Biology of *Bactrocera dorsalis* (DIPTERA: TEPHRITIDAE): Implications on population dynamics and pest management

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A Dissertation/Thesis Submitted to the College of Science in Partial Fulfilment of the Requirements for the Award of the Degree of Master of Science in Biological Sciences (Applied Entomology) of BIUST

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CERTIFICATION

The undersigned certifies that he/she has read and hereby recommends for acceptance by the Faculty of Science a dissertation/thesis titled: **Biology of *Bactrocera dorsalis* (DIPTERA: TEPHRITIDAE): Implications on population dynamics and pest management** in fulfilment of the requirements for the degree of Master of Science in Biological Sciences (Applied Entomology) of BIUST.

Dr C. Nyamukondiwa (Supervisor)

Signature:

Date: 03 February 2018

DEDICATION

I dedicate this thesis to my mother (Patricia Gobuamang Motswagole)

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ABSTRACT

Fruit production is one of the fastest growing sectors in Botswana. As one of the agricultural sectors, fruit production is very crucial as it contributes immensely towards income generation and employment opportunities, especially for vulnerable rural livelihoods. Despite all the benefits, one of the biggest hurdles in the fruit production industry is disease and insect pests. Chief among these insect pests is the *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Bactrocera dorsalis* is a worldwide economic insect pest of fruit and vegetables that has spread its geographical range to many African countries including Botswana. It was first detected in Botswana in 2010 in the Chobe district. However its spread and establishment around the country following its first detection is largely unknown. Furthermore despite this pest being of economic importance and a biosecurity threat, its response to prevailing Botswana microclimates and global change remained unknown. My study therefore investigated (i) presence and (ii) seasonal population dynamics of *B. dorsalis* in Botswana. Furthermore I investigated the thermal tolerance of *B. dorsalis* by measuring its different thermal low and high temperature activity traits *vis a vis* Critical thermal limits (CTLs), Lethal temperature assays (LTAs), and Supercooling points (SCPs), in order to understand how temperature largely impact this specie's activity and thus population dynamics, abundance and consequently invasive potential. My seasonal monitoring results indicate that *B. dorsalis* is now established in the Chobe district, (its first area of detection) as shown by its continued presence all year round and high average monthly trap catches (<0.1) as compared to other districts. . Furthermore the insect pest has been detected in other districts south of Botswana, including Kgatleng, Kweneng, South-east, and Southern. This indicated that since 2010, the insect pest has spread down south of the country, with potential negative effects on fruit industries in those areas. Nevertheless, records of this insect pest in some of the areas (South-East and Kgatleng) were erratic, symbolizing that the areas did not have 'resident breeding populations' for *B. dorsalis* but rather repeated introductions from 'endemic' or highly infested areas. Laboratory thermal activity experiments showed there was an improvement in critical thermal maxima (CT_{max}) at higher ramping rate across all the two developmental stages, indicating the lack of potential to shift high temperature tolerance for the two developmental stages at short timescales. The average high temperature of activity (CT_{max}) for adults and larvae were 46.16°C and 45.23°C respectively. However, there was an improved critical thermal minimum (CT_{min}) for larvae at slower ramping rate, indicating potential to improve low thermal tolerance at slower ramping rates, otherwise termed rapid cold hardening, the average low temperature for activity (CT_{min}) for adults and larvae 9.10°C and 7.3°C respectively. The results for lower- and upper lethal temperature assays (LLTs and ULTs respectively) revealed a reduction is survival at all the developmental stages as severity and duration increased, affirming the notion mortality is a function of temperature duration and severity. The ULTs and LLTs for adults, larvae and pupae ranged from 39-45, -6– 4°C; 38-41, -4–2°C and 39-45, -8–4°C respectively at 0.5 to 4hrs treatments. Pupae were the most temperature tolerant compared to other mobile stages. The SCPs of *B. dorsalis* developmental stages were -16.5 (adults), -16.6 (pupae) and -12.18˚C (larvae). SCP's were significantly affected by developmental stages with pupae and adults having relatively depressed SCPs compared to larvae. General microclimatic temperatures recorded here versus experimentally derived thermal limits to activity imply that both high and low temperatures may not limit *B. dorsalis* establishment in the short term, and that the species may thrive upon introduction to the thermal environments investigated. The results of this study are of major implications to the management and enforcement of quarantine regulations of *B. dorsalis.* Knowledge of thermal biology is highly critical in the development of phytosanitary measures as well as in the forecasting of its ability to spread and establish in novel areas. This data may help in the development of mechanistic models of *B. dorsalis* invasion potential.

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CHAPTER 1

General Introduction

1.1 Fruit flies and their economic significance

Fruit flies (Diptera; Tephritidae) are the most significant insect pests in the fruit industry (White and Elson Harris 1994), with many species being polyphagous causing serious damage to different fruit crops (White and Elson Harris 1994; Clarke *et al.* 2005). Not only do they cause direct damage to fruit crops but also have impacts on trade with countries suffering from loss of markets (Follet and Neven 2006). The losses have been estimated to cause an annual economic damage of USD 42 million in Africa and USD 1 billion worldwide (STDF 2010). The most destructive species belong to the genera *Bactocera, Ceratitis, Dacus, Rhagoletis* and *Anastrepha* (White and Elson Harris 1994).

Amongst these species the most important insect pests of fruit and vegetables belong to the Dacine group Macquart (Diptera: Tephrtitidae), with the predominant genus being *Bactocera* (Clarke *et al.* 2005). They are polyphagous in nature and in addition, they are highly invasive insect pests, but none has gained attention as *Bactocera dorsalis* first described as *Bactocera invadens*, a highly multivoltine and polyphagous fruit fly (White and Elson Harris 1994; Vayssières *et al.* 2009). Larval fruit fly development takes place in the seed bearing part of the plant and seldom in other plant parts such as the flowers and stems (White & Elson-Harris, 1992). It is an aggressive invasive fly known to out-compete native insect pests when introduced in novel environments (Ndiaye *et al* 2008; Ekesi *et al* 2009). This fruit fly has a large host range of over 40 species (Ekesi and Billah 2007 with the primary host being mango, *Magnifera indica* L. (Anacardiaceae) (Rwomushana *et al*. 2008a). Because of these reasons, it has successfully dispersed to novel environments, where it affected several fruit crops and thus contributing to significant economic and trade losses.

1.2 Bactocera dorsalis life cycle

The duration of *B. dorsalis* life cycle is about 25 days at 28°C (Ekesi 2006). Females deposit white ‘banana’ shaped eggs in clusters hatching in 38 hours at 28 °C (Bateman 1972, White and Elton Harris 1992). A single female can lay >1000 over her life time, 55% of which can develop into adults (White and Elton Harris 1992). Rwomushana *et al.* (2008b) indicated that there is a linear relationship between *B. dorsalis* development and temperature. Developmental time for eggs was 5.71 and 1.24 days at 15 and 35˚C respectively; larval developmental time was 35.95

and 6.64 days at 15 and 35°C while pupal development took 34.08 days at 15°C and no adults eclosed at 35°C (Rwomushana *et al*. 2008b). Ekesi *et al.* (2006) previously reported that *B. dorsalis* successfully completed development at 28°C which lie between the optimum ranges of 20-30°C which are preferred by the species. Upper pupal developmental threshold for *B. dorsalis* was found to be 35°C while lower developmental thresholds were recorded as 8.8, 9.4 and 8.7°C for eggs, larva and pupae respectively with the respective corresponding thermal constants of 31, 168 and 177 DD (Rwomushana *et al*. 2008b). Ekesi *et al*. (2006) also estimated development time of *B. dorsalis* at 25 days; egg-larva development time at 1.5 days; larval development 11.1 days and a pupal development time of 12.4 days 28C°. At 30°C, *B.dorsalis* generation time takes only 17 days (Ekesi *et al* 2006), indicating the significant correlation between temperature and rate of insect development. Moreover, *B. dorsalis* adults thrive at variable and wide temperature ranges (~15-35°C), making them highly adaptable to novel heterogeneous thermal environments (Ekesi 2011). This, together with its polyphagous and multivoltine nature, may contribute to the invasion potential of this fruit fly species.

At benign temperatures (28°C) *B. dorsalis* reach sexual maturity within 7 days after which they lay eggs between days 8-22 (Rwomushana *et al* 2009). Egg viability/survival was estimated at 87% (35°C); 97% (20°C); larval survival was estimated at 84% (35°C) and 99% (25°C); while pupal eclosion was estimated 0% and 96% at 35 and 25°C respectively (Rwomushana *et al* 2009). At optimum environmental conditions (65% RH 28°C and 12:12 light: dark cycles) eggs hatch into larvae after 2/3 days , the larvae will develop into 1st to 3rd instar in a period of 7 days (Bateman, 1972 White and Elson Harris (1992). The 1st instar will migrate deeper into the fruit to feed on the pulp of the fruit (Pena *et al.* 1998), and predisposes the fruit to secondary pathogens (Pena and Mohyuddin 1997). During the 3rd instar (hopers), the larvae will molt two times into a maggot which will jump out of the fruit into the soil where it pupates 2-5cm beneath the host plant (see figure 1 for complete life cycle). Pupal development may vary from 8-14 days depending on temperature, and adult life expectancy is also variable, ranging from 75.1 days in females and 86.4 days in males with, average net fecundity and fertility of 794.6 and 608.1 for females and males respectively (Ekesi *et al* 2006).

