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Thermal biology, population fluctuations and implications of temperature extremes for the management of two globally significant insect pests

Casper Nyamukondiwa [a,1], Christopher W. Weldon [b,c], Steven L. Chown [d], Peter C. le Roux [b,e,2], John S. Terblanche [a,*]

[a] Centre for Invasion Biology, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
[b] Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
[c] Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa
[d] School of Biological Sciences, Monash University, Victoria 3800, Australia
[e] Department of Geosciences and Geography, University of Helsinki, P.O. Box 64, FI-00014, Finland

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ABSTRACT

The link between environmental temperature, physiological processes and population fluctuations is a significant aspect of insect pest management. Here, we explore how thermal biology affects the population abundance of two globally significant pest fruit fly species, *Ceratitis capitata* (medfly) and *C. rosa* (Natal fruit fly), including irradiated individuals and those expressing a temperature sensitive lethal (*tsl*) mutation that are used in the sterile insect technique. Results show that upper and lower lethal temperatures are seldom encountered at the field sites, while critical minimum temperatures for activity and lower developmental thresholds are crossed more frequently. Estimates of abundance revealed that *C. capitata* are active year-round, but abundance declines markedly during winter. Temporal autocorrelation of average fortnightly trap captures and of development time, estimated from an integrated model to calculate available degree days, show similar seasonal lags suggesting that population increases in early spring occur after sufficient degree-days have accumulated. By contrast, population collapses coincide tightly with increasing frequency of low temperature events that fall below critical minimum temperatures for activity. Individuals of *C. capitata* expressing the *tsl* mutation show greater critical thermal maxima and greater longevity under field conditions than reference individuals. Taken together, this evidence suggests that low temperatures limit populations in the Western Cape, South Africa and likely do so elsewhere. Increasing temperature extremes and warming climates generally may extend the season over which these species are active, and could increase abundance. The sterile insect technique may prove profitable as climates change given that laboratory-reared *tsl* flies have an advantage under warmer conditions.

1. Introduction

Knowledge of thermal biology has proven especially useful for understanding insect demography, population fluctuations and in forecasting pest outbreaks (e.g. Kingsolver, 1989; Bale, 2010). Low temperatures associated with winter generally suppress insect population growth rates, through reductions in development, suppression of activity, indirect chilling injury, and/or direct chilling mortality (Chown and Nicolson, 2004; Bale, 2010). Insects may cope with winter conditions by either behavioural or physiological compensation, arrested development (e.g. diapause) or some combination thereof (Denlinger and Lee, 2010). Physiologically, insects can adjust thermal tolerance over either the short-term (hardening) or longer-term (acclimatization in the field and acclimation under controlled laboratory conditions). A common example of acclimatization is the suite of physiological changes occurring at the onset of winter, typically providing improved low temperature tolerance (Hoffmann et al., 2003). Knowledge of overwintering biology, including physiological responses, is therefore often critical for assessments of pest insect population dynamics (Bale, 2010). For many economically significant pest insects, seasonal variation in physiology or overwintering biology is poorly

* Corresponding author. Tel.: +27 21 808 9225; fax: +27 21 808 2356.
E-mail address: jst@sun.ac.za (J.S. Terblanche).
[1] Present address: Department of Earth and Environmental Sciences, Botswana International University of Science and Technology (BIUST), Private Bag BO 041 Bontleng, Gaborone, Botswana.
[2] Present address: Department of Plant Sciences, University of Pretoria, Private Bag X20, Hatfield 0083, South Africa.

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elucidated. Improving this situation is important not only for pest management over the short term, but is also significant in the context of rapidly changing climates globally (Bale and Hayward, 2010).

This is true for two of the most significant pests of commercially-grown fruit crops: the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and the Natal fruit fly, *C. rosa* Karsch. Both are multivoltine, polyphagous tephritid flies that result in millions of dollars of losses annually (Malacrida et al., 2007; De Meyer et al., 2008). Demographic analyses of the two species suggest a very high net reproduction rate while young and a lack of diapause (Carey, 1984; Manrakhan and Addison, 2007). *Ceratitis capitata* probably originated from sub-Saharan East Africa and has become widely distributed in most continents with tropical and temperate climates (Malacrida et al., 2007). By contrast, *C. rosa* has a restricted African distribution (De Meyer et al., 2008; De Villiers et al., 2012), but is considered a significant biosecurity threat. In consequence, both species represent a burden to agriculture and act as barriers to economic transformation of rural communities through direct crop losses, the costs of control practices, and reduced market access. Comparatively little is known however about the relationships between their demography, abundance and thermal biology, and this is not a typical consideration in their management.

In addition to chemical control (pesticides including malathion), the Sterile Insect Technique (SIT) is one of the main methods used to control *C. capitata* (Klassen and Curtis, 2005). SIT involves releasing many males that have been rendered reproductively sterile by radiation, with the intent that they will mate with wild females and reduce the number of viable offspring (Knipling, 1959). Strains of *C. capitata* currently used in SIT campaigns possess a 'temperature sensitive lethal' (*tsl*) mutation that makes females homozygous for the *tsl* gene more susceptible to high temperature mortality (Franz, 2005). Application of a heat stress to eggs kills female embryos and permits large-scale, male-only releases. The release of a male-only strain improves the efficacy of SIT because only wild females will be present in the target area (Caceres, 2002). Females homozygous for the *tsl* gene remain sensitive to high temperatures throughout their lifetime such that sustained temperatures of over 28 °C lead to significant adult mortality within only a few days (Franz, 2005). It remains unknown however, whether the *tsl* mutation or irradiation affects the thermal tolerance of released male *tsl C. capitata* under field conditions. Such data have direct implications for ongoing SIT control programmes in South Africa and other parts of the world where *C. capitata* is considered a major pest. By contrast, control of *C. rosa* does not yet involve the release of sterile males, but the future of large-scale SIT campaigns could benefit from knowledge of radiation effects on the thermal tolerance of this species.

The overall aim of this work is therefore to determine aspects of the thermal environment that influence the population fluctuations of these two key horticultural pests to inform management and control practice. Specifically, to understand the influence of *tsl* and climate on demography or population fluctuations more generally, especially given expectations of future warming climates around the globe (Archer and Rahmstorf, 2010), we examined the extent to which thermal physiology accounts for population variability in both species. We determined the key thermobiological traits, including plastic responses in the field, across both species and in irradiated/unirradiated and untreated vs. *tsl* strains of *C. capitata*, and complemented these investigations with assessments of longevity of adults in the field. These data were then used in conjunction with microclimate information to estimate which physiological parameters, including developmental rates and limits, estimated from other studies, might be most significant in affecting population fluctuations, how SIT treatments might affect them, and what a warmer future might mean for the management of these species.

2. Materials and methods

We focused on traits likely to modify the impact of temperature on population growth. Our primary focus here was to understand factors influencing the field population abundances of wild flies and for this reason these traits were scored on untreated flies which were assumed to be representative of wild flies. These included upper and lower lethal temperature (ULT, LLT), supercooling point (SCP), functional activity limits (or critical thermal limits) recorded as critical thermal minima (CT_{min}) and maxima (CT_{max}), and, where possible, variation among key life-stages (see Nyamukondiwa et al., 2010). Using previously established low temperature thresholds for development (LDT) and the accumulation of development time above these thresholds (sum of effective temperatures, SET) (Duyck and Quilici, 2002; Grout and Stoltz, 2007), combined with microclimate data gathered in four major fruit production areas, we then reviewed variation in population abundance to consider potential explanatory traits. A secondary aim of this work was to inform current management practises and better understand how extrinsic (e.g. season) and intrinsic factors (e.g. irradiation, *tsl* mutation) or species differences might influence the outcomes of this work and we therefore also sought to compare these factors, particularly under field conditions. To this end, we used a combination of species and strains. Each experiment is outlined below with additional details given in Electronic Supplementary Material (ESM).

2.1. Origin of study animals

Unless otherwise indicated, *C. capitata* and *C. rosa* used in all experiments came from a large outbred culture, maintained in high numbers under buffered, variable temperatures (25 ± 4 °C), and has been in the laboratory for *c*. 200 generations. The culture is regularly supplemented with wild flies caught during summer, by mating wild-caught males to cultured females, to minimise inbreeding effects. Relative humidity is not strictly controlled throughout rearing, but is typically ~65–75% in culture containers.

