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ABSTRACT: Since the first detection of *Bactrocera dorsalis* in Botswana in 2010, the establishment, spread, and response to prevailing Botswana microclimates under rapidly changing environments remain unknown. This study investigated the presence, seasonal population abundance, and thermal biology of *B. dorsalis* in Botswana. We measured *B. dorsalis* thermal tolerance *vis* critical thermal limits (CTLs) and lethal temperature assays (LTAs) to understand how temperature largely impacts on fitness and hence invasive potential. Seasonal monitoring results indicated *B. dorsalis* establishment in the Chobe district (its first area of detection). Trap catches showed continuous adult flies' presence all year round and high average monthly trap catches as compared with other districts. Furthermore, *B. dorsalis* was detected south of Botswana, including Kgatleng, Kweneng, South-east, and Southern districts. Critical thermal maxima (CT_{max}) to activity for adults and larvae were 46.16°C and 45.23°C, whereas critical thermal minima (CT_{min}) to activity for adults and larvae were 9.1°C and 7.3°C, respectively. Moreover, we found an improved CT_{min} for larvae at a slower ramping rate, indicating potential rapid cold hardening. The lower lethal temperature (LLT) and upper lethal temperature (ULT) assays revealed a reduction in survival at all the developmental stages as severity and duration of both temperature extremes increased. Microclimatic temperatures recorded in Botswana showed that environmental temperatures fall within the thermal breath of *B. dorsalis* activity measured here, indicating a potential conducive climate niche for the insect pest across the country, albeit other factors, e.g., host availability, play a significant role. These results therefore suggest that Botswana microclimatic temperatures aided *B. dorsalis* activity and invasion pathway are thus significant in mapping invasions and pest risk analysis, and may also aid in designing pest management strategies.

KEYWORDS: Asian fruit fly, climate change, invasive insect species, thermal tolerance, population dynamics, insect phenology

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Introduction

Fruit flies (Diptera: Tephritidae) are the most significant insect pests in the fruit industry,[1] with many species being polyphagous and causing serious damage to different fruit crops.[1,2] Apart from direct damage to fruit crops, these insects also have impacts on trade because of their quarantine significance.[3,4] The losses have been estimated to cause an annual economic damage of US$42 million in Africa and US$1 billion worldwide.[5] The most destructive species belong to the genera *Bactrocera, Ceratitis, Dacus, Rhagoletis*, and *Anastrepha*.[1] Among these species, the most important insect pests of fruit and vegetables belong to the Dacine group Macquart (Diptera: Tephritidae), with the predominant genus being *Bactrocera*.[2] They are polyphagous in nature and are highly invasive insect pests. Undoubtedly, within this genus, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) has gained most attention. First described as *Bactrocera invadens*,[1,6,7] the species has shown an exponential expansion in its African range over the past decade.[8,9] It is an aggressive invasive fly known to outcompete native insect pests when introduced in novel environments.[10,11] This has been clear from its environmental competitiveness and invasive advantage,[8] hence raising questions on its environmental niche breadth[12] and possible climate change responses.[13] When insects are accidentally introduced in novel environments, different fitness traits such as reproduction, survival, and longevity under different temperature variations are important factors to consider. This is because they form critical filters in the invasion pathway, survival of which forms a critical part of the invasion process.[14] Moreover, understanding the invasion potential, that is, where an insect pest is most likely to invade (based on, e.g., bioclimatic modeling), and knowledge on its taxonomy are related questions that are vital to efficacious management of invasive species.[15]

Insect population dynamics are governed by several biotic (living entities) and abiotic (non-living entities) factors and their interactions.[16] Understanding these factors especially for pests of economic concern is important in coming up with effective management strategies.[17] Tephritid abundance and distribution are notably dependent on several abiotic and biotic factors.[7] Among these factors, temperature is the most dominant abiotic factor that affects insects' development, range survival, and abundance.[18–20] Indeed, high temperature extremes may have diverse negative impacts on insects either indirectly through, e.g., limiting key physiological activity traits[21] or directly through temperature-related mortality or sterility associated with irreversible cell and tissue damage.[19,22] Furthermore, low temperature extremes may also have direct consequences[23] and may affect insect population dynamics either through reduced growth rates, or through a reduction in development rate, as well as suppression in feeding, activity, and mating.[19,24,25] As such, investigating thermal tolerance is the first step toward determining whether a

population may establish following introduction in novel environments,[25] and may help explain the role of temperature in driving insect population abundance.[26]

Insects may cope behaviorally or compensate physiologically to differences in temperature, at various temporal and spatial scales.[19,22,27–29] Behavioral adjustments act as a first line of defense and are dependent on the opportunities available on the insects' habitat.[30] If unfavorable temperatures persist, physiological responses are employed as critical mechanisms to ensure survival.[28,31] Physiologically, insects have developed mechanisms like diapause, or sensitivity to photoperiod to reset and maintain the seasonal clock,[32] or use short- to long-term phenotypic plasticity.[19] Indeed, the ability to shift phenotypes has been documented to aid survival in insects[19,33] including other fruit fly species.[25] However, it has been reported that short-term responses to temperature may not likely explain the currently observed *B. dorsalis* invasion potential.[14] Similar reports have documented that phenotypic plasticity alone may not adequately provide complete compensation against rapidly changing environments,[34] suggesting a critical role of other mechanisms, e.g., behavioral modification and evolution.