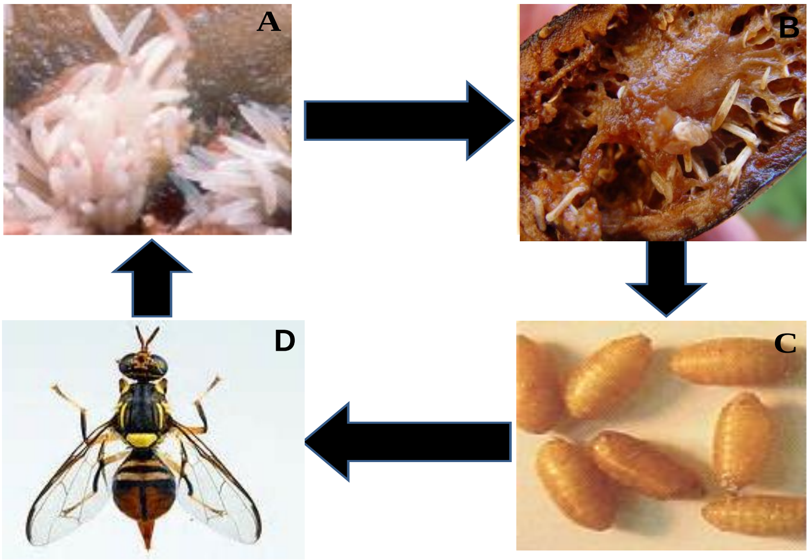

Figure 1: Generalized life cycle of *Bactocera dorsalis* (A) eggs (B) larvae (C) pupae (D) adult

1.3 Climate change and insects

The ongoing climate change, partly due to increased atmospheric CO_2 and other greenhouse gases has led to a significant impact on the natural environment (Peng *et al.* 2004; IPCC 2014). Current estimates predict a global mean annual temperature increase of 1^oC by 2025 and 3^oC by the end of the 21st century (UNFCCC 2007; IPCC 2014). Climate change has indirect and direct effects on insect's abundance and distribution (Porter *et al.* 1991). It indirectly affects the insect's hosts (e.g. plants and prey animals), competitors and natural enemies (Porter 1991; Mutamiswa *et al*. 2017a), and directly by affecting the insect's reproduction, development and fitness (Vargus *et al.* 2000). The impact of climate change may be revealed through insect's phenology, distribution and activity (Bale *et al.* 2002). Amongst the parameters of climate change, temperature may be the most significant environmental factor affecting insect behavior, distribution, reproduction and survival (Yamamura and Kiritani 1998).

Environmental temperature is undoubtedly one of the most significant factors affecting insect geographic distribution and abundance (Cossins and Bowler 1987; reviewed in Chown and Nicolson 2004). Temperatures coupled with relative humidity are the key abiotic factors affecting insect population dynamics (Angilletta *et al.* 2002; Bowler and Terblanche 2008), temperature and relative humidity determine the phenology of insect species and consequently their abundance (Duyck and Quilici 2002; Vera *et al*. 2002; Grout and Stoltz 2007). Because temperature and humidity vary in space and time, and variability altered through global change, it is significant for insects to withstand or adapt to variations in abiotic stressors, at both spatial and temporal scales (Overgaard and Sørensen 2008). Temperature has the greatest effect on insect development (Taylor 1981, Pedigo 1989); it affects characteristics such as sex ratio (Zheng *et al*. 2008), adult life span, survival fecundity and fertility (Yang *et al*. 1994; Dreyer and Baumagrtner 1996; Infante 2000). Ultimately temperature thus dictates insect abundance, colonization, distribution, behavior, fitness, and above all reproductive sterility and mortality (Kang *et al*. 2009). Thus to a greater degree, physiological tolerance to climatic stress (temperature and humidity) may dictate whether an invasive species may thrive upon introduction into a new habitat? (Chown 2007).

1.4 Why are insects especially vulnerable to temperature

Because insects are ectotherms, their internal body temperature is always in equilibrium with environmental temperature, at least for small organisms <1g (Chown and Nicolson 2004). In addition insects have a short generation time and high reproductive rates hence they are more likely to respond much faster to climate change (Bale *et al* 2002). As a result, external ambient environmental temperatures thus affect insect growth (Jawaski and Hilszczanski 2013), and in all likelihood, increases in temperature associated with climate change may affects insect pest population in many ways by impacting their physiology and development directly and indirectly (Bale *et al* 2002). This may have been witnessed in many ways including pest resurgence (Skervin and Felon 2003), invasions (Mutamiswa *et al*. 2017b) and failure of efficacy for biological control (Mutamiswa *et al*. 2017a).

1.4.1 Direct influence of temperature on insects

Temperature fluctuations may affects insects life history traits e.g. change in sex ratios as in thrips (Lewis 1997). Temperature also directly affect reproduction rates (Moore, Allard 2008; Netherer, Schopf 2010), rates of metabolism and consequently development resulting in increased populations (Moore and Allard 2008; Netherer and Schopf 2010). Insects generally have an activity and developmental thermal window, and an optimum temperature whereupon all activities are maximal. Any deviation from the optimal has negative consequences on development and fitness traits affecting insect survival (Parsons 1979; Marchand and McNeil

2000). Adverse effects of temperature may cause insects to display adaptive physiological responses, phenotypically and genotypically hence altering their ability to perform normally (Cossins and Bowler 1987). These adaptive responses are dealt with through capacity and resistance adaptations to lethal temperatures (Cossins and Bowler 1987). The effects of temperature on insects in general and activity/performance may be described using the thermobiological scale (Figure 2) as proposed by Vannier (1994) and the performance curve (Figure 3) respectively.

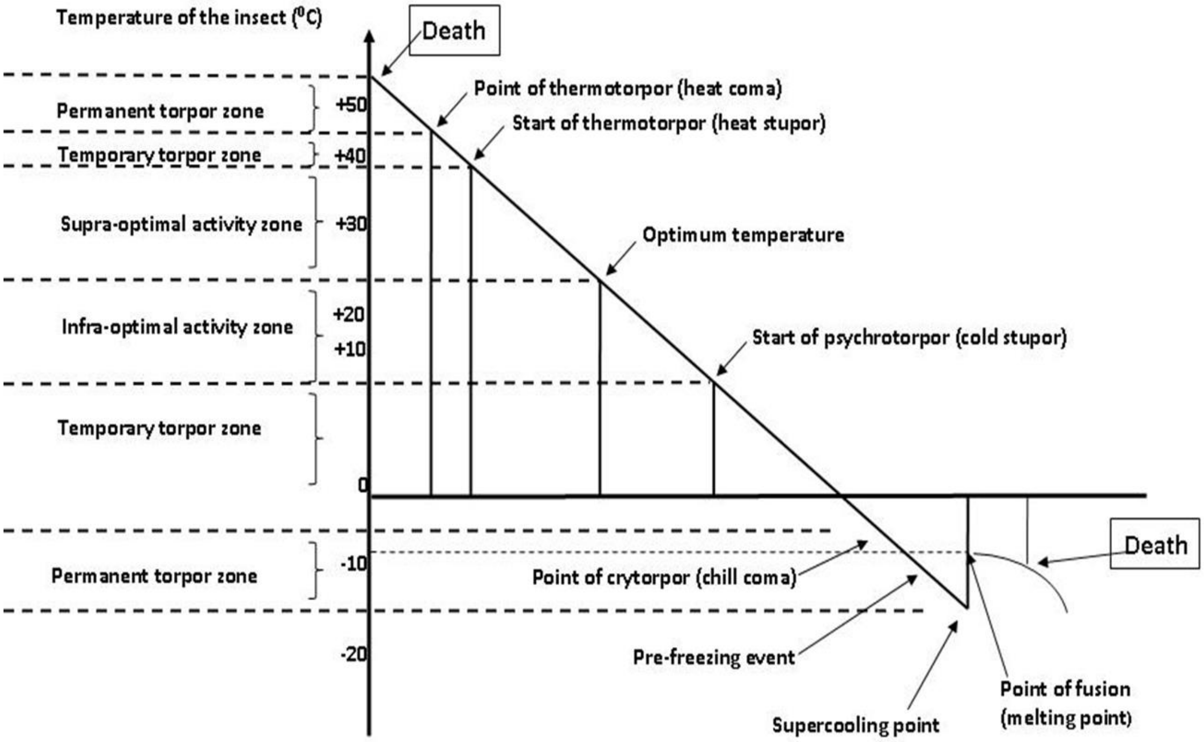

Figure 2: The thermobiological scale showing insect responses to temperature. Redrawn from Vannier (1994).

Extreme temperatures influence physiological responses in insects by affecting their life sustaining functions (Cossins and Bowler 1987). As a function of both severity and time of exposure temperature can be lethal to insect’s survivability (Cossins and Bowler 1987). Extended exposure usually results in increased mortality (Salt 1961; Asahina 1969), as a result of irreversible continued damage to tissue due to exposure to stressful conditions. At low extremities, the continued variation in temperature first results in knockdown (stupor) followed by coma, then trauma and finally death (Chown and Nicholson 2004) (Figure 1). At optimum temperature which differs among insects' species in space and time, activity and performance are at maximum allowing for enhanced survival, reproduction, growth and evolutionary fitness. Insects show a wider range of responses at both sublethal and lethal low temperatures than they do at high temperatures (Chown and Nicholson 2004). At temperatures above the optimum insects enter a supra-optimal activity zone, followed by a temporary torpor then permanent torpor and finally death. These responses are ways in which insects alter the relationship between the temperature they are experiencing and survival probability to better their chances of overcoming the adverse temperature variations they are exposed to (Chown and Nicholson 2004).

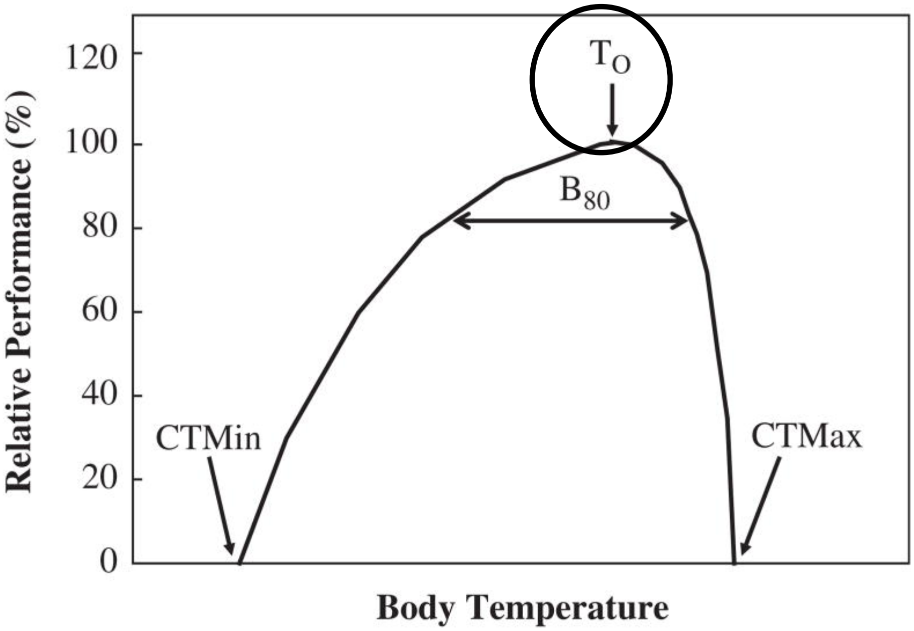

Figure 3: Relationship between body temperature and performance in ectotherms, (T_o) representing the optimum temperature for performance, (B_{80}) the 80% performance breath and CT_{min} and CT_{max}. (Redrawn from Angilletta *et al* 2002).