2.2. Supercooling points and lower lethal temperatures

The supercooling point of three stages was examined for each *Ceratitis* species: third instar larvae, pharate (pink eye stage) pupae, and adults of mixed sex that were ~5–6 days old. Mixed-sex cohorts were tested because no sex effect could be detected in pilot trials. To determine supercooling points (SCPs), sixteen individuals of each species in each stage were individually loaded into 0.65 ml microcentrifuge tubes. Each insect was placed in contact with the tip of a type-T copper-constantan thermocouple (36-AWG; Omega, Laval, Canada), inserted through the tube's lid and both the insect and thermocouple held in place using cotton wool. Each tube was placed into a plastic bag and then into ethanol that was regulated at experimental temperatures by a circulating programmable refrigeration bath (Huber CC410WL, Offenburg, Germany). Thermocouples were connected to one of two 8-channel Picotech TC-08 (Pico Technology, Cambridge, UK) thermocouple interfaces and temperature recorded at 1 Hz using PicoLog software. Experiments began at 10 °C, at which flies were held for 10 min to allow equilibration, before the ethanol in the bath was cooled at 0.5 °C min^{-1} (and see ESM). Variation in cooling rate was not however, a major factor influencing SCP (Table S1). SCP for each individual was determined as the lowest temperature recorded prior to a spike in temperature associated with the latent heat of crystallisation.

To determine the extent of pre-freezing/chilling injury, we examined lower lethal temperatures (LLTs) of larvae, pupae (pharate stage) and adults (~2 days old) of *C. rosa* and *C. capitata*. A plunge protocol was adopted to determine LLT. A range of low temperatures (5, 1, −3, −7, and −11 °C) applied for 8 h was used to cover the full survival range (0–100%) of each life stage and species. Survival was assessed 24 h after the end of low temperature exposure (full details in ESM).

Frequency distributions of SCP showed unimodal or slightly skewed distributions, which did not respond strongly to transformation. Species, developmental stage and cooling rate effects on SCPs were analysed using a generalized linear model (GLZ) which is more robust to violations of parametric assumptions, and run assuming a normal distribution and an identity link function in SAS (v. 9.3 SAS Institute, Cary, NC, USA). Developmental stage effects on LLT were examined using a GLZ assuming a binomial distribution and a logit link function with corrections for overdispersion (proc genmod). Non-linear curve-fitting procedures were used in SAS (proc probit) to determine the low temperature which was lethal for 50% of the population (LLT_{50}).

2.3. Critical thermal limits and upper lethal temperatures

Critical thermal limits (CTLs) were estimated using a dynamic protocol undertaken by a single observer (see Terblanche et al., 2011). Because estimates of CTLs may in some cases differ from lethal limits taken over longer time-scales, and especially since temperature-related mortality is typically the outcome of time–temperature interactions (Chown and Nicolson, 2004), we also determined survival across a range of static conditions of varying duration (upper lethal temperatures, ULT).

Survival was determined at one of three durations (4, 6 and 8 h) and a range of temperatures (35 to 40 °C at 1 °C intervals) used to obtain a full range of 0–100% mortality conditions to estimate ULT for both adult *C. capitata* and *C. rosa*. In all ULT assays, five replicate 60 ml vials (N = 10 adult flies per vial, 2–5 days old) were used for each treatment. Following high temperature treatment, vials were transferred to a climate chamber (25 ± 1 °C) before scoring survival 24 h post-treatment (see ESM for further details). Tests for significance of temperature, duration of exposure, species and their possible interactions on ULT were undertaken using a GLZ (binomial distribution, logit link function) in SAS with corrections for overdispersion. Non-linear curve-fitting procedures were used to determine the high temperature which was lethal for 50% of the population (ULT_{50}) (proc probit).

To compare the different strains and treatments of *Ceratitis* (see Introduction for rationale) we also assayed CTLs for these groups in the laboratory. This also allowed comparisons of the relative rank order of thermal sensitivity between laboratory and field cage trials (see below) to be made. In all cases, CTLs were determined from a start temperature of 25 °C (ramp rate: 0.25 °C min^{-1}) and were defined as the temperature at which each individual lost co-ordinated muscle function, consequently losing the ability to respond to gentle physical stimulation. Before analysing the results for CTLs, data were checked for normality and equality of variances using the Shapiro–Wilk and Hartley–Bartlett tests, respectively, and in all cases assumptions were met. To determine the effect of species/strain on CTLs, both CT_{max} and CT_{min} were analysed using one-way ANOVA followed by Tukey–Kramer's post-hoc tests to identify homogenous groups.

2.4. Field responses to variable conditions

To determine if *C. capitata* and *C. rosa* rapidly alter thermal tolerance in response to natural variations of temperature (and photoperiod), and if any such response varies across seasons, we measured thermal tolerance of both species when exposed to diurnal temperature variation in late austral winter/early spring (September 2010) and summer (February 2011). This was undertaken on adult flies (5–6 days old) of five separate treatments/strains: (1) untreated laboratory-reared *C. capitata*, (2) unirradiated *tsl C. capitata*, (3) irradiated *tsl C. capitata*, (4) untreated laboratory-reared *C. rosa* and (5) irradiated laboratory-reared *C. rosa*. All 'untreated' flies ([1] and [4]) were obtained as first instar larvae from Citrus Research International, Nelspruit, South Africa (and are from the same stock culture as in other thermal tolerance experiments, see Section 2.1). Upon arrival in Stellenbosch, larvae were kept at 25 ± 1 °C (65 ± 10% relative humidity [R.H.]) until pupation. The pupae were then transferred into insect rearing cages (32.5 × 32.5 × 32.5 cm) in the laboratory (12:12 h L:D photoperiod), at room temperature (25 ± 3 °C) (65 ± 10% R.H.). All flies had access to food and water *ad libitum*, and bananas for oviposition. All cages were held at similar low densities (±1000 flies/cage) to avoid stressful crowding effects that may affect thermal tolerance estimates. Irradiated and non-irradiated *tsl C. capitata* were obtained from SIT Africa (Stellenbosch, South Africa). The *tsl C. capitata* (Vienna 8 strain) were treated with high temperature at the egg stage to remove all females. Irradiation (for both *tsl C. capitata* and *C. rosa*) was performed during the pupal 'pink eye stage' using ^{60}Co at 110 Gy for 44 min. All thermal tolerance assays were undertaken using mixed sex flies since this does not seem to have large effects in either species (Nyamukondiwa and Terblanche, 2009).

Twenty-five cages (400 insects per treatment/strain spread equally among 5 cages) were transferred to an outside, shaded environment close to the laboratory. The flies were introduced outside in the afternoon (in winter) and morning (in summer) to minimise initial temperature difference between the laboratory (25 °C) and the ambient temperature (T_a). After 1 h, the first subsample was collected from each of the 25 cages and then subsequently every 6 h for 24 h following modifications from Overgaard and Sørensen (2008). Cold and heat shock was assayed following Nyamukondiwa et al. (2010). Insect body temperature ranging between 5 and 30 °C equilibrates with T_a within 5 min of direct exposure to −5 and 41 °C (see Terblanche et al., 2011). For all cold and heat shock tolerance experiments, five replicate 60 ml vials of 10 insects each were exposed directly to 41 °C for 2 h or −5 °C for 2 h in programmable waterbaths (Huber). Flies were then returned to a 25 °C climate chamber for 24 h before scoring survival. Survival was defined as a coordinated response to gentle prodding, or any other normal behaviour such as walking, feeding, mating and flying. During the entire field experiment, ambient temperature was recorded using one shaded iButton (Dallas Semiconductors, Model DS1920; 0.5 °C accuracy; 5 min sampling frequency) located inside a randomly chosen cage.

To examine the effects of treatment, species and season (winter and summer) on fly cold or heat shock survival, treatment groups were compared using a GLZ, assuming a binomial distribution and with a logit link function in SAS (proc genmod) with corrections for overdispersion. For both heat and cold shock survival, treatment was not significant; hence the model was rerun using only temperature and season as variables. Overlap in 95% confidence limits was used to identify statistically homogeneous groups.