Extreme temperatures degrade and destabilize many of the insect's molecular components such as carbohydrates and membranes, and therefore insects cannot tolerate an infinite range of temperatures.[31] Temperatures which are lethal to insects are a function of both duration and severity of exposure to the particular temperature.[19,29,34] Over long periods, temperature affects seasonal phenology and evolutionary responses.[16,35] Nevertheless, temperature at short timescales also plays an important role as it is a driver of insects' key life activities, population dynamics, and consequently species abundance and biogeography.[36,37] As a result, it is imperative that insects remain in environments with benign temperatures, key for optimum life history activities.[19,38]

Since its first detection in Africa, Kenya in 2003,[6,8] *B. dorsalis* was first reported in 2010 for the first time in the Chobe district. Following its detection, a delimiting survey to measure the extent of its spread was performed in 2013 (MoA, personal communication). Nevertheless, since then, no work has been done, to assess the extent of spread south of Botswana and the role of biotic/abiotic microenvironments in facilitating the invasion process. Here, we therefore investigated the presence and spatiotemporal seasonal population abundance of *B. dorsalis*, to determine its establishment and spread in Botswana post detection. Second, we investigated *B. dorsalis* thermal biology to help explain how Botswana temperature microenvironments may help shape its population dynamics and invasion pathway.

Materials and Methods

Detection and population abundance of B. dorsalis

Surveillance. A country-wide survey to determine the distribution of *B. dorsalis* was performed in the districts of Botswana over a period of 2 years (2015-2017). Yellow Chempac bucket traps (Chempac, South Africa) baited with methyl eugenol pheromone lure, placed at the bottom of the trap and acting as a sex attractant to *B. dorsalis* were used. The bucket traps were hung 1.5 m above the ground on fruit trees with a minimum required trap density of 1 trap per km^2 or 1 trap per hectare.[39] An insecticide block dichlorvos (DDVP) was added in each bucket to kill the trapped flies and prevent them from escaping. The bucket rims and hanging wires were coated with vaseline (Petroleum jelly; Unilever, South Africa) to prevent ants from getting inside and feed on trapped flies. All the traps were labeled for easy identification, and for each trap, location, district, farm name, trap number, date trap set, and GPS coordinates were recorded. The lures were changed fortnightly and flies caught were placed in 60-mL plastic vials with 70% ethanol for preservation. These were taken to the laboratory for identification using gross morphology using a stereomicroscope (BestScope BS3060BT; Hangzhou Scopetek Opto-Electric Co., China) and a fruit fly electronic identification system (Citrus Research International, South Africa).[40]

Fruit sampling. Both cultivated and wild fruits showing signs of damage within each of the sampling areas and districts were collected. Over-ripe and ripe fruits were collected from the ground and from the trees to maximize chances of getting fruit-fly-infested fruit specimens. The cultivated fruits were collected during the fruiting austral summer seasons (November-March) for the period 2015/2016 and 2016/2017. The samples were placed in a closed plastic container (3500 cm^3) with fine mesh on the lid to allow for aeration. These containers were filled with a layer of sterilized soil ~5 cm at the base as the pupation medium. Samples were then incubated in the lab at 28°C, 65% relative humidity (RH), and photoperiod maintained at (12:12) light:dark cycles in a climate chamber (HPP 260; Memmert GmbH+ Co.KG, Germany). The samples were regularly checked every 24 hours for fly eclosion. Emerging fruit flies were collected, preserved in 70% alcohol, and stored in 60-mL vials before morphological identification.[40]

Thermal tolerance assays

Insect culture. The initial colony of *B. dorsalis* was obtained from the International Center for Insect Physiology and Ecology (ICIPE) in Kenya and then cultured in the laboratory using artificial diet.[41] The culture was widely outbred with regular supplementation with wild flies to prevent loss of fitness likely caused by stress associated with overcrowding and laboratory adaptation.[42] For adult eclosion, the pupae were kept in 240 mm^3 BugDorm cages (BugDorm-BD43030F; MegaView Science Co., Ltd, Taiwan) in the laboratory under (12:12 L:D photoperiod; 28°C ± 1°C and 65% RH) in Memmert climate chambers. On adult eclosion, flies were fed on a sugar diet, water, and yeast (for protein) *ad libitum*. Thermal tolerance experiments were performed using 4- to 7-day-old adults of

mixed sex, as sex appears not to have a significant effect on thermal tolerance in similar Tephritid fruit flies.[21]