As illustrated in Figure 2 insects performance is bounded by their Critical thermal limits (CTLs) (CT_{min} and CT_{max}), the minimum and maximum temperature allowing insect activity respectively (Huey and Stevenson 1979). Activity in this case is defined as any activity that affects insect fitness and survival e.g. locomotion, prey finding, host searching, mate finding, mating and others. In consequence, insects at their CTLs lose components of their fitness traits

(Nyamukondiwa and Terblanche 2009) and thus deleterious to survival. The graph gradually increases from CT_{min} until optimal temperature where insects performance (development, locomotion, flight) is at maximum (T_o), then it drops rapidly until it reaches CT_{max} (Huey and Stevenson 1979). However acclimation may shift the performance curve by change in position or shape (Chown and Nicolson 2004) and this may in turn enhance the evolutionary fitness of insects for example by prolonging their flight activity under unfavorable conditions and also improve flight performance in more favorable conditions (Chown and Nicolson 2004).

1.4.2 Indirect influence of temperature on insects

Changes in temperature may result in differential responses of parasitoids and host insect populations (Coviella and Trumble 1999; Rouault *et al* 2006; Mutamiswa *et al.* 2017a). If the host population emerges earlier than the natural enemy this reduces the window of parasitism (Coviella, Trumble 1999; Rouault *et al.* 2006; Mutamiswa *et al.* 2017a), resulting in increased population of the insect pest (Coviella and Trumble 1999; Rouault *et al.* 2006). Temperature has a negative effect on plants physiology and development hence increasing their susceptibility to phytophagous and invasive insect pests ultimately increasing their population (Ayres and Lombardero 2000; Rouault *et al*. 2006; Moore and Allard 2008; Netherer and Schopf 2010). Many of the phytophagous insect pests' development are directly related to the host plant phenology that is dependent on temperature. Temperature and humidity may result in different responses of host and the insect pest hence disrupting host plant synchronization (Szujecki 1998; Bale *et al*. 2002). Higher temperature may cause a rapid development of natural enemies (Netherer and Schopf 2010) and not their hosts, thus possible asynchrony at the two trophic levels (Netherer and Schopf 2010). Furthermore, increased temperatures may also affect insect pests by disrupting their chemical responses such as in aphids, weakening the alarm pheromones which are produced during predator attacks making them susceptible to attack (Awmack *et al*. 1997; see discussions in Chidawanyika *et al.* 2012).

1.5 Relative Humidity (RH)

Environmental temperature and humidity affects the development of insects as well as evapotranspiration through insect body surface (Guarneri *et al*. 2002). Due to their small size (having high surface area to volume ratio) insects are more vulnerable to water loss (Gibbs 2002) than larger vertebrate animals. Insects' body water content is limited by their body (Willmer, 1982; Raghu *et al* 2004), hence they need to keep their water content at a certain threshold. This is in turn influenced by a number of factors including the insect's degree of cuticle permeability and behavioral osmoregulation (Willmer, 1982; Raghu *et al* 2004). Insects survival is influenced by their ability to tolerate fluctuations in RH (Romoser and Stoffolano, 1998). Relative humidity contributes to the lubrication and cuticular softness of insects, hence may prevent embryo development and hatching (Guarneri *et al.* 2002). Furthermore, RH above threshold may not be conducive for some insects, and may trigger fungal attach and fouling (Gullan and Cranston, 2005). This has a bearing on the oviposition, longevity, egg laying, egg hatchability and development (Gullan and Cranston, 2005). Dehydration occurs at low relative humidity due to loss of moisture, in eggs it leads to contraction and shrinking hence high mortality (Norhisham *et al*. 2013).

1.6 Adaptation of insects to changing climate

Insects are able to respond to change in temperature by modifying their morphology, behavior and physiology; this can take place at different time scales: seconds, hours or even days (Lutterschmidt and Hutchison 1997; Hoffmann *et al*. 2003). Insects react first to climate change by acute response by change in their metabolic rate which occurs immediately after exposure (Hochachka and Somero 2002), or chronic which occurs after long periods of exposure to temperature increase or decrease (Tattersall *et al*. 2010). In addition to acute and chronic response evolutionary adaptation can also evolve through successive generations (Tattersall *et al*. 2010).

1.6.1 Thermal plasticity adaptation

After exposure to temperature variability, insects respond through thermal phenotypic plasticity which gives insects the ability to adjust their phenotype either on short term or long term adaptation to the new environment (David 2004; Angilletta 2009; Austin and Moehring 2013). In essence phenotypic plasticity is the capacity of a genotype to exhibit a range of phenotypes in response to environmental variations and it is common amongst insects and highly adaptive (Whitman and Ananthakrishnan 2009). Phenotypic plasticity plays an important role in an insect's fitness, in a variety of changing environments leading to diverse phenotypic variation observed in nature (Scheiner, 1993; Via and Lande, 1985; Price *et al*. 2003; Fordyce, 2006). This may be achieved through acclimatization in the environment and acclimation under controlled laboratory conditions (Angilletta 2009). Phenotypic plasticity exist in two forms, it may be irreversible or reversible. The latter also known as phenotypic flexibility (Hazel 1995; Seebacher 2005) allows insects to change their physiology temporarily as an immediate response to chronic temperature changes through membrane changes or different isozymes (Hazel 1995; Baldwin and Hochachka 1970; Tattersall *et al.* 2010). Similarly, phenotypic plasticity can also be irreversible or permanent examples include increased body size in cooler temperatures (Angilletta 2009; Tattersall *et al*. 2010; Austin and Moehring 2013) and increased desiccation resistance in dry environments (Bubliy *et al*. 2012).

1.6.2 Acclimation/Hardening

A form of phenotypic plasticity that enhances the survival of insects to temperatures that are normally lethal, by pre-exposure to some form of sublethal temperatures are known as Acclimation/Hardening (Whitman and Ananthakrishnan, 2009; Bubliy *et al*. 2012), and this allows for physiological and biochemical adjustments so as to cope with thermal stress at low and high temperatures (Whitman and Ananthakrishnan, 2009; Bubliy *et al*. 2012). Short term exposures to sublethal temperatures are commonly referred to as hardening (Cossins & Bowler 1987; Chown and Nicolson 2004), this treatment often leads to reversible physiological changes (Hoffman *et al*. 2003). Acclimation on the other hand may be referred to as long term exposure to sublethal conditions resulting in both reversible and irreversible physiological changes (Hoffman *et al*. 2003). Due to variations in abiotic factors like temperature, light and humidity insects must be able to respond to these to maximize their fitness (Liefting and Ellers 2008). Phenotypic plasticity is an adaptive behavior, and evolved to increase fitness under stressful environmental conditions following preconditioning at the same stressor, synonymous to Beneficial acclimation hypothesis (BAH) (Kristensen *et al.* 2008; Angilletta 2009; Cooper *et al.* 2010), BAH is a process that is meant to benefit insects by assisting them to compensate for the effects of environmental change, henceforth acclimation gives a performance leverage in the acclimated environments than non-acclimated ones (Leroi *et al*. 1994; Huey *et al.* 1999; Deere and Chown 2006; Deere *et al*. 2006).

Some insects exhibit rapid physiological thermal responses that enable them to survive adverse thermal environments, Rapid Heat hardening (RHH) and Rapid cold Hardening (RCH) are some form of thermal responses that allow insects to survive temperature extremes (Nyamukondiwa and Terblanche 2011). Rapid cold hardening is a form of phenotypic plasticity, and is defined as the ability of insects to cope with extreme cold temperatures after brief exposure to sublethal temperatures therefore improving their survival (Sinclair and Chown, 2003), the survival is achieved at low temperatures that would otherwise be lethal (Lee *et al*. 1987; Lee and Denlinger 2010). RCH allows both freeze-tolerant and freeze-intolerant insects to survive potentially low temperatures (Kelty and Lee 1999).

Short periods of exposure to non-lethal but warm temperatures tend to increase subsequent temperature resistance (Bahrndorff *et al*. 2009; Fischer *et al*. 2010; Sobek *et al*. 2011),The effects of hardening maybe enhanced by repeated pre-exposure to non-lethal temperatures (Krebs and Loeschcke 1994) therefore increasing the insects ability to temperatures that would be deleterious to them on normal circumstances, this form of thermal response is known as RHH, much of this response is mediated by heat-shock proteins that are up-regulated during pre-exposure (Dahlgaard *et al*. 1998; Benoit *et al*. 2011). Indeed improvement in temperature tolerance have been reported in several studies among Drosophila species (Nyamukondiwa and Terblanche 2010; Nyamukondiwa *et al*. 2011; reviewed in Chown and Nicolson 2004; Lee and Denlinger 2010).