Insects can respond rapidly to temperature change but trade-offs between heat and cold shock survival can be present (Hoffmann et al., 2003). To determine if this is the case in *Ceratitis* an approach similar to that described in Overgaard and Sørensen (2008) was used. The correlation between mean proportion cold/heat shock survival and average T_a recorded 2 h prior to sampling was analysed using robust regression in R v. 2.11.1 statistical software (R Development Core Team, 2010). Initially data were analysed for each season and species/treatment separately, but then

also with both seasons pooled. Only the latter results are presented as the major conclusions are not affected by pooling data.

2.5. Field longevity

To determine the survival of different *Ceratitis* species/strains under semi-natural field conditions in mid-summer, field longevity of five different treatments of *Ceratitis* was assessed. Twenty-five cages (five replicate cages of 200 insects for each treatment) were introduced to an outdoor, shaded environment when the flies were ~5 days old. This was done at a time when T_a approximated the rearing temperature (25 °C) (~mid-day). Mortality in each cage was recorded the following morning and then subsequently every 24 h until extinction, defined as <1% of the original fly population in each cage. Ambient temperature and humidity were recorded using iButtons randomly attached inside five of the replicate cages.

A Cox proportional hazards model for censored observations from the 'survival' library of R was used for survival analysis. The main effects of treatment, replicate and average or maximum temperature were included in the model. Untreated *C. capitata* was designated as the reference category for treatment (using the function 'relevel'). To account for time-dependent changes in T_a, we also included the interaction of mortality time with either average or average maximum T_a (Fox, 2002). The model did not violate the assumption of constant hazards (verified using 'cox.zph'). Hazard ratios were used to assess differences in the rate of extinction among treatments/strains.

2.6. Calculation of development time/rate

Microclimate data records obtained from four geographic sites in the Western Cape province, South Africa, and data for thermobiological traits generated by this study were used to predict development time and rate of *C. capitata* and *C. rosa*. These sites comprised Hex River Valley (33.46609°S; 19.66304°E; 459 m a.s.l.), Villiersdorp (33.88279°S; 19.36793°E; 262 m a.s.l.), Ceres (32.98865°S; 19.30354°E; 947 m a.s.l.) and Klein Swartberg (33.28360°S; 22.35425°E; 302 m a.s.l.). The Western Cape province is climatically diverse, with distinct micro- and macroclimates resulting from topography and the influence of the Indian and Atlantic oceans. The Province has a predominantly Mediterranean climate. For each site, three farms were selected and the total fruit fly trap catch per month was determined. The temperature at each site was recorded at hourly intervals using iButtons. Temperature time series from each site was used to calculate the total duration when temperatures were below (or above) each species lower (or upper) developmental threshold (LDT or UDT). The time required for the accumulation of adequate degree hours (i.e. sum of effective temperatures; SET) for each species complete development from egg to adult was also calculated: when temperatures were within the thresholds for development (i.e. higher than LDT and lower than UDT), the difference between the temperature and the LDT was multiplied by the interval until the next temperature record to calculate the degree hours associated with that specific record. For each record in the time series, the degree hours were then summed across consecutive temperature records until the SET was met or exceeded, giving the developmental period required for an egg laid at that specific time. This calculation was repeated across the whole time series until inadequate records remained for calculations (i.e. at the end of the time series). Where more than 2 h were missing from the time series, missing values were interpolated using sinusoidal regression, estimating the daily temperature cycle from the 120 records before and after the missing data (where fewer records were available all available records were used). Sum of effective temperatures, and developmental and lethal thresholds for the two species were extracted from Duyck and Quilici (2002) and Grout and Stoltz (2007), and all time-series calculations were run in R 2.11.1 statistical software (R Development Core Team, 2010). A temporal autocorrelation of fortnightly trap captures for each site with the mean development time estimates for the trapping period was calculated.

2.7. Population abundance

Abundance of *C. capitata* and *C. rosa* was monitored fortnightly at four sites in the major deciduous fruit producing areas of the Western Cape (South Africa) fortnightly using yellow bucket traps baited with a synthetic three component Biolure® attractant (Chempac, Paarl, South Africa) that attracts both sexes of *C. capitata* and *C. rosa*. Traps were serviced fortnightly and specimens returned to the laboratory for identification. For areas in which SIT programmes are operational, *tsl* flies can be easily distinguished as micronized fluorescent powder mixed with pupae is trapped in the head capsule (Manrakhan and Addison, 2007); UV light screening therefore ensured that only wild *C. capitata* were recorded. Monitoring was conducted over 2009 and 2010 in the aforementioned four sites. These data were then related to the expected population size given requirements for development above the LDT and the SET, and the physiological traits determined in the present study. Time series autocorrelation analyses were performed in STATISTICA v. 10 (AX, Statsoft, Tulsa, OK, USA).

3. Results

3.1. Supercooling points and lower lethal temperatures

Supercooling point was similar across both species, but differed among life stages (Fig. 1A; Fig. S1). Further differentiation of animals into those removed just prior to and those removed several degrees after the SCP showed no evidence of freeze tolerance (ESM). Cooling rate had no significant effect on SCPs ($\chi^2 = 0.19$, $df = 3$, $P > 0.97$) (Table S1).

LLT varies among stages and species (Table 1) but it is clear that pre-freeze mortality is significant with complete mortality by −3 to −7 °C (Fig. 1B and C). Exposure at temperatures ⩾1 °C for 8 h was not lethal to any developmental stages of either species. In the 8 h low temperature exposures, LLT_{50} was estimated as −0.61 °C (95% Lower CL–Upper CL: −1.16 −0.07 °C) for *C. capitata* and 0.69 °C (95% LCL–UCL: −0.01–1.62 °C) for *C. rosa* across all life-stages. The temperature at which 90% mortality occurred (LLT_{90}) varied from the most cold tolerant pupae (*C. capitata*: −6.25 [−7.73 to −5.18 °C]; *C. rosa*: −4.91 [−6.73 to −3.66 °C]), to least tolerant but similar adults and larvae (*C. capitata* adults: −2.58 [−3.65 to −1.84 °C]; *C. rosa* adults: −3.01 [−4.21 to −2.18 °C]; *C. capitata* larvae: −3.01 [−4.23 to −2.18 °C]; *C. rosa* larvae: −2.93 [−4.34 to −1.96 °C]).

3.2. Upper lethal temperatures and critical thermal limits

Species, temperature and duration of exposure significantly affected high temperature survival. Longer exposure duration and higher temperatures resulted in a reduction in survival (Fig. 2a and b; Table 2). ULT_{50} across all durations tested was similar for both species (*C. capitata*: 38.02 [37.88–38.30]; *C. rosa*: 37.85 [37.80–37.93] °C) although a significant species × temperature effect highlights different high temperature survival responses between the species under specific treatment conditions. For example, *C. capitata* has higher survival at 37 °C for 8 h than *C. rosa* (~20 vs 0% respectively) but is similar for many of the other treatments examined.

Species/strain affected both CT_{max} ($F_{4,95} = 21.39$, $P < 0.001$; Fig. 2c) and CT_{min} ($F_{4,95} = 23.65$, $P < 0.001$; Fig. 2d). Unirradiated