Critical thermal limits. Critical thermal limits (CTLs) were assayed as outlined by Nyamukondiwa and Terblanche.[43] Third instar larvae and 4- to 7-day-old adults of *B. dorsalis* were individually placed in the organ pipe connected to the water bath (Lauda Eco Gold; Lauda Dr. R. Wobser GmbH & Co. KG, Germany). Critical thermal limits started at a set-point temperature of 28°C for 10 minutes to allow for equilibration, then ramped up (for CT_{max}) or down (for CT_{min}) until each organism reached its limit to activity.[34,43] Three different rates (0.12°C/min, 0.25°C/min, and 0.50°C/min) were used for both CT_{min} and CT_{max}. A mixture of propylene glycol and water at a ratio of 1:1 was used to enable the water bath to operate at subzero temperatures. A thermocouple (type K, 36SWG) digital thermometer connected to a digital thermometer (53/54IIB; Fluke Cooperation, USA) was inserted into the organ pipe control chamber to record the temperature experienced by the flies. In this study, CTLs were defined as the temperature at which *B. dorsalis* lost coordinated muscle function.[43]

Lethal temperature assays. Lethal temperature assays (LTAs) were measured using standardized plunge protocols, with necessary modifications,[44,45] using a programmable water bath (Systronix; Scientific Engineering (Pty) Ltd, South Africa) filled with 1:1 propylene glycol and water. Upper lethal temperatures (ULTs) and lower lethal temperatures (LLTs) were determined for a range of time (from 0.5 to 4 hours). A total of 10 insects were put in 3 replicate 60-mL polypropylene vials, and the vials were placed in a ziplock bag for each temperature × time treatment until a range of 0% to 100% mortality was recorded. To avoid desiccation-related mortality, during ULT experiments, a piece of moistened cotton was suspended from the perforated lids of the vials. Temperatures in the vials were verified using digital thermometers (Fluke 53/54IIB, Fluke Cooperation).[46] Following ULT and LLT experiments, treatment vials containing the assayed *B. dorsalis* were placed in a Memmert climate chamber at 28°C ± 1°C and 65% RH for 24 hours (to allow insect recovery) before survival was recorded. Survival was defined as a coordinated response to external stimuli such as prodding or normal behaviors like feeding, flying, and mating for adults; ability to pupate for larvae; and ability to eclose for pupae.[47]

Statistical analyses

Critical thermal limits met the linear model assumptions of constant variance and normal errors, so the effects of ramping rate on CTLs were analyzed using one-way analysis of variance (ANOVA) in STATISTICA version 13 (StatSoft, USA) with the dependent variable being either CT_{max} or CT_{min} and categorical predictor was the ramping rate (0.12°C/min, 0.25°C/min, and 0.50°C/min). Tukey-Kramer post hoc tests were used to separate statistically heterogeneous groups. Lethal temperature assays (LLT and ULT) were analyzed using a generalized linear model (GLM) assuming binomial for LTAs and a logit link function in R statistical software. Temperature-time graphs were computed in OriginPro 8. To determine the effect of environmental temperature on seasonal *B. dorsalis* population abundance in the field, shaded microclimatic temperatures were recorded in all regions where trapping was done, using Thermochron iButtons (model DS1920; Dallas Semiconductor, USA) (0.5°C accuracy; 1 hour sampling frequency). The microclimatic data were then linked with the average trap catches and also combined with thermal tolerance estimates to establish the effect of diurnal fluctuating temperatures on seasonal *B. dorsalis* population abundance.