1.6.3 Evolutionary adaptation

Insects under climate change tend to avoid deleterious environmental consequences through plastic responses and evolutionary adaptation (Williams *et al.* 2008), by either shifting their geographical distribution timing and reproduction thus altering the composition of the natural habitat (Bale *et al.* 2002). The chances of evolutionary changes are high in insects as they grow quickly, have high reproductive rates and have short generation time (Parmesan 2006). Evolutionary adaptation may be rapid in both invasive species in new environments and native species (Hendry *et al.* 2008). Evolutionary change may be an important adaptation strategy for insects (Whitney *et al.* 2008), that gives insects a potential leverage and ability to conquer unfavorable novel environments (Bell and Collins 2008). Climate change will alter the selection pressures within a particular population (Stillman 2002), because most insect population are adapted to their local environment to a certain degree (Mendez 2007), hence traits that convene survivability to the existing environment might not be successful in the new environment and evolutionary responses might take place (Mendez 2007). Climate change is also expected to shift insect’s distribution from unsuitable unstable environments and moving into new climatic areas by range expansion at the cooler altitudinal and latitudinal limits (Hughes 2000). Phenological changes also play a vital role in insect's adaptation to climate change (Root *et al*. 2003, Root and Hughes 2005) and as a result of temperature increase insects will advance their developmental phases becoming adults earlier and also increasing their length of flight period, as in butterflies which have been reported to increase their flight period by 2-10 days for every $1^{o}C$ increase in temperature (Roy and Sparks 2000).

1.7 History and geography of *Bactocera dorsalis*

Africa is home to several native economically important and damaging fruit flies (Lux *et al*. 2003). The results of several surveys in the Eastern and Southern Africa (ESA) countries show that mango is attacked by many fruit flies species such as among others *C. cosyra, C. rosa, and C. anonae* (Lux *et al*. 2003). On cucurbits several native Dacus species (*D. bivittatus, D. lounsburyi, D. ciliatus, D. puntatifrons, D. frontalis, D. vertebratus* also cause considerable damage to crops (White and Elson-Harris, 1992; De Meyer et al., 2002; Ekesi and Billah, 2007). Although Africa is known worldwide to be the origin of several fruit fly establishments and introductions, the continent has also become vulnerable to novel invasive species. For example, the recent invasion of *Bactocera zonata* in Egypt (De Meyer *et al*. 2007) *B. invadens* now described as *B. dorsalis* in Africa (Drew *et al*. 2005), and *B. latifrons* Tanzania (Mwatawala *et al*. 2007). Amongst all the native and exotic fruit flies known, *B. dorsalis* is thought to be responsible for causing major economic losses ever since it was detected in Africa (French 2005). Nevertheless, this economic damage varies in space, as other areas are not particularly vulnerable to the insect species.

The oriental fruit fly, *B. dorsalis* Hendel (Diptera: Tephritidae) is an economically significant and invasive fruit fly native to Asia (Drew *et al*. 2008). Since it was first detected in Kenya 2003 (Lux *et al*. 2003) and in Tanzania (2003) (Mwatawala *et al*. 2004) *B. dorsalis* has been rapidly spreading throughout Africa and currently it has been reported in 28 African countries including the Comoros and Cape Verde, where it affected over 40 host species including mango. *Bactocera* is a large genus with over 400 species (White and Elson-Harris 1994). *B. dorsalis* is highly multivoltine and polyphagous, (White and Elson Harris 1994; Vayssières *et al*. 2009), and so are the other species in the *B. dorsalis* complex. Because of this reason, it has successfully dispersed to novel environments where it affected several fruit crops and thus contributing to economic losses. More so, the invasive potential for this species may also come from its ability to tolerate novel heterogeneous environments, as has been reported for other fruit fly species (e.g. Nyamukondiwa *et al.* 2010).

In 2007 Botswana began its national detection surveys of fruit flies. Traps baited with different lures were mounted in different strategic areas around the country to detect fruit fly species occurring in the country. *Bactocera dorsalis* was first detected in 2010 in the Chobe district and by 2013 it had been detected in other two districts (North east and the central district). Following its detection a delimiting survey to measure the extent of its spread was carried out in the affected districts. Quarantine of affected areas, field/orchard sanitation, supervised inspection of host material and chemical control using male annihilation technique (MAT) were carried out in order to manage the pest (Pers comm MoA). Nevertheless, since then, no work has been done, to check its extent of spread south of Botswana, and the potential for it thriving elsewhere within the country, based on differences in biotic/abiotic factors in space.

1.8 Rationale of the study

Agriculture played a significant role in the economy of Botswana until the 1967 when diamonds were discovered (CSO 2008). Since then the contribution of the agricultural sector to the Gross Domestic Product (GDP) drastically declined (Seleka 1999). Despite the farming industry's declining share of the GDP, it still remains vitally important in the economy (MoA 1996). The horticultural sector faces many challenges with the main ones being weak farmer associations, poor agricultural infrastructure, poor management practices and insect pests and pathogen management (MoA 1996). One of the recent devastating insect pests is the fruit fly particularly *B. dorsalis.* Outbreaks of this fruit fly has resulted in direct losses with females laying eggs in the skin of fruits, the infested fruits and vegetables quickly rot and fall to the ground and becoming inedible. The direct damage caused by *B. dorsalis* threatens the livelihood of families that depend upon selling fruits. Furthermore, it also has major impacts on food security and income of several countries. For example Botswana was funded up to US$219,000 in 2014 for the management of this fruit fly (MoA 2014). Besides the direct damage indirect losses is associated with quarantine restrictions because infestation or mere presence of these flies in a country could also restrict free trade and export of horticultural products in lucrative markets abroad, hence rejection of European markets is on the increase to any African country where *B. dorsalis* has been detected. For example in 2008 Kenya lost US$1.9 million due to *B. dorsalis* quarantine restrictions imposed by South-Africa on avocado export. Similarly, South Africa has also closed its market to Mozambique on the export of bananas and mangoes due to the presence of *B. dorsalis* (Cugala *et al.* 2009).

To our knowledge, most research on *B. dorsalis* focused on cold tolerance/pretreatment meant for postharvest disinfestation for export produce (Grout *et al*. 2011). Nevertheless, knowledge of thermal biology is highly critical in explaining the observed and future trends in the distribution of invasive insect species and development of phytosanitary measures. Basic thermal biology data and its plasticity can help explain some of the observed trends in the current distribution of *B. dorsalis*. This involves determining critical thermal limits to activity (CTLs), which are the temperatures determining functional ranges for key life history traits e.g. locomotion, mate finding, host searching and others (Nyamukondiwa and Terblanche 2009). lethal temperatures (upper lethal and lower lethal), which are the temperatures required to kill a certain proportion of the population in a specified time period (Cossins and Bowler, 1987). Critical Thermal Limits are an ideal standard of evaluating insects' thermal requirements and physiology (Lutterschmidt and Hutchison, 1997). They can be measured easily and rapidly in the laboratory (Chown and Nicolson, 2004) and are typically evaluated using simple dynamic (changing temperature at constant rate) or static (temperature held constant while duration varies or vice-versa) methods (Lutterschmidt and Hutchison, 1997).

Insect population dynamics are governed by several biotic (living entities) and abiotic (non-living entities) factors and their interactions (Walner 1987). Understanding these factors especially for pests of economic concern is important in coming up with management strategies and policies (Baskauf 2003). Therefore knowledge of *B. dorsalis* basal thermal biology and its plasticity to low and high temperature thereof, may be critical in explaining *B. dorsalis* invasive capacity, since temperature tolerance, may be one of the many geographic and environmental variables/barriers that an animal has to go through before becoming established and consequently invasive upon introduction into a novel habitat. This research was aimed at (1) establishing the current distribution of *B. dorsalis* in Botswana as a way of mapping out the population dynamics and the extent of its spread south since introduction north of the country in 2010 and (2) assess *B. dorsalis* thermal tolerance and explain how these traits of thermal biology likely shape population dynamics and biogeography of this invasive and economic fruit fly in Botswana. I hypothesise that *B. dorsalis* did not spread south following its introduction north of Botswana and that temperature tolerance may not impact on population dynamics and biogeography of *B. dorsalis.* This information may be useful in phytosanitary regulations which have implications on plant and plant product movements, prediction models forecasting of pest incidence outbreaks and may help in insect pest risk analysis.

1.9 References

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CHAPTER 2

Seasonal population phenology and distribution of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) in Botswana

2.1 Introduction

Insect populations fluctuate as a result of varying effects of both intrinsic and extrinsic factors (Price 1997). This is because there is synchrony between seasonal patterns of insect abundance with availability and quality of resources which vary temporary with the climate (Wolda 1978; Bale *et al.* 2002) and in time and space. Maintaining the appropriate seasonality is a basic ecological requirement for all organisms, as critical life history events must be synchronized to appropriate seasonal cycles to avoid lethal temperatures or other environmental extremes (Menzel and Fabian 1999). With the ongoing global warming associated with climate change, the close association between environmental and body temperature of ectotherms means that their abundance and distribution will be greatly affected, (Parmesan and Yohe 2003; Colwell *et al*. 2008; Chown *et al*. 2010). The seasonal effects of climatic changes will directly lead to modifications in insect development and dispersal, involving the alteration of developmental rates, voltinism and survival of insects subsequently acting upon size, density and extent of host plant exploitation (Bale *et al*. 2002).

Insects' abundance can change over time due to a variety of reasons including changes in macro- and microclimatic environments (Wolda, 1988; Pateman *et al*. 2015). Tephritid abundance and distribution are notably dependent on several abiotic factors (temperature, relative humidity and rainfall) and biotic factors (host plants and natural enemies) (Vayssières *et al*. 2008). Amongst these factors temperature is the most dominant abiotic factor that directly affects insects' development, range survival and abundance (Aluja *et al*. 2012). Insects therefore have to cope physiologically or compensate behaviorally to differences in temperature, at various temporal and spatial scales (e.g. Huey and Pascual 2009; reviewed in Chown and Nicolson 2004; Denlinger and Lee 2010). Physiologically insects have developed mechanisms like diapause (hibernation or state of arrested development) or sensitivity to photoperiod to re-set and to maintain the seasonal clock (Danks 1987). At high temperatures, insects are able to perform faster and efficiently, for example, they can develop, feed, reproduce and disperse in warm climates although their life span is short-lived (Drake, 1994). For many insects, lower temperatures associated with winter are thought to lower insects growth rates, either through reduction in development rate, suppression in feeding, activity and mating (Chown and Nicolson 2004; Bale 2010). In temperate climates for example fruit flies are seasonal in abundance and multivoltine species such as *Ceratitis capitata* increase their population up to a peak in summer and early autumn then decline rapidly in winter (Rodonjic *et al*. 2013). This timing of population peaks and dips ought to be known, as they dictate when control measures ought to me prioritized, thus optimizes control.