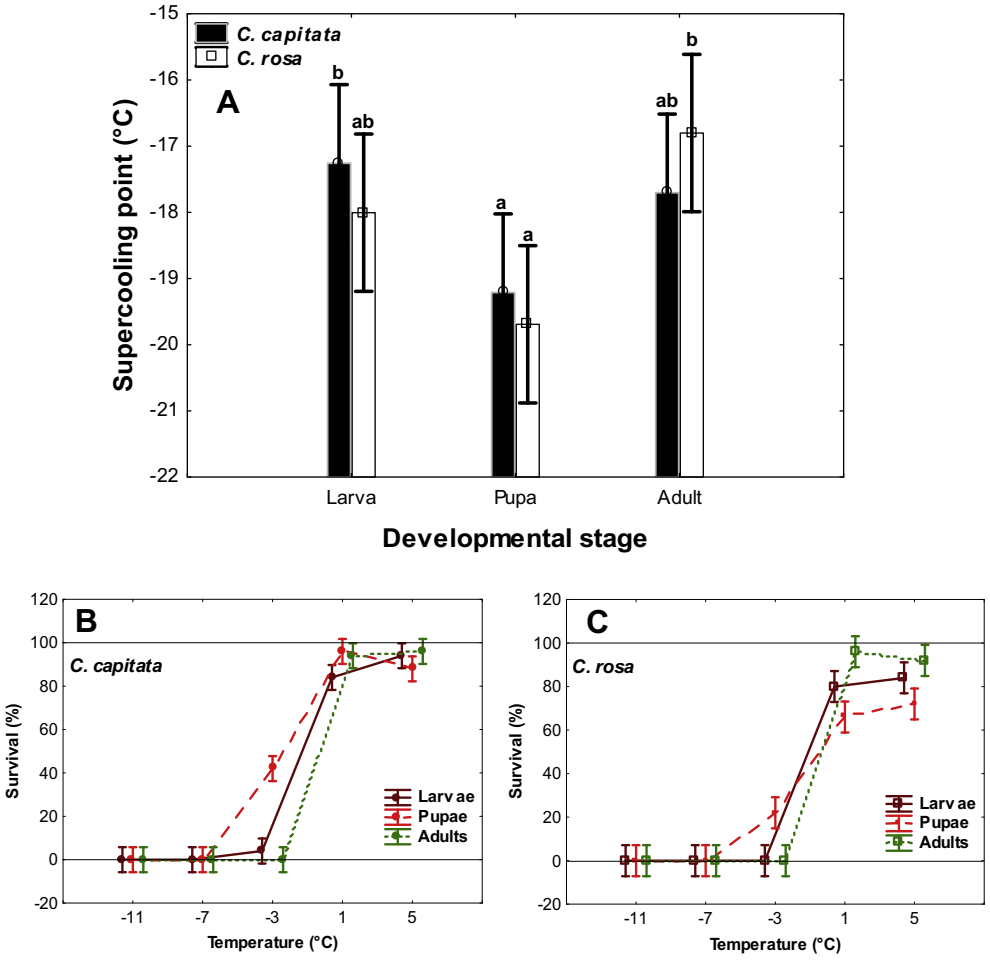

Fig. 1. (a) The effect of developmental stage on supercooling points in *C. rosa* and *C. capitata*. Errors bars denote 95% CLs ($N = 16$ per group). Developmental stage significantly influenced SCPs in both species ($\chi^2 = 15.45$, $df = 2$, $P = 0.0004$) although there were no differences between species ($P > 0.82$) or species × life-stage effects ($P > 0.33$). Means with the same letter are not statistically different. Mean survival (±95% CLs) of different developmental stages of (b) *C. capitata* and (c) *C. rosa* at low temperatures for 8 h. Each data point represents an average of 5 replicates, $N = 10$ individuals per replicate/treatment/life-stage.

Table 1
Summary results of a generalized linear model showing the effects of developmental stage, *Ceratitis* species, temperature and their interactions on low temperature survival [lower lethal temperature (LLT)] in *C. rosa* and *C. capitata*. DF = degrees of freedom.

Trait	Effect	DF	Wald χ^2	*P*
LLT	Developmental stage	2	8.56	0.0022
	Species	1	23.55	<0.0001
	Temperature	4	4792	<0.0001
	Developmental stage × species	2	21.20	<0.0001
	Developmental stage × temperature	8	156.79	<0.0001
	Species × temperature	4	16.25	0.0060
	Developmental stage × species × temperature	8	20.59	0.0182

and irradiated *tsl* flies had significantly higher CT_{max} than the other three treatments, which did not differ from each other (Fig. 2c). Irradiated *C. rosa* had significantly higher CT_{min} than all the other treatments, and untreated *C. capitata* had significantly lower CT_{min} than the other groups. The remaining species/strains were not statistically different from each other. The rank order of longevity under semi-field conditions matched the rank order of CT_{max} but not CT_{min} (cf. Table 4 and Fig. 2c, d).

3.3. Field responses to variable conditions

There were significant season and temperature × season effects for both cold and heat shock survival (Table 3). Treatment (which included the effect of species) however was not significant for either cold or heat shock survival (Table 3). Similarly, none of the other interactions were significant for both heat and cold shock survival. Cold shock survival was significantly higher in winter (16.9% on average) than in summer (10.9% on average; Fig. S2; Table 3), while heat shock survival was significantly higher in summer (17.7% on average) than in winter (13.7% on average; Fig. S2) although in both cases with high variation among replicates within each season. There was a significant positive relationship between cold shock survival and ambient temperature in winter ($r^2 = 0.2488$, $P = 0.0252$; Fig. 3A) but not in summer ($r^2 = 0.0474$, $P = 0.3566$; Fig. 3B). However, the relationship between heat shock survival and ambient temperature was not

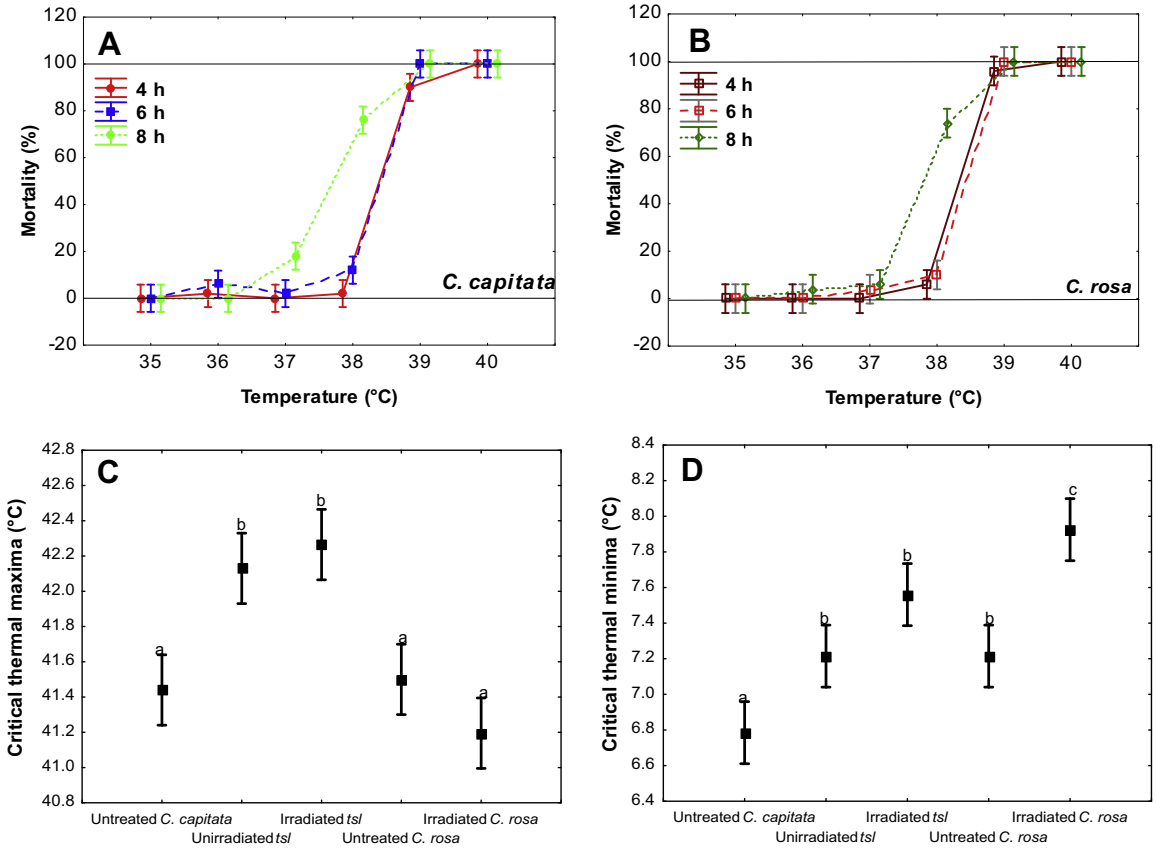

Fig. 2. The effect of temperature and duration on survival in (a) *C. capitata* and (b) *C. rosa*. Each data point represents an average of 5 replicates, $N = 10$ individuals per replicate/treatment (error bars: standard error of the mean). (c) CT_{max} and (d) CT_{min} in different *Ceratitis* species/strains. CT_{max} and CT_{min} experiments started at 25 °C (ramp rate: 0.25 °C min^{-1}). ($N = 20$ per strain/treatment group; means ±95% CLs).

Table 2
Summary results showing the effects of *Ceratitis* species, temperature, duration of exposure and their interactions on high temperature survival (upper lethal temperature, ULT) in *C. rosa* and *C. capitata*. Treatment groups were analysed using a generalized linear model assuming a binomial distribution and a logit link function with corrections for overdispersion. DF = degrees of freedom. Significant effects are shown in bold.