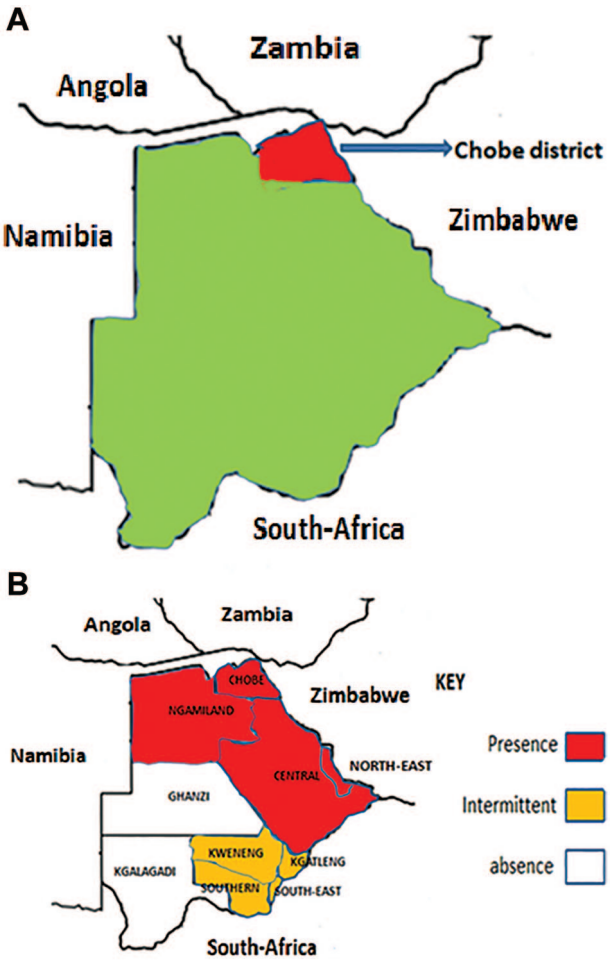

Figure 1. Maps of Botswana showing the presence of *B. dorsalis vis* Chobe district, the area of first invasion and detection (A) and the current state of *B. dorsalis* distribution within the country (B).

Results

Detection and spatiotemporal population abundance of B. dorsalis

Since its first detection in the Chobe district in 2010 (Figure 1A), our results show a southward spread of *B. dorsalis*, with detection in 7 other separate districts apart from its area of introduction (Figure 1B and Table 1). *B. dorsalis* adults were caught in traps placed in cultivated mango, orange, and guava fruit orchards.

Table 1. Monthly average number of *B. dorsalis* adults emerging from damaged cultivated fruits collected during the fruiting season (November-March) for the period 2015 to 2017.

DISTRICT	FRUIT TYPE	MONTHLY AVERAGE NUMBER OF *B. DORSALIS* ADULTS FROM FRUITS DURING FRUITING SEASON (NOVEMBER-MARCH)		
		2015	2016	2017
Chobe	Mango	17	25	30
	Orange	14	10	12
Ngamiland	Mango	23	10	17
	Orange	8	9	4
Central	Mango	10	14	9
	Orange	5	6	9
North-east	Mango	14	17	25
	Orange	8	12	10
Kgatleng	Mango	3	5	1
	Orange	2	2	1
Kweneng	Orange	1	2	1
	Guava	8	3	2
Southern	Mango	2	0	0
	Guava	1	1	1
South-east	Orange	1	0	0

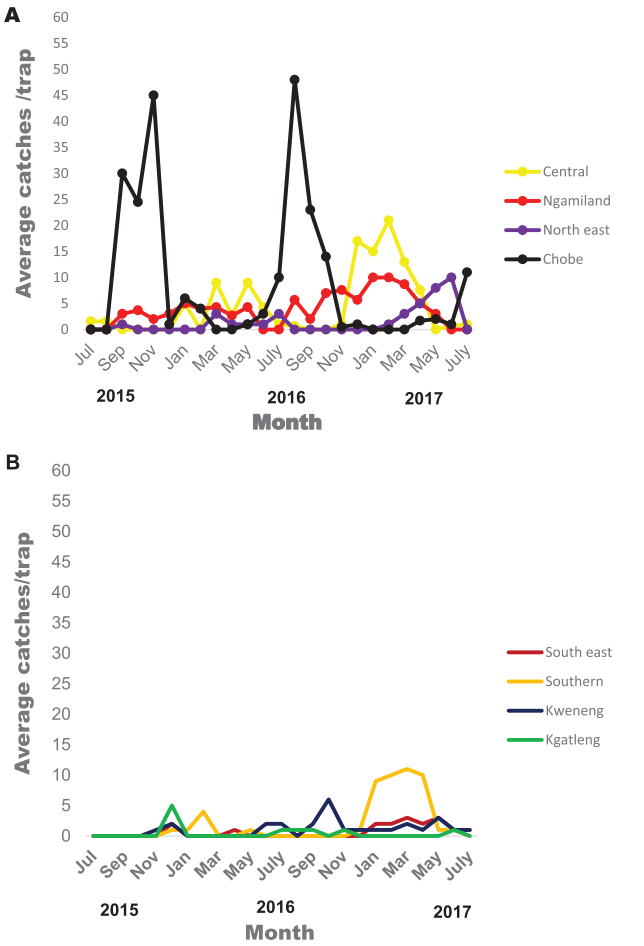

Figure 2. Population abundance of *B. dorsalis* in (A) Chobe, Ngamiland, Central, and North-east and (B) South-east, Southern, Kweneng, and Kgatleng districts. Adults were baited using a pheromone lure in yellow Chempac bucket traps containing methyl eugenol pheromone lure.

However, results from direct fruit sampling showed recovery of more flies from mango relative to other fruit hosts, suggesting that mango may be a more preferred host for the species (Table 1).