Although climate has a great influence on the seasonal abundance and distribution of insects, many other factors such as interspecific competition, parasitism, predation and distribution of resources at a particular time of the year appear to act together with climate to mold the distribution and abundance of insects (da Silva *et al*. 2011). Adult emergence may vary from one location to the other because even though locations may be geographically close to each other, they may also be subjected to different microclimatic conditions (Randall, 1982). In a study conducted by Ekesi *et al.* (2006) on field infestation of *Bactrocera invadens* in Kenya, there was a clear indication that there was a high level of infestation at low elevation signifying that *B. invadens* was well adapted to warmer climates. In southern Africa although feral fruits and vegetables are important sources of supplementary nutrition and acting as a source of income (Mausse and Bandeira 2007), they also act as alternative host for economically important fruit fly pests. In Tanzania for example the presence of *B. dorsalis* is linked with the presence of guavas and mangoes (Copeland *et al*. 2006; Mwatawala *et al*. 2006). *Bactrocera dorsalis* has a wide host range across 28 families, comprising both wild and cultivated fruits. The abundance and distribution of *B. dorsalis* acceptable resources determines their availability in space and time (Courtney, 1985, 1986, Bozer *et al*. 1996, Eggert and Wallace 2003). Resources are typically unevenly distributed in space and time therefore this requires that organisms be present at the time and place when resources will be most available (Courtney, 1985, 1986, Bozer *et al*. 1996, Eggert and Wallace 2003). Most insects overcome this by (i) diapausing during periods of unfavorable temperature conditions or (2) migrating to more favorable environmental conditions (both biotic [food, shelter] and abiotic [temperature and relative humidity]). As a consequence, insects follow some natural cycles, where life histories of many species are synchronized with periods when host resources and nutritional value are most available (Fenny 1970, Valey and Grawdwell 1970, Lawrence *et al*. 1997).

The association between environmental temperature, physiological processes and population dynamics plays an important role in insects’ ecology, evolution and pest management (Bale 2010; Hill *et al*. 2011; Sherriff *et al*. 2011). An understanding of environmental temperature and thermal tolerance under field conditions is important in forecasting insect outbreaks and predicting insect abundance variations and subsequent crop damage (e.g. Sherriff *et al*. 2011; reviewed in Bale 2010). In addition thermal thresholds have been used to predict population dynamics of insects (reviewed in Bale 2010). Here, I therefore investigate the demography of *B. dorsalis*, a pest native to Asia and destructive to many fruits and vegetables (Ekesi 2007). I specifically looked into its (1) current distribution relative to its first area of detection and (2) how climate and availability of resources likely explains its current distribution. In addition, I also investigated the relationship between temperature and abundance of *B. dorsalis* in Botswana. Since its first detection in Africa (2003) (Drew *et al*. 2005) and first report in Botswana's Chobe district in 2010 (Ministry of Agriculture Pers Comm, 2015), *B. dorsalis* remains a significant biosecurity threat to agro-based communities. Nevertheless, since 2010, no work has documented *B. dorsalis* establishment and its further invasion South of Chobe district, and Botswana at large. Such information remains critical in developing phytosanitary regulations, and may help inform pest management. The data will assist in optimization of monitoring and control techniques, in that early detection improves the chances of eradication and containment before the pest population reaches an economic injury level. In addition, it may also assist in determining areas of high infestation, hence advise policy for management.

2.2 Methods and materials

2.2.1 Surveillance

A country-wide survey to determine the distribution of *B. dorsalis* was carried out in the districts of Botswana over a period of two years (2015-2017) by placing traps (yellow chempac bucket traps) (Fig. 1) baited with Methyl eugenol pheromone lure placed to the bottom which acted as an attractant to *B. dorsalis*. Methyl eugenol is a pharapheromone used to attract males of several species from the Genus *Bactrocera* (White and Elson-Harris, 1994). The buckets were hung 1.5m above the ground on fruit trees with a minimum required trap density of 1 trap per km^2 or 1 trap per hectare. 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 also coated with Vaseline 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 any flies caught were placed in 60ml plastic vials with 70% alcohol for preservation, labeled according to location, date of collection and trap number. These were taken to the laboratory for identification using gross morphology using a stereomicroscope (Bestscope BS3060BT, Hangzhou Scopetek, Opto Electric Co, Hangzhou, China) and a fruit fly electronic identification system from the Citrus Research International (CRI South-Africa).

Figure 1: Yellow Chempac bucket traps (Chempac Pvt. Ltd., Paarl, South Africa) used in adult *Bactrocera dorsalis* trapping placed 1.5m above ground.

2.2.2 Fruit sampling

Fruits showing signs of damage within each of the sampling areas and districts were collected (Fig. 2A, B and C, Fig 5). Some fruits (over-ripe and ripe) were collected from the ground and from the trees to maximise chances of getting infestations. The cultivated fruits were collected during the fruiting seasons that is from November to March during (2015-2017). The samples were placed in a plastic container containing sterilized soil to allow for pupation (Fig. 2D). Samples were then incubated in the lab at 28°C, 65% RH and photoperiod maintained at L12: D12 in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) (Fig. 2E). The samples were regularly checked for any fly eclosion of the *B. dorsalis*. Emerging fruit flies were collected, identified and preserved in 70% alcohol and stored in 60ml vials.

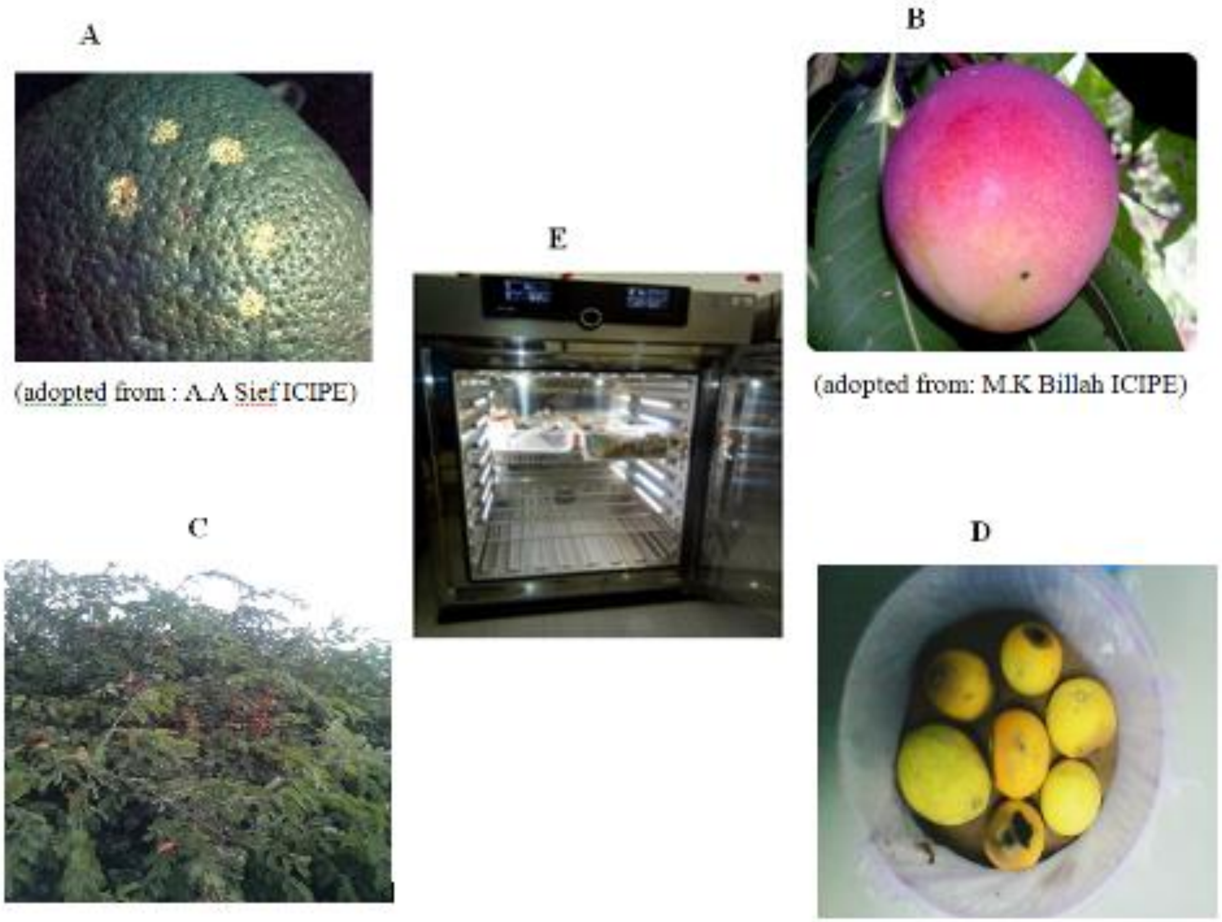

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Figure 2: Fruits with signs of fruitfly damage, and incubation of sampled fruits in a climate chamber (E). (A) Egg laying marks by fruit flies on an orange fruit following oviposition, (B) Mango fruit fly damage, (C) *Capparis tomentosa* with fruits showing signs of fruit fly damage. (D) fruit samples stored in a plastic container with sterilized soil and (E) climate chamber to allow incubation of the sampled fruits.

2.2.3 Microclimatic data

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 Thermocron iButtons (Dallas Semiconductors, Model DS1920) (0.5°C accuracy; 1 h 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 fluctuating temperatures on seasonal *B. dorsalis* population abundance using Origin Pro 8.

2.3 Results

2.3.1 Field seasonal monitoring of Bactrocera dorsalis

Apart from, Chobe district (first detection district) represented in red (Fig. 3A), *B. dorsalis* was found in four other districts south of the country (Fig. 3B). However, there were variations in abundance in these districts, with Chobe, Ngamiland and Central (represented in red) showing all year round consistent population whilst Kgatleng, Kweneng, Southern and South-East districts (represented in yellow) indicated sporadic introductions. Kgalagadi and Ghanzi districts (represented in white) indicated absence of *B. dorsalis* (Fig. 3B).