Effect	DF	Wald χ^2	*P*
Species	**1**	**4.89**	**0.0271**
Temperature	**1**	**249.30**	**<0.0001**
Duration	**2**	**12.13**	**0.0023**
Species × temperature	**1**	**4.86**	**0.0274**
Species × duration	2	3.43	0.1803
Temperature × duration	**2**	**11.08**	**0.0039**
Species × temperature × duration	2	3.41	0.1820

significant in winter ($r^2 = 0.0835$, $P = 0.2165$; Fig. 3C) but significant and positive in summer ($r^2 = 0.5262$, $P = 0.003$; Fig. 3D). Across pooled treatments, laboratory-assayed cold shock survival and ambient temperature were negatively related in winter ($r^2 = 0.2488$, $P = 0.03$; Fig. S3), but not in summer ($r^2 = 0.0474$, $P = 0.36$). Laboratory-assayed heat shock survival and ambient temperature were however, not significantly related in winter ($r^2 = 0.0835$, $P = 0.22$), but were positively related in summer ($r^2 = 0.5262$, $P < 0.01$). After pooling seasons, there was no significant relationship between heat and cold shock in any treatment or when all treatments were pooled ($P > 0.43$ in all cases; Fig. S4).

3.4. Field longevity

Individuals from all *Ceratitis* species/strains survived >14 days from the day they were introduced into the semi-natural environment in summer (Fig. 4). High mortality was recorded on day 6 and 7 coincident with relatively high average maximum T_a (36.1 and 34.7 °C respectively; Fig. 4). In these 2 days absolute maximum reached 40.7 °C in the cages, a temperature close to the mean CT_{max} for both species. Overall, survival was significantly higher in unirradiated and irradiated *tsl* flies, and least in irradiated *C. rosa*

Table 4
Summary results of survival analysis of five treatments using Cox proportional hazard model in R. Untreated *C. capitata* was used as the reference category; a hazard ratio of <1 or >1 indicates a higher or lower probability of survival, respectively, relative to the reference category. CL = Confidence Limits. Significant effects are shown in bold. Letters indicate homogeneous subsets ascribed by overlap of lower and upper 95% confidence levels for parameter estimates.

Treatment	Parameter estimate	95% Lower CL	95% Upper CL	Wald χ^2	*P*	Hazard ratio
Untreated *C. capitata*	0.000					1.000 b
Unirradiated *tsl*	−0.178	−0.221	−0.100	33.238	<0.001	0.663 a
Irradiated tsl	−0.054	−0.122	0.003	2.863	0.091	0.805 b
Untreated *C. rosa*	0.069	−0.001	0.132	4.114	0.043	1.230 c
Irradiated *C. rosa*	0.301	0.234	0.359	89.032	<0.001	1.552 d

Table 3
Summary results of the effects of field average temperature recorded 2 h prior to sampling, treatment (untreated *C. capitata*, unirradiated *tsl*, irradiated *tsl*, untreated *C. rosa* and irradiated *C. rosa*), season (winter and summer) and their interactions on cold and heat shock survival. Cold and heat shock survival were tested for 2 h at −5 and 41 °C respectively. Both the full and minimum adequate models are presented here. Statistically significant effects are indicated in bold. DF = degrees of freedom.

Trait	Effect	DF	Wald χ^2	*P*
Cold shock survival	Temperature	1	3.30	0.0693
	Treatment	4	0.77	0.9429
	Season	**1**	**9.07**	**0.0026**
	Temperature × treatment	4	0.18	0.9961
	Temperature × season	**1**	**9.48**	**0.0021**
	Treatment × season	4	1.38	0.8475
	Temperature × treatment × season	4	1.31	0.8592
	Temperature	1	3.29	0.0696
	Season	**2**	**10.81**	**0.0045**
	Temperature × season	**1**	**8.95**	**0.0028**
Heat shock survival	**Temperature**	**1**	**20.57**	**<0.0001**
	Treatment	4	3.66	0.4537
	Season	**1**	**13.02**	**0.0003**
	Temperature × treatment	4	4.96	0.2917
	Temperature × season	**1**	**8.92**	**0.0028**
	Treatment × season	4	1.26	0.8674
	Temperature × treatment × season	4	1.63	0.8028
	Temperature	**1**	**21.61**	**<0.0001**
	Season	**2**	**101.08**	**<0.0001**
	Temperature × season	1	8.53	0.0035

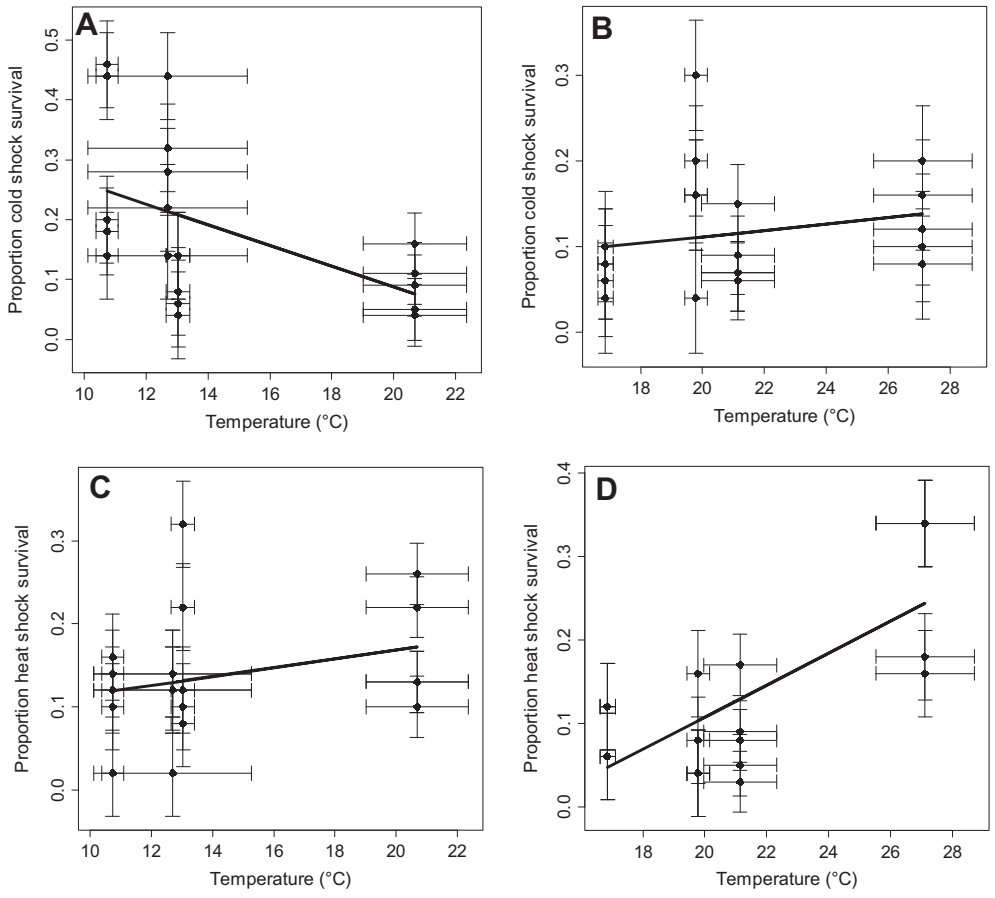

Fig. 3. Scatterplots of mean cold shock survival (±SE) as a function of the corresponding mean ambient average temperature (±SE) recorded 2 h prior to sampling for all treatments/strains (untreated *C. capitata*, unirradiated *tsl*, irradiated *tsl*, untreated *C. rosa* and irradiated *C. rosa*) pooled together in (A) winter and (B) summer and the relationship between mean heat shock survival and mean ambient temperature 2 h prior to sampling in (C) winter and (D) summer. Note that axes of the graphs differ for clarity.