High numbers of *B. dorsalis* adults were captured from the Chobe, Ngamiland, Central, and North-east districts (Figure 2A), whereas Kgatleng, Kweneng, Southern, and South-east districts had sporadic populations of less than 5 adults/trap/month (Figure 2B). The highest abundance of the insect pest in the Chobe district was during the months August to December (Figure 2A), coincident with increased temperatures and host availability following austral winter season. However, in the other 3 districts (Ngamiland, Central, and North-east), the insect pest was at its highest during the months November to April, also coinciding with the conducive biotic and abiotic environments. From the sampled fruits, high numbers of *B. dorsalis* emerged in fruits collected from Chobe, Ngamiland, Central, and North-east than the rest of the other districts (Table 1).

Thermal biology of B. dorsalis

Critical thermal limits (CTLs)

Ramping rate and developmental stage had significant effects on CT_{max} (Table 2; Figure 3A). Using a ramping rate of 0.25°C/min, the CT_{max} of *B. dorsalis* was 46.16°C for adults and 43.23°C for larvae (Figure 3A), whereas the CT_{min} was 9.1°C for adults and 7.3°C for larvae (Figure 3B). Interaction between developmental stage and ramping rate was not significant for CT_{max} but was however significant for CT_{min}. For both developmental stages, it appeared that the slowest ramping rates (0.12°C/min) compromised CT_{max}, whereas the fastest rate (0.50°C/min) enhanced high-temperature tolerance or CT_{max} (Figure 3A). Conversely, for CT_{min}, it appeared that the 2 slowest ramping rates improved low-temperature tolerance (CT_{min}), compared with the higher ramping rate (0.50°C/min) (Figure 3B). Generally, developmental stage did not affect low-temperature tolerance (Figure 3A) but had a significant effect on high-temperature tolerance (Table 2; Figure 3B).

LTAs. An increase in duration and severity at both high and low temperatures resulted in increased adult and larvae mortalities (Table 3 and Figure 4). In ULT assays, 100% survival was observed at 39°C at all durations in adults (Figure 4A), whereas for the larvae 100% survival was observed at 38°C (Figure 4B). Similarly, both adults and larvae recorded 0% survival at 45°C across all time interactions (Figure 4A and B).

Table 2. Summary results of one-way ANOVA showing the effects of ramping rate, developmental stage (larvae and adults), and their interaction effects on *B. dorsalis* critical thermal limits (CT_{max} and CT_{min}).

EFFECT VALUE	SS	D.F.	M.S	*F*-VALUE	*P*-VALUE
CT_{max}					
Ramping rate	151.5	2	75.7	28.97	.032669
Developmental stage	12.2	1	12.2	4.68	<.0001*
Ramping rate × developmental stage	15.5	2	7.8	2.97	.055298
CT_{min}					
Ramping rate	25.235	2	12.618	6.817	<.001*
Developmental stage	63.948	1	63.948	34.550	<.000*
Ramping rate × developmental stage	8.274	2	4.137	2.235	.111630

Abbreviations: ANOVA, analysis of variance; d.f., degrees of freedom; MS, mean sum of squares; SS, sum of squares.
*Significant effect.

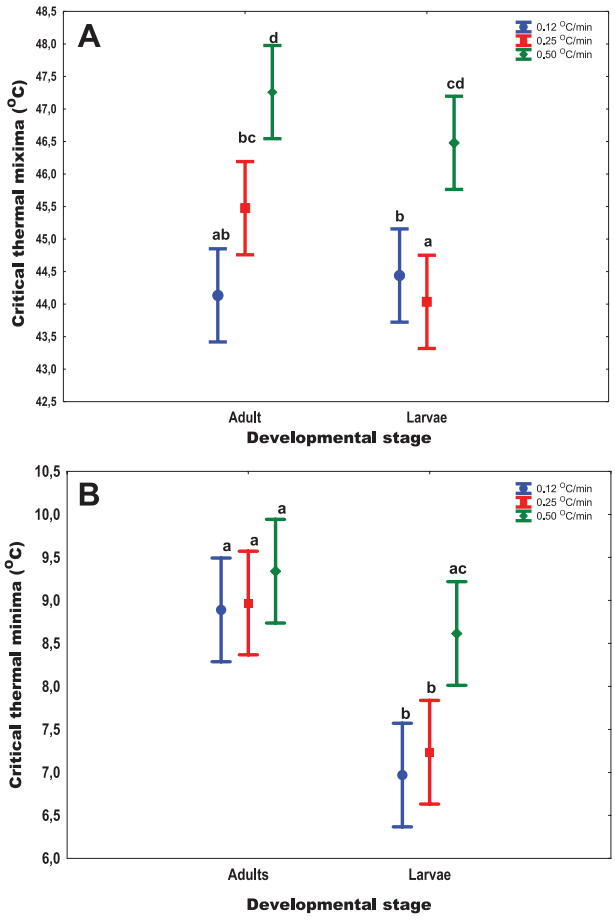

Figure 3. The effect of different ramping rates and developmental stage (larvae and adults) on *B. dorsalis* critical thermal limits (A) CT_{max} and (B) CT_{min}. Means with the same letter(s) are not statistically significant. Each point represents mean ± 95% CL. Tukey-Kramer post hoc tests were used to separate statistically heterogeneous groups. CL indicates confidence level.