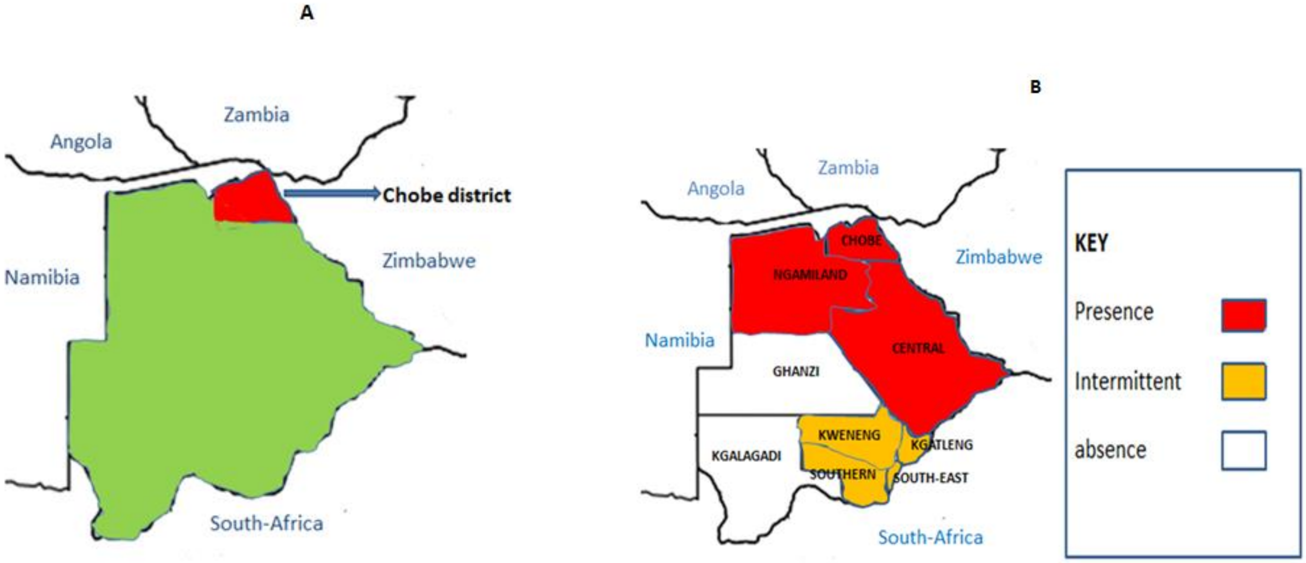
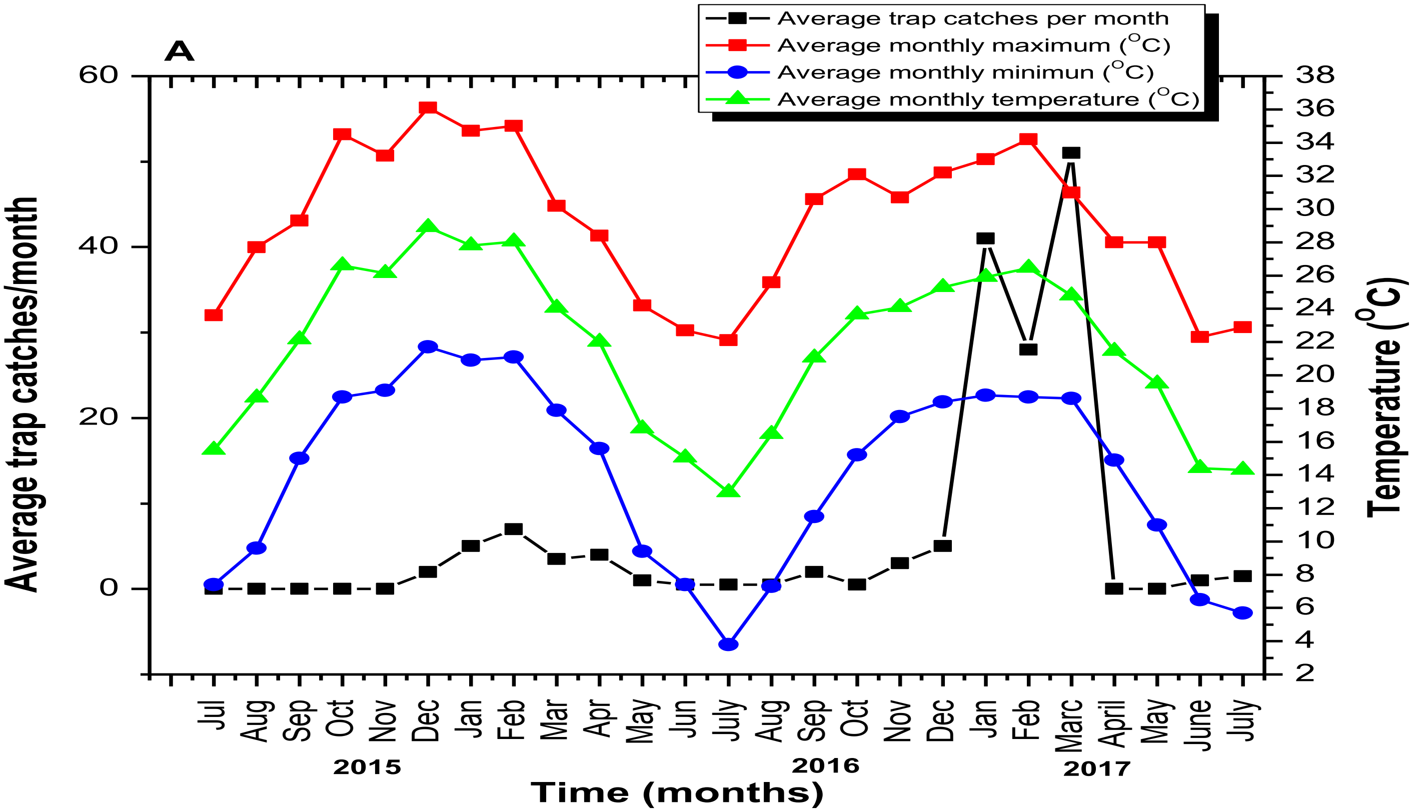


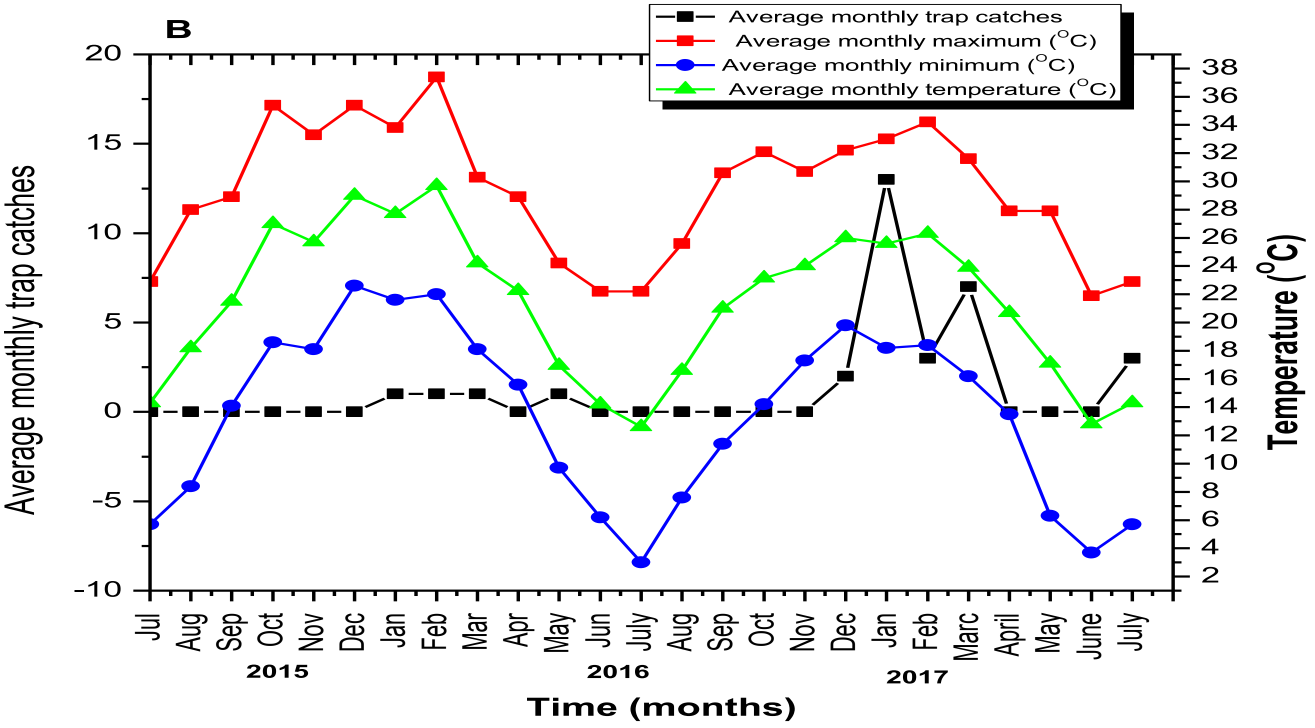
Figure 3: Maps of Botswana (A and B) showing the demography of *B. dorsalis*. (A) shows the area on first invasion by *B. dorsalis* (Chobe district, in red). And (B) showing the current state of *B. dorsalis* distribution, with red symbolising presence (with all year round distribution, yellow represents intermittent presence, while white indicates absence.

2.3.1 Seasonal population size

The seasonal abundance of *B. dorsalis* increased with increasing temperatures, and conversely decreased with decreasing temperature in all the districts where *B. dorsalis* was recorded *vis a vis* Kweneng, Kgatleng, Southern, South-East, Chobe, Central, Ngamiland and North-East. Districts Chobe, Central, Ngamiland and North-East had all year round persistent populations while

Kweneng, Kgatleng, Southern, South-East showed sporadic and not so persistent populations around the year (Fig. 4). From November trap catches started increasing, peaking around January (coincident with high temperatures), February and March and decreasing from the onset of winter April/May, again, the dip coinciding with low temperatures and consequent prolonged developmental time.