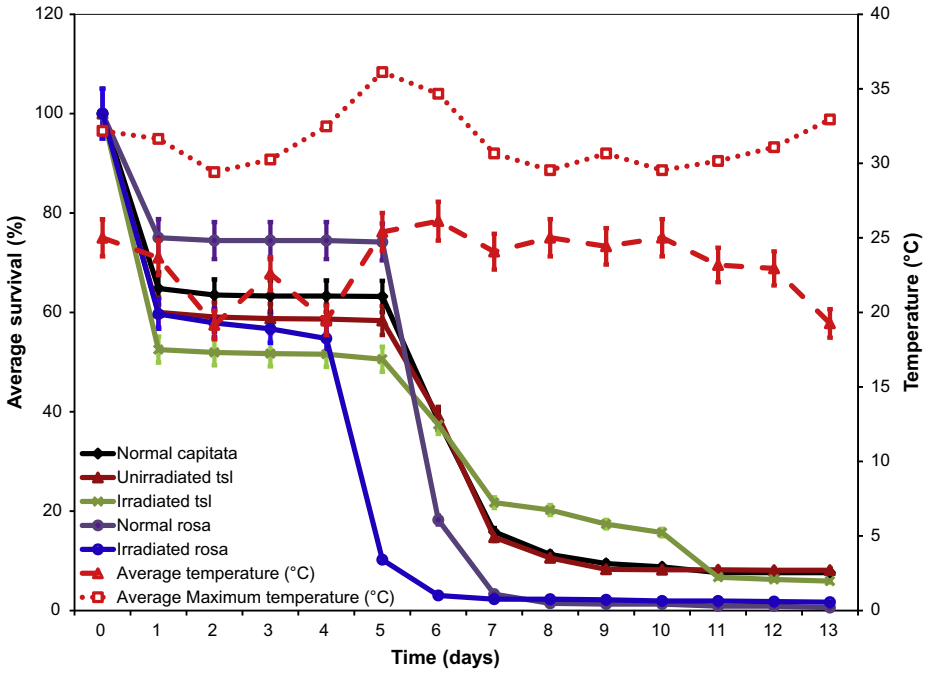

Fig. 4. Estimates of longevity (averaged over 5 replicate cages) in adult *C. rosa* and *C. capitata* during semi-field conditions in summer (error bars denote ±95% CLs). Five replicates of 1000 flies each per treatment were used and extinction was defined as <1% of the original population. Mortality was recorded every day for 14 days. Survival analysis of the different strains/treatments during this experiment was analysed using Cox proportional hazard models (Table 4). Avg max temp represents an average of the six highest temperature readings obtained during the day.

(rank order of survival = unirradiated *tsl* > irradiated *tsl* > untreated *C. capitata* > untreated *C. rosa* > irradiated *C. rosa*; Table 4; Fig. 4).

3.5. Population abundance

At all four sites only *C. capitata* was captured in pheromone traps. Owing to concerns about establishment risk in the region (Manrakhan and Addison, 2007) however, we report on potential significance of thermal variation at the study sites for *C. rosa* too. In both 2009 and 2010, relative abundance of *C. capitata* increased from January and reached a peak in April/May. With the onset of winter however, trap catches decreased from June through to December, peaking again in April/May (Fig. 5). With the exception of Klein Swartberg, ambient temperatures never exceeded the CT_{max} of *C. rosa* and *C. capitata*. Winter temperatures did not fall below the lower lethal temperature (LLT) of adult flies but frequently dropped below LDT and CT_{min} (see Fig. 6 for Hex River Valley and Fig. S5 for the rest of the sites).

When expressed as the frequency of occurrence, temperatures $<CT_{min}$ and <LDT were much more common than those $>CT_{max}$ (illustrated in Fig. 6 for both species in the Hex River Valley; Fig. S5 rest of the sites). Indeed, at all sites in 2009 and 2010, there were insufficient day degrees $month^{-1}$ between May–September to allow development of a single generation of *Ceratitis*. Fortnightly estimates of relative population abundance and estimated development time (Fig. S6) were significantly autocorrelated in most sites, indicating that seasonal lags in relative population abundance generally matched the seasonal lags in development time (Fig. 7 for Hex River Valley and Fig. S7 for other sites), providing further evidence for low temperature suppression of the *C. capitata* population. Despite this, and as evidenced by the large increase in population size in the warmer months of the year, the model predicted the thermal environment in 2009 and 2010 was sufficient for 5–6 generations per year of *C. capitata* in Ceres, 7 generations per year in Hex River Valley and Villiersdorp, and 8 generations per year in Klein Swartberg. For *C. rosa*, the model predicted a total of 5 generations per year in Ceres, 6 generations per year in Hex River Valley and Villiersdorp, and 6–7 generations per year in Klein Swartberg.

4. Discussion

Insect populations fluctuate as a consequence of intrinsic and extrinsic factors such as changing abiotic and biotic conditions (Price, 1997). In the case of this study system, resources are abundant nearly year round given the timing of fruit production and the practise of leaving unharvested and fallen fruit in the orchards (Manrakhan and Addison, 2007). The agroecosystem setting implies low predation rates and the use of SIT has little significant impact on parasitoid populations (Wong et al., 1992; Vargas et al., 2001). Moreover, populations decline at a time when rainfall is increasing in this winter-rainfall region (Tyson and Preston-Whyte, 2000), suggesting that water availability, a limiting factor in the dynamics of some insect populations (Chown and Nicolson, 2004) is not especially significant. Rather, it seems likely that the predominant extrinsic factor influencing demography is thermal variation.

Adult *C. capitata* and *C. rosa* are capable of surviving temperatures up to 37 °C for 8 h (Fig. 2A and B), and can maintain activity up to at least 40 °C (Nyamukondiwa and Terblanche, 2010). While ULT_{50} estimates are lower than CT_{max} estimates, this is likely a consequence of the longer durations involved compared with the total duration of heat stress in the CT_{max} assays (see Chown and Nicolson, 2004 for discussion). *Ceratitis capitata* are also capable of adjusting their heat shock survival capability over the short term in the laboratory (e.g. Kalosaka et al., 2009; Nyamukondiwa and Terblanche, 2010), and in the field in our trials in the summer months (Fig. S4), when heat shock is likely to be encountered. Laboratory studies have further confirmed that CT_{max} of both species may vary as a consequence of thermal history within a single generation (range of variation: ~0.5 °C; Nyamukondiwa and Terblanche, 2010). In consequence, temperatures need to approach

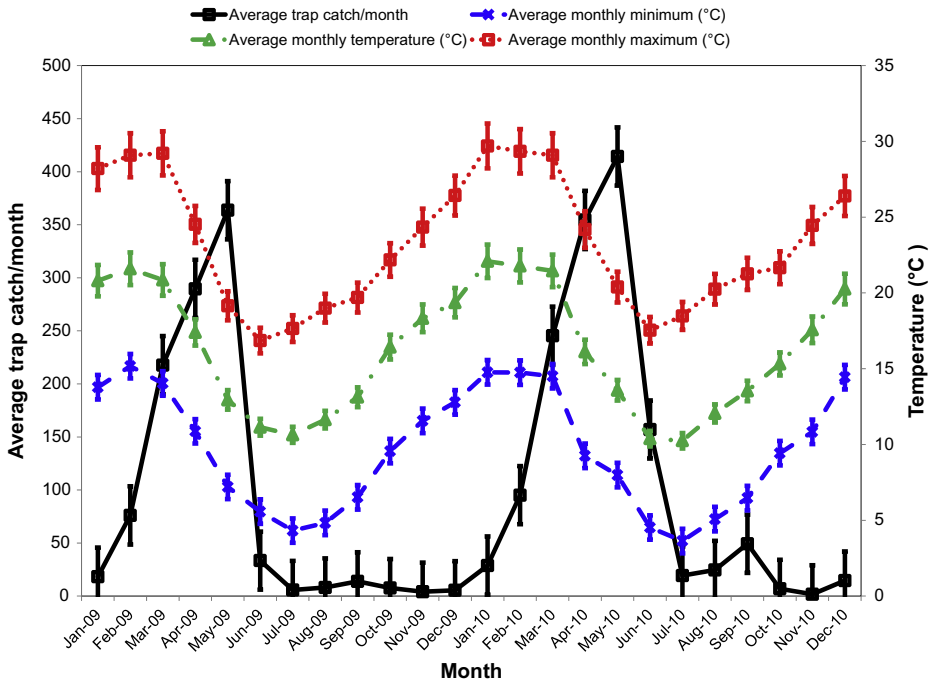

Fig. 5. Seasonal population phenology of *C. capitata* across four sites in the Western Cape Province as a function of average monthly, average minimum and average maximum temperature during 2009 and 2010. *Ceratitis rosa* was not caught in any traps so the results are omitted. Each point represents the mean trap catch obtained from the average of four trapping sites (±95% CLs). Traps were serviced every fortnight and average *C. capitata* caught/trap/month was calculated. Population abundance was then averaged across the four sites.

these limits before mortality is experienced (Fig. S3), and this rarely happens in the field sites examined in this study (Fig. S3; Fig. 6). Thus, high temperature is unlikely to have a significant effect on the demography of this species in most years (and supported by field microclimate recordings, e.g. Fig. 6), though recognising that heat wave conditions may sometimes prevail over the Western Cape (Tyson and Preston-Whyte, 2000), and that extreme temperature events are becoming more common globally (Hansen et al., 2012). Given similarities in their thermal responses, it seems likely also that if *C. rosa* were to be introduced to the region, its populations would not be limited by high temperatures.