Adults showed a narrower temperature activity range at stressful temperatures (39°C - 43°C for the 2- and 4-hour durations), whereas the larvae on the other hand exhibited a higher survival/activity for the same time × temperature treatments (Figure 4A and B). This result indicates that larvae are more tolerant to heat than adults. Lower lethal temperature assays showed 100% survival for *B. dorsalis* adults at 0°C (Figure 4C) and for larvae at 2°C (Figure 4D). Similarly, 100% mortality was achieved at −6°C and −8°C for adults and larvae, respectively, indicating that larvae were more cold tolerant than adults.

Field microclimatic data recorded from Kgatleng district (S24.61224; E25.97326; 977) showed summer monthly average temperatures of 28.24°C, whereas the mean monthly average temperature for the winter season is 13.73°C. Considering the CT_{max} values of 46.16°C and 45.23°C (adults and larvae, respectively) and ULT of 45°C, and the CT_{min} values of 9.1°C and 7.3°C (adults and larvae, respectively) and LLT of approximately −6°C to −8°C, it appears that *B. dorsalis* may be under no high-temperature stress and little low-temperature constraint, the latter limited to low winter temperatures (see Figure 5).

Discussion

Key determinants of invasion success for insect species include climatic suitability, propagule pressure, and availability of suitable hosts.[48,49] Climate change influences the distribution and abundance of invasive insects by altering where species and hosts can occur, through changes in population growth rates, propagule pressure, and spread.[50] We found that, since its detection in 2010 in the Chobe district, *B. dorsalis* has consistently spread and is now established in areas outside its first detection zone (Chobe district; see Figures 1 and 2). Seasonal population monitoring showed that the pest species is now established in Chobe, Ngamiland, North-east, and Central districts of Botswana, and that populations in South-east, Southern, Kweneng, and Kgatleng districts range from intermittent to persistent, again confirming the progressive spread

Table 3. Summary effects of temperature severity and duration of exposure on the survival of *B. dorsalis* adults, pupae, and larvae.

DEVELOPMENTAL STAGE	PARAMETER	X^2	D.F.	*P*-VALUE
Upper lethal temperatures				
Adults	Time	77.91	3	<.0001*
	Temperature	396.14	3	<.0001*
	Time × temp.	22.79	9	<.001*
Larvae	Time	54.11	3	<.000*
	Temperature	371.72	3	<.000*
	Time × temp.	2.33	9	<.9852*
Lower lethal temperatures				
Adults	Time	104.44	3	<.0001*
	Temperature	645.76	5	<.11
	Time × temp.	19.61	15	.1873
Larvae	Time	104	3	<.0001*
	Temperature	392.84	3	<.0001*
	Time × temp.	4.75	9	.8555

Abbreviations: d.f., degrees of freedom; LLT, lower lethal temperature; ULT, upper lethal temperature.
Analysis was done using generalized linear model (GLM) assuming binomial distribution with a logit link function in R software 3.3.0. Three replicates of 10 individuals were used for both high and low temperatures (ULT and LLT).

of the invasive fruit fly species. The high populations of adult were consistent (see Figure 2A and B) peaking in summer, coincident with the fruiting season (November-March) and favorable high temperatures. This suggests that temperature and host availability are behind this pest establishment and may drive *B. dorsalis* population abundance in natural landscapes. Temperature is the most important factor limiting the development of ectotherms. An increase in temperature has been linked to the accumulation of degree days resulting in shorter developmental time of insects. For example, as the temperature increased, larval development of *B. invadens* decreased from 35.95 days at 15°C to 6.64 days at 35°C.[51] When the developmental time is shortened, this will result in more generations in a year, hence high propagule pressure, which enhances pest establishment. Moreover, temperature is a key driver that determines field fitness.[21,52] As such temperature drives many key life history traits including locomotion,[21] mating, feeding, oviposition, and flight, among other traits.[53]

It also appears that host availability, e.g., the cultivation of mango fruits, may also play a part in the reported *B. dorsalis* establishment. Our results showed high abundance of the insect pest species in mango orchards, symbolizing its preference for this host. Indeed, reports documented mango as the most preferred host by *B. dorsalis* and movement of adult flies has been linked to host availability.[40] Furthermore, it is reported that when hosts are abundant and diverse, tephritids are non-dispersive.[54] This is especially true for a polyphagous invasive fruit fly species like *B. dorsalis*.[55] Thus, potentially limited host resources in dry arid to semi-arid Botswana environments may also partly account for the rapid spread and establishment of this pest species, in search of new and diverse host plants. Our results document that the pest species has spread south-east of the country, in Ngamiland, Central, and North-east districts with consistent all year round pest abundance (see Figure 2A), albeit sporadic and with low population abundance as we go south of Botswana *vis* Kgatleng, Kweneng, Southern, and South-east districts (Figure 2B). The latter may suggest that these areas may not have all year round host plants, and as such, may not support consistent *B. dorsalis* breeding. In consequence, intermittent populations recorded here may be sustained through repeated introduction of propagule pressure,[48,49] or that the species may be behaviorally compensating for the temporal stressful biotic and abiotic environments through, e.g., diapause.[56]