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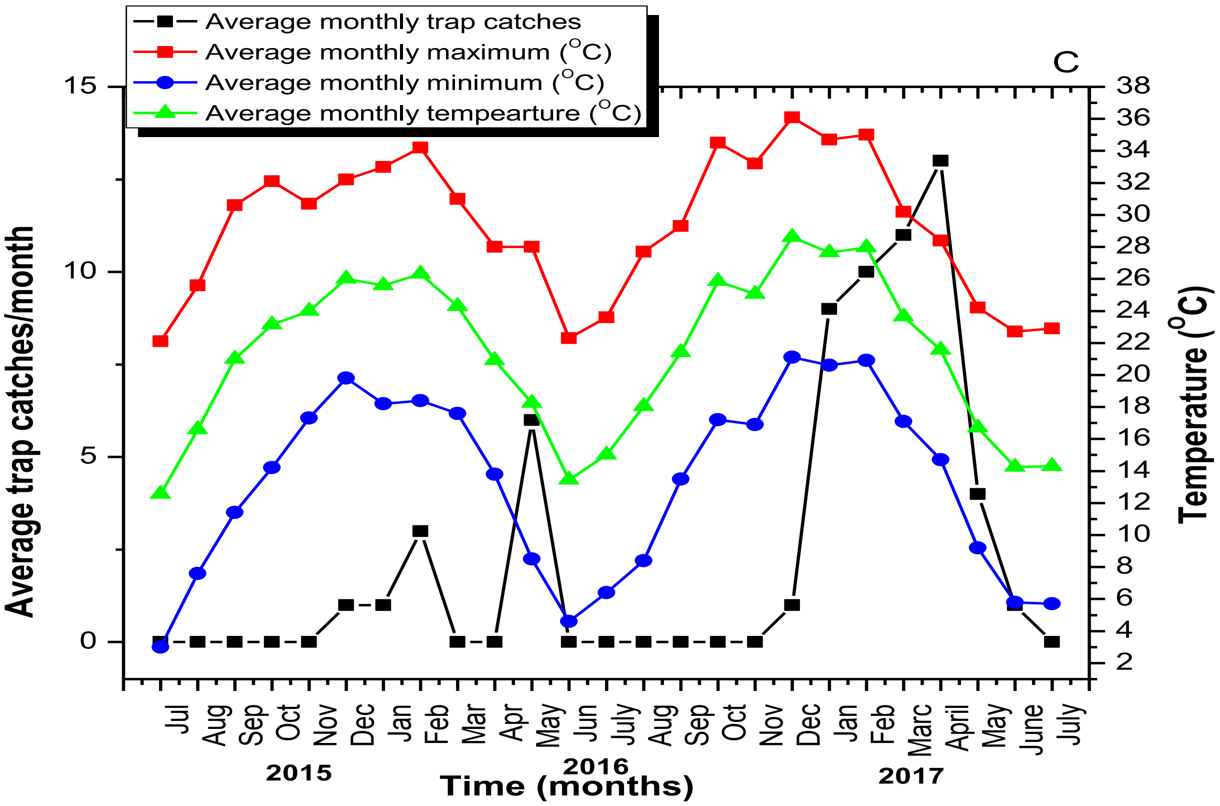
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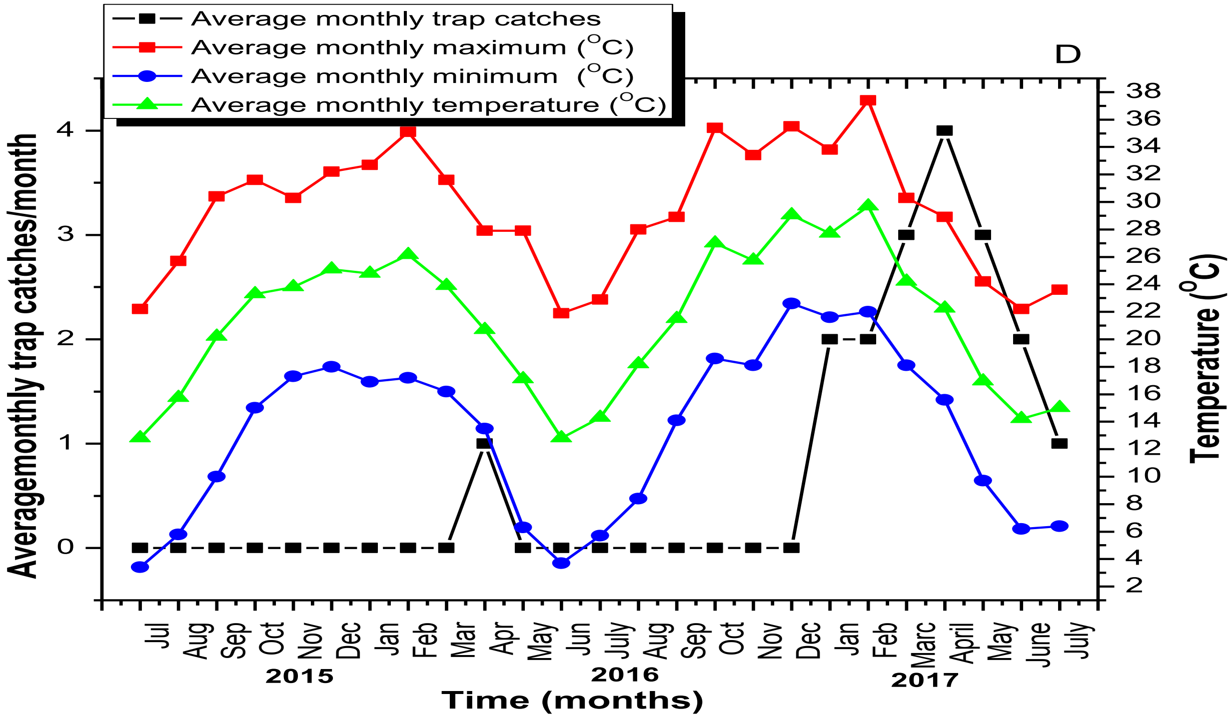
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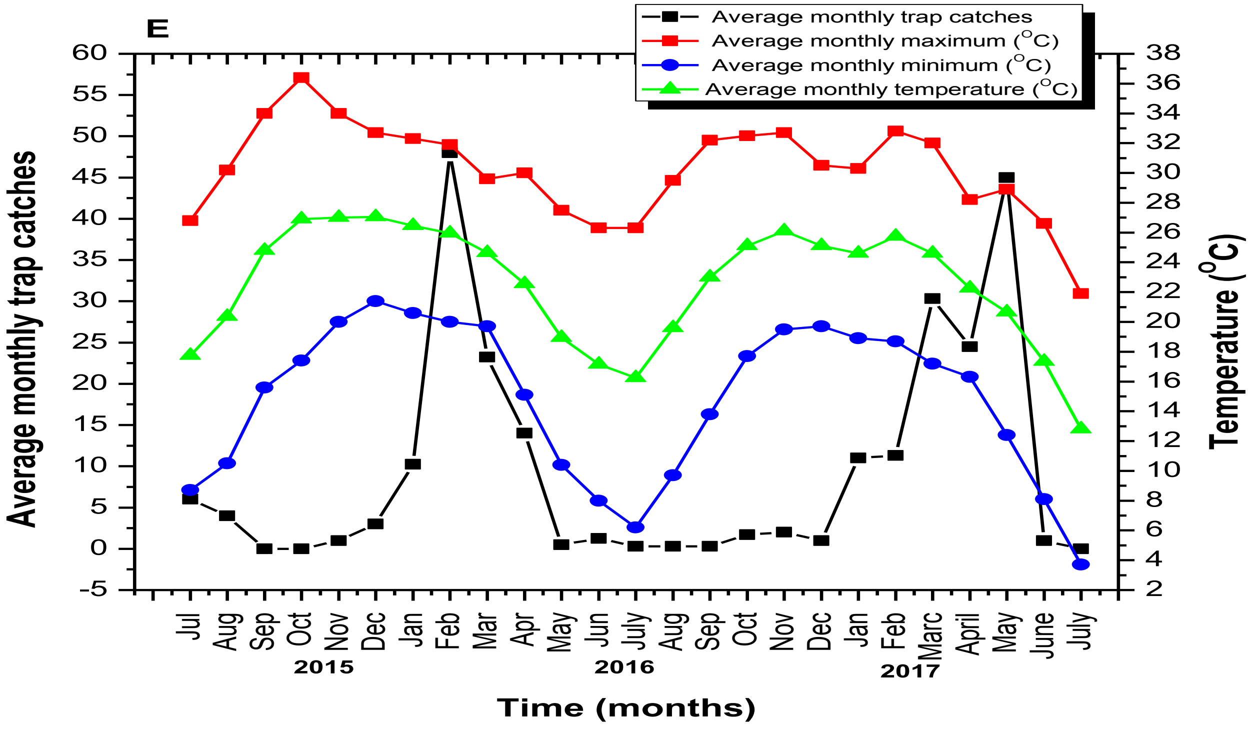
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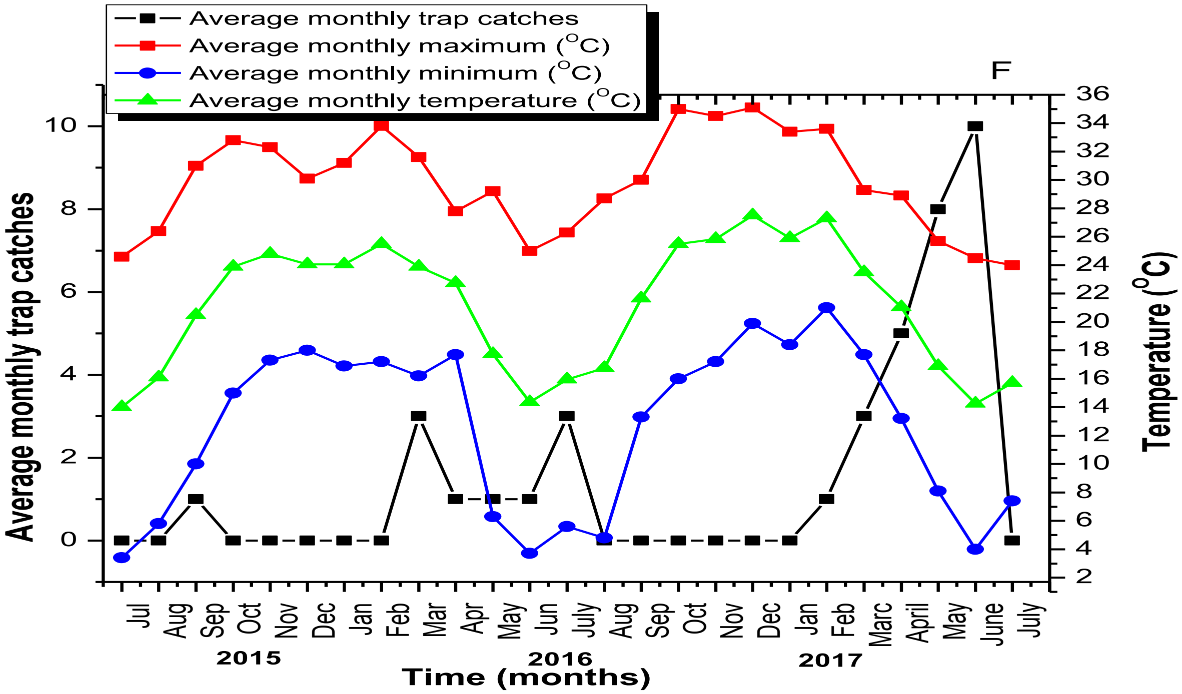
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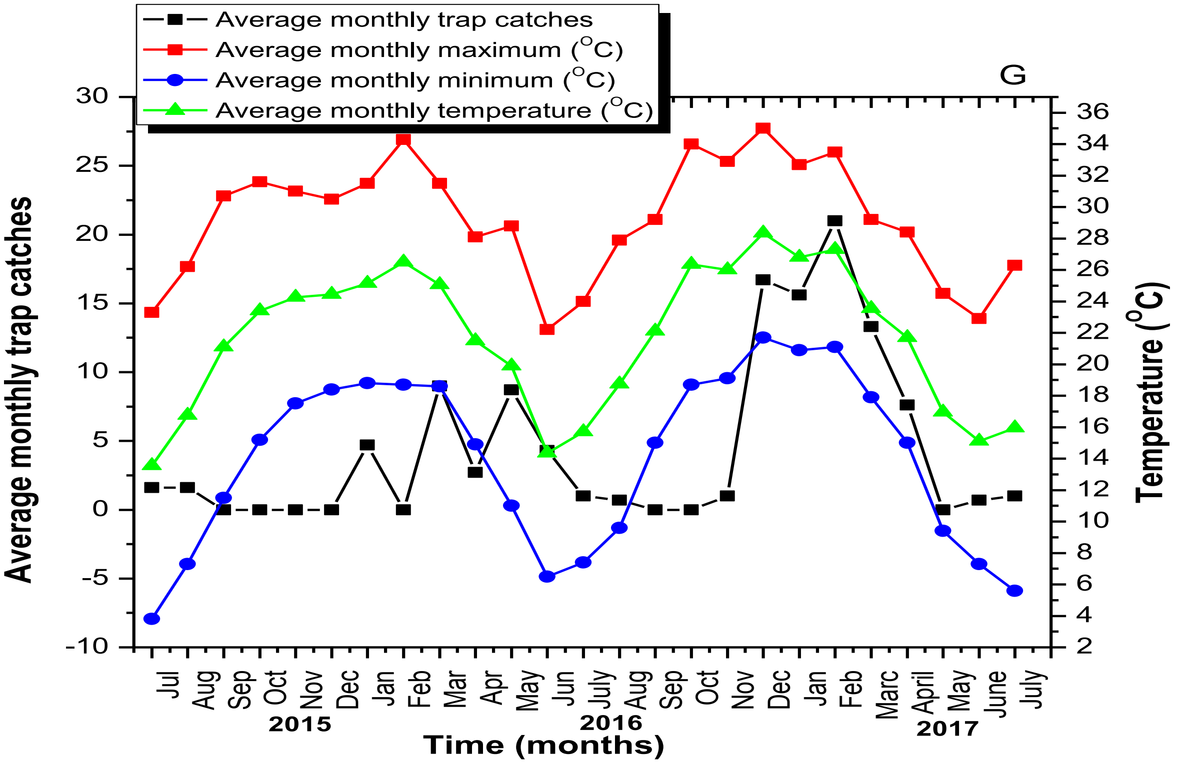
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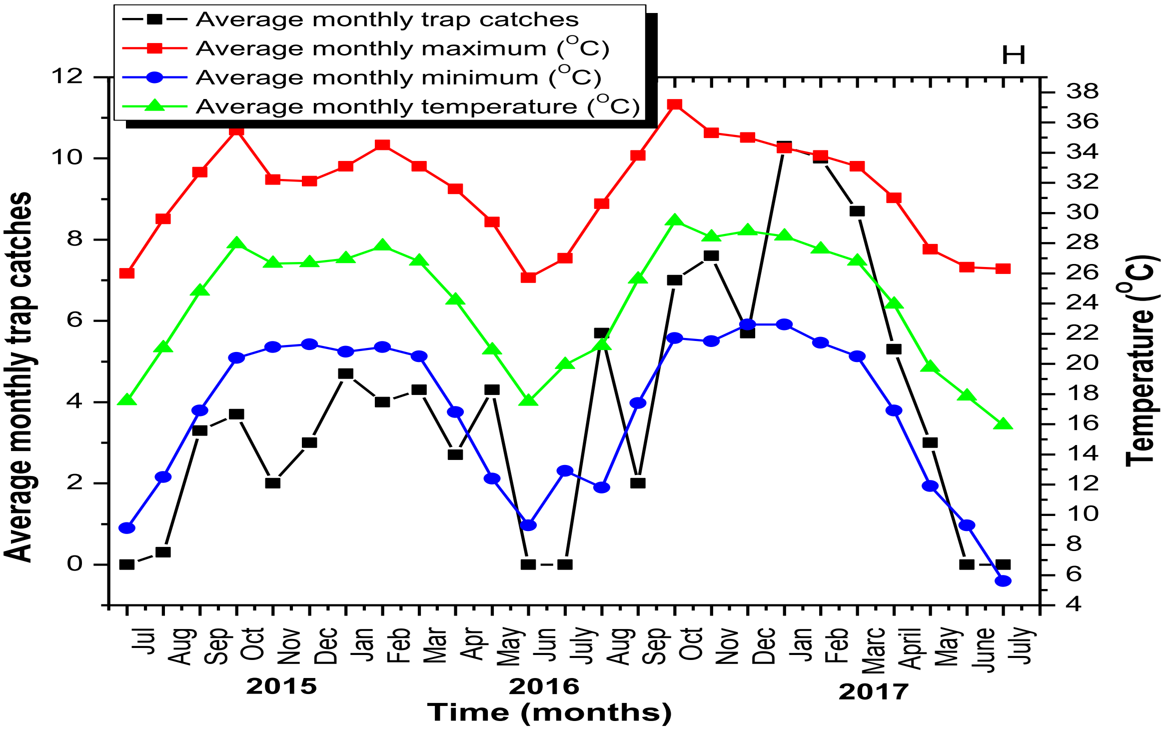