By contrast, low temperature is much more likely a candidate for influencing the demography of *C. capitata*. Despite relatively low freezing points, larvae, pupae and adults have LLT_{50} of *c.* −3 °C (8 h). This is perhaps unusually high compared with other chill susceptible species, but is largely in keeping with results for several drosophilids (Denlinger and Lee, 2010). It also compares well with other tephritid flies: the Queensland fruit fly, *Bactrocera tryoni*, and the lesser Queensland fruit fly, *B. neohumeralis*, experience 50% mortality when held at −4 °C for almost 7 h (Meats, 2006). Adult activity generally also ceases before 6 °C (the mean CT_{min}; Nyamukondiwa and Terblanche, 2010), and the lower developmental threshold (LDT) for the species' complete life cycle is 11 °C (Grout and Stoltz, 2007). Temperatures below these various thresholds are common across all of the sites investigated from May until September (Fig. 6; Fig. S5). Moreover, given the duration of temperatures below the LDT, insufficient time for development is available for the same period. Thus, a decline in the ability of the adults to remain active (despite some effects of acclimation on CT_{min} which may influence it by ∼1.5 °C; Nyamukondiwa and Terblanche, 2010), an increase in the mortality of all stages (despite some ability to improve cold shock survival given changing ambient temperatures), and preclusion of development all conspire to mean a dramatic decline in population density in winter (June to July). It seems unlikely that SIT is responsible for this decline because the number of flies released is insufficient to have an impact (SIT: wild fly ratio of <0.1 for Hex River and Riebeek Kasteel in summer) and areas which are under control have similar (or higher) population abundances and similar phenology to those not under SIT control (Manrakhan and Addison, 2013).

Low temperatures are likely to continue to suppress the population throughout winter. The increase in abundance with rising temperatures in summer appears to be delayed, which is a typical pattern observed in fruit fly species that over-winter in temperate climate regions (Fletcher, 1974; Papadopoulos et al., 1996; Maelzer et al., 2004). The larval life stage is most likely involved in over-winter survival in *C. capitata* (Papadopoulos et al., 1996), so this delay likely reflects the time taken for the first spring and early summer cohorts to reach the adult stage. Indeed, similar autocorrelation profiles for population abundance and development time (Fig. 7; Fig. S7) support such an interpretation. Again, given similarities between the thermal biology and thermal sensitivity of development of *C. capitata* and *C. rosa*, similar population dynamics of the latter might be expected were it to overcome biosecurity measures and colonise the region (and see De Villiers et al., 2012).

The model discussed here focused only on adult *Ceratitis* fruit flies. Adult *Ceratitis* represent the real risk in terms of fruit infestation and contribute directly to population growth through oviposition. Thus adults are responsible for most economic damage, are closely monitored and are commonly subject to control practices and (Manrakhan and Addison, 2007). Nevertheless, it is also worthwhile to consider other *Ceratitis* developmental stages in future studies. Temperature data for our field experiments was collected using iButtons that were placed in shaded trees at ∼1.5 m height, typical of the habitat of adult fruit flies. Microclimate use and behavioural thermoregulation by different fruit fly developmental stages may however also be significant factors in explaining how temperature affects *Ceratitis* population dynamics (Kaspari and Yuval, 1999).

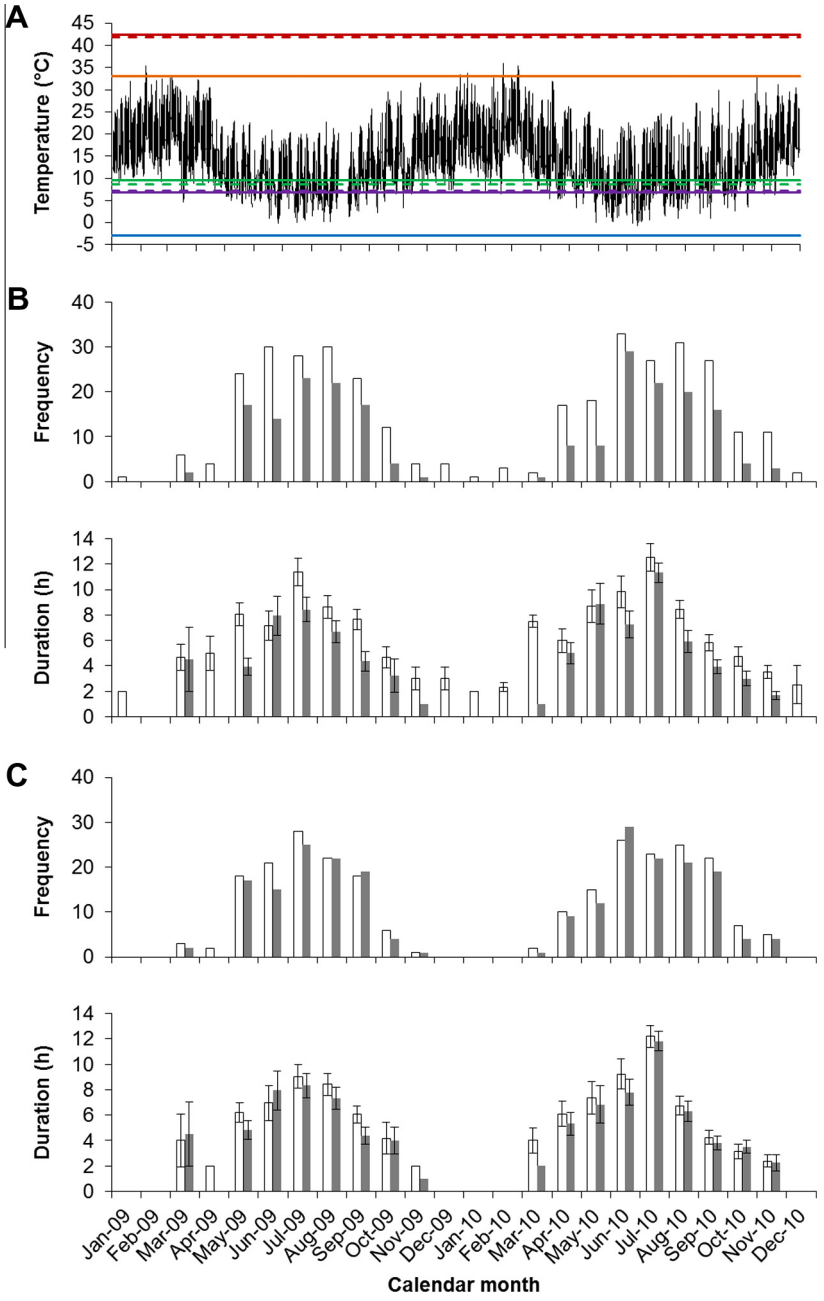

Fig. 6. Microclimate temperature recorded from (A) Hex River Valley over 2 years (2009–2010). Ambient temperature was recorded using iButtons (0.5 °C accuracy; 1 h sampling frequency). Solid and dotted horizontal lines represent for *C. capitata* and *C. rosa*, respectively, the lower lethal temperature (blue), critical thermal minimum (purple), lower developmental temperature (green), upper developmental temperature (orange), and critical thermal maximum (red). (B) Frequency distribution and duration of suboptimal temperature conditions recorded over 2 years (2009–2010) in Hex River Valley for *C. capitata* and (C) *C. rosa*. Shaded bars represents temperatures $<CT_{min}$ while blank bars represents temperatures <LDT. CT_{min} was 7.2 and 6.9 °C while CT_{max} was 41.5 and 41.4 °C for *C. rosa* and *C. capitata*, respectively. LDT was 7.9 and 9.6 °C for *C. rosa* and *C. capitata*, respectively (see Grout and Stoltz, 2007). LDT = lower developmental threshold. Error bars denote ±95% CLs.