Thermal activity physiological thresholds examined here also suggested that *B. dorsalis* may not be under significant temperature-related physiological strain indicating a near-conducive climate niche across the country. The highest temperatures allowing activity (CT_{max}) for adults and larvae were 46.16°C and 45.23°C, respectively. These thresholds of activity were far above the summer average monthly maximum temperature of 28.24°C giving an optimal ambient temperature environment

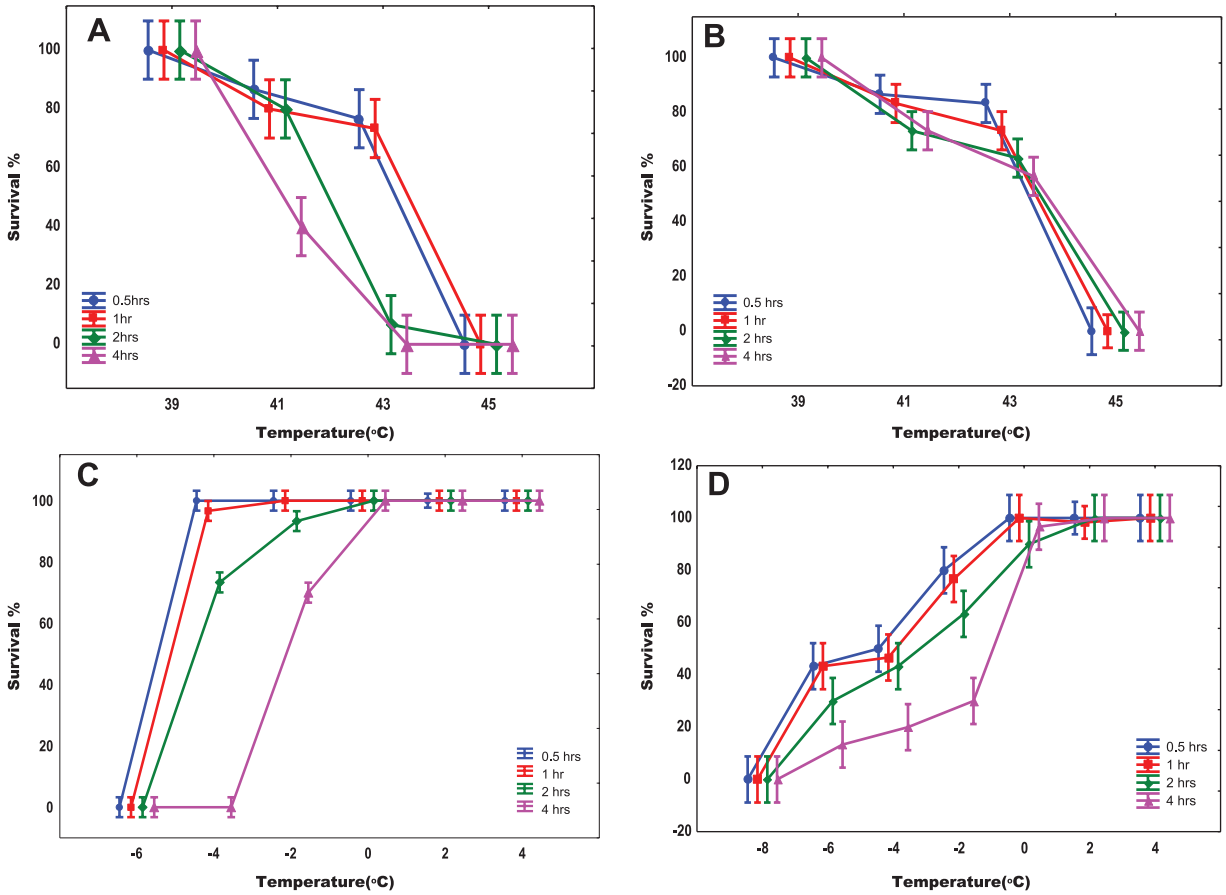

Figure 4. Summary results of the effect of high and low temperatures on the survival of different developmental stages of *B. dorsalis* at different temperature × time interactions: (A, B) upper lethal temperature assay results for adults and larvae, respectively; (C, D) lower lethal temperature assay results for adult and larvae, respectively. Error bars represent 95% CL. CL indicates confidence level.

