee *Megachile rotundata*. *J Insect Physiol* **133**:104275 (2021).
 - 32 Lehmann F-O, The constraints of body size on aerodynamics and energetics in flying fruit flies: an integrative view. *Fortschr Zool* **105**:287–295 (2002).
 - 33 Clusella-Trullas S, Terblanche JS and Chown SL, Phenotypic plasticity of locomotion performance in the seed harvester *Messor capensis* (Formicidae). *Physiol Biochem Zool* **83**:519–530 (2010).
 - 34 Steyn VM, Mitchell KA and Terblanche JS, Dispersal propensity, but not flight performance, explains variation in dispersal ability. *Proceedings of the Royal Society B: Biological Sciences* **283**:20160905 (2016).
 - 35 Frazier MR, Huey RB and Berrigan D, Thermodynamics constrains the evolution of insect population growth rates: “warmer is better”. *Am Nat* **168**:512–520 (2006).
 - 36 Loeschcke V and Hoffmann AA, The detrimental acclimation hypothesis. *Trends Ecol Evol* **17**:407–408 (2002).
 - 37 Hoskins JL, Janion-Scheepers C, Ireland E, Monro K and Chown SL, Constant and fluctuating temperature acclimations have similar effects on phenotypic plasticity in springtails. *J Therm Biol* **93**:102690 (2020).
 - 38 CABI, *Bactrocera dorsalis* (oriental fruit fly). *Invasive Species Compendium* 2023 (2023).