4.1. Ecological and management implications

In terms of the future potential cost to agriculture in the region, *C. capitata* and *C. rosa* (were it to colonise) are both likely to show increases in population abundance in response to climate change given that a major signal of such change is a faster increase in winter than in summer temperatures (Archer and Rahmstorf, 2010). Indeed, in any areas where the species are not likely to be limited by resource or water availability, which includes large parts of Africa, southern Europe, Americas, and some parts of Australia (De Meyer et al., 2008), the short-term future appears to be one of an increase in the severity of impacts of these species in the absence of any further control measures. Over the longer term, however, rising thermal maxima (Hansen et al., 2012; Kruger and Sekele, 2012) may pose substantial challenges, so possibly reducing abundance. In this regard, the significantly higher CT_{max} values of the *tsl* flies relative to outbred conspecifics may prove to be advantageous. The 'Vienna 8' *tsl* strain of *C. capitata* is used routinely in the Western Cape in SIT programmes that aim to suppress wild populations during development and harvest of deciduous crops. Efficacy of SIT is dependent on the ratio of sterile males to wild females in the population (Knipling, 1959), which is reliant on the survival of sterile males after release into the field. If *tsl* males maintain higher survival in the

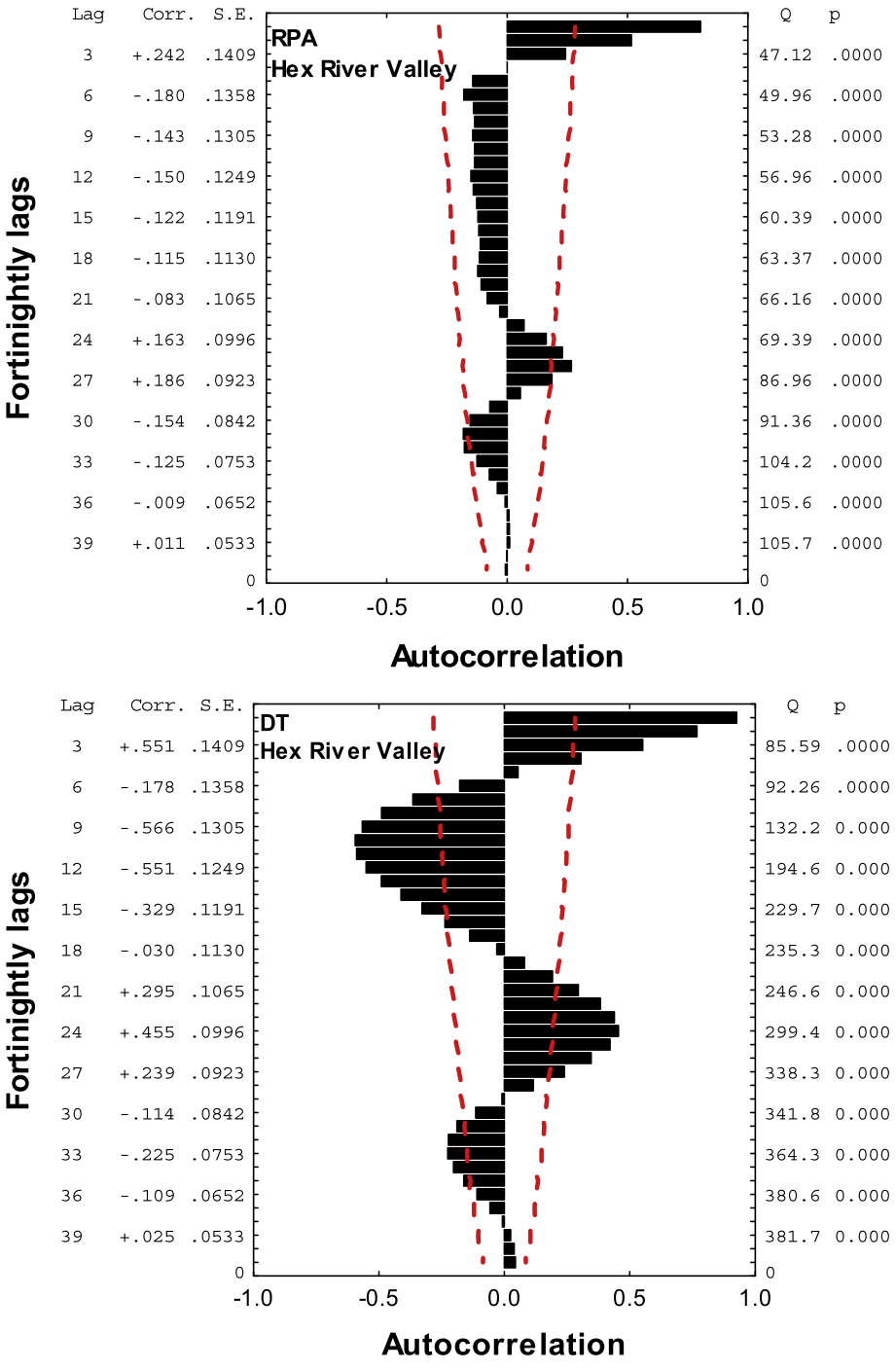

Fig. 7. Autocorrelation plot for fortnightly relative population abundance (RPA, top panel) and development time (DT, bottom panel) for *Ceratitis capitata* recorded over 2 years (January 2009–December 2010) in the Hex River Valley, Western Cape Province, South Africa. The dashed lines on each figure represent the 95% confidence intervals, while the values reported to the right of the lags on the *Y*-axis are the autocorrelation coefficients and their standard errors. RPA represents average *C. capitata* trap capture over 2 weeks at a site while estimated DT represents the time required to complete development from egg to adult as a function of the accumulation of adequate degree hours.

field, then they may prove to be preferred mates under extreme temperature conditions resulting in more effective control. For this reason, understanding the mechanisms underlying the improved thermal tolerance of the *tsl* flies is important. The precise reason why *tsl C. capitata* survived better and showed increased thermal tolerance is unclear. Two possible explanations for this result are (i) that *tsl C. capitata* are in better condition owing to high-quality diet and optimal rearing conditions or (ii) that the *tsl* mutation (possibly coupled with the heat treatment required to sort males from females during the egg stage) has given the flies an overall thermal tolerance advantage. Further work is required to distinguish between these two possibilities. In summary, the current results show that irradiation may negatively affect field survival times and thermal tolerance in *C. rosa* but has little or no apparent detrimental effect on *C. capitata*. This is in keeping with the literature on radiation effects more generally, in which trait- or species-specific effects may be detected (e.g. Collins et al., 2008).

More generally, by combining information on thermal traits for these *Ceratitis* species, field-collected temperature data, and abundance data from routinely inspected traps, our study provides support for several key ideas. First, we have shown that the various physiological responses mounted by insects to deal with thermal variation may have significant consequences for population dynamics. For example, rapid responses in the field that enhance shock survival may be sufficient to improve adult survival of occasional high temperatures and improve survival of the longer, low temperature events, given that tolerances and critical thermal minima are close to values typically encountered in the field. Rapid physiological responses to ambient temperature change have been documented in a variety of species, but clear indications of association between these responses and survival have been forthcoming for a few species only (e.g. Kelty, 2007; Overgaard and Sørensen, 2008; Basson et al., 2012), and even fewer from simultaneous investigations of animals in the field and laboratory (e.g. Kristensen et al., 2008; Chidawanyika and Terblanche, 2011).

Second, when taken in the context of other work on the species, the current study shows that the data required for mechanistic models of species abundance (which can readily be converted to estimates of distribution; e.g. Kearney et al., 2010) are not as onerous as is often made out. Given the costs of these particular species to the global agricultural economy (e.g. California, USA: US$1300-1900 million per year; Chile: US$78 million per year; Mexico: US$230 million per year; Enkerlin 2005), the investment required in obtaining such data is relatively small. By contrast, the insights achieved from such approaches can be considerable. For example, in this particular case, orchard practices such as fruit removal during the summer months may substantially reduce populations, and into the future, as temperatures continue to rise both here and elsewhere (Archer and Rahmstorf, 2010; Hansen et al., 2012). Further development of control practises may be required, although sterilized males may be favoured by rising extremes, at least from an activity perspective.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2013.09.004.

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