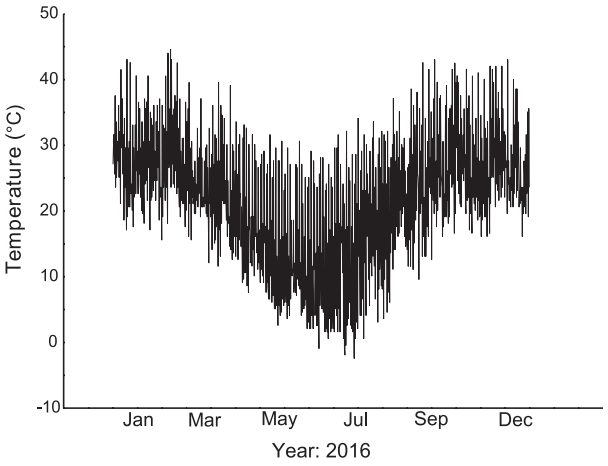

Figure 5. Shaded microclimatic data from Kgatleng district (S24.56449; E0.26.16940) recorded during January - December 2016. Temperature was recorded using Thermochron iButtons (model DS1920; Dallas Semiconductor, USA) (0.5°C accuracy and 1 hour sampling frequency).

supporting the progressive spread and establishment of *B. dorsalis* across Botswana and an enhanced temperature safety margin.[57] Furthermore, our CTL results revealed improved CT_{max} following faster ramping rates for both developmental stages and generally improved CT_{min} for only larvae following slower ramping rates. Results for the effects of ramping rate on CT_{max} suggest that *B. dorsalis* may not adjust its high-temperature tolerance in the short term. This may suggest that *B. dorsalis* invasion potential may not be aided by phenotypic plasticity of high-temperature tolerance, otherwise rapid heat hardening.[14] However, the improvement of low-temperature tolerance (CT_{min}), following slower ramping rates in larva, indicates the ability of this developmental stage to adjust its phenotype at short timescales, termed rapid cold hardening (RCH). This RCH result in larvae contrasts the observations by Pieterse et al[14] who found no RCH in *B. dorsalis* adults. Nevertheless, this may point to differential plastic responses across different developmental stages in this fly species.[19] Indeed, RCH is induced by diurnal temperature cycles[23,58,59] and, in some scenarios, these plastic responses have been reported to enhance invasion potential.[14,25] However, the role of this small plastic response to low temperature in larval *B. dorsalis* in the invasion success of this fly species is unknown. Field microclimatic data revealed that the upper (~45°C) and lower (approximately −8°C to −6°C) temperature limits are seldom encountered in nature (see Figure 5). However, the duration of these suboptimal thermal conditions remain unknown. Nevertheless, exposure to these lethal and sublethal temperatures plays an important role

ecologically as it determines traits of fitness, e.g., activity, time required for thermal stress knockdown, and possibly time required for recovery following stressful thermal conditions.[19] Our experiments used standardized laboratory colonies and protocols where insects are confined in the test environment. However, in nature, insects may use many behavioral modifications to cope with changing stressful temperature conditions, e.g., seek microclimatic or shade environments. Thus, although our results report that stressful temperatures are seldom reached, organisms may be able to compensate behaviorally, and thus prevailing ambient temperature conditions in Botswana may not be a limiting factor for *B. dorsalis* establishment.

The present results reveal that, following first invasion in 2010, *B. dorsalis* has spread and is now established in Botswana. Thermal biology assays reveal a wide thermal breath for *B. dorsalis*, and a comparison of these thermal traits and prevailing ambient temperature conditions suggest that *B. dorsalis* may thrive in most parts of the country. This basal temperature tolerance, coupled with morphological, behavioral, and physiological adjustments may help facilitate the invasion potential of *B. dorsalis*.[14] Such attributes are critical for invasive species and likely shape their invasion potential under climate change. Whereas our observations recorded *B. dorsalis* in almost all districts of Botswana, in some of the areas populations were not persistent, probably owing to erratic host availability. Nevertheless, if hosts are available, it seems that the temperature in Botswana may not likely offset *B. dorsalis* invasion and consequent establishment. We suggest a wider range of trait conditions be tested before solid conclusions are drawn on the relationship between thermal biology and *B. dorsalis* invasion potential. Nevertheless, current results are significant for pest risk analysis, developing phytosanitary regulations and informing pest management strategies for this invasive species in a globally changing environment. Future work may also consider optimization of monitoring and control techniques for efficacious *B. dorsalis* management and the potential of parasitoids in managing insect pest populations.

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Author Contributions

CN contributed in project conceptualization and management, formal analysis, visualization and validation, writing, review, and editing. RM contributed in data curation, formal analysis, investigation, visualization and validation, writing - original draft, writing, review and editing. NG contributed in formal analysis, visualization and validation, writing, review, and editing.

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