Figure 4**:** Seasonal population phenology of *B. dorsalis* across eight different districts Kweneng (A), Kgatleng (B), Southern (C), South-East (D), Chobe (E), Central (F), Ngamiland (G) and North-East (H), as a function of average maximum, average monthly and average minimum temperatures during 2015-2017. Flies were baited using a pheromone lure in yellow chempac bucket traps (Fig. 1) and the traps were serviced after every four weeks, and the average trap catches calculated as catch/trap/month.

2.3.2 Fruit sampling and fruit fly percentage composition

From the sampled fruits, four different fruit flies were identified namely *B. dorsalis*, *C. rosa, C. cosyra* and *C. capitata* (Table 1). The flies were collected from the mango, orange and guava fruits in different districts that were sampled. *B. dorsalis* was the most dominant fly infesting mango with 76.5% infestation in Chobe, 65.2% in Ngamiland, 100% in Central, Kgatleng, North-east, and Southern having the lowest percentage infestation in Kweneng followed by *C. cosyra* with 17.7% in Chobe and 43.5% in Ngamiland.

Table 1: Percentage composition of fruit flies that emerged from sampled fruits (mangoes, oranges and guavas) in different districts (Chobe, Ngamiland, Central, North-east, Kgatleng, Kweneng, Southern, South-east) during the fruiting seasons (November-March) 2015-2017.

Location	**fruits**	**Total number of Fruit flies**	**% emergence**			
			B. dorsalis	*C. rosa*	*C. capitata*	*C. cosyra*
Chobe	Mango	17	76.5	0	5.9	17.7
	Oranges	14	64.3	35.7	0	0
Ngamiland	Mango	23	65.2	0	30.4	43.5
	Oranges	8	0	0	37.5	62.5
Central	Mango	9	100	0	0	0
	Oranges	5	20	0	0	80
North-East	Mango	3	100	0	0	0
Kgatleng	Mango	3	100	0	0	0
	Oranges	2	0	100	0	0
Southern	Mango	1	100	0	0	0
	Guava	2	100	0	0	0
Kweneng	Oranges	26	26.9	53.8	11.5	0
	Guava	22	50	0	9.09	40.9

Figure 5: *Capparis tomentosa* a wild fruit in the Chobe district showing signs of fruit-fly damage.

2.4 Discussion

The link between environmental temperature, physiological processes, and population fluctuations is a significant aspect in insect pest management (Nyamukondiwa *et al*. 2013), since climate is one of the determining factors of insect population fluctuations and or seasonal patterns (Torres and Madi-Ravazzi 2006). Climate change may influence the biology of insects particularly ectotherms (Deutsch *et al*. 2008), hence insects may respond to climate change in different ways such as shift in distribution (Dillon *et al*. 2010). This may be achieved by adaptation, physiologically or behaviorally otherwise insects will face extinction (Bradshaw and Holzapfel 2001; Calosi *et al*. 2008; Chevin *et al*. 2010; reviewed in Chown and Nicolson 2004; Angilletta 2009). Low temperatures associated with winter generally affect insects population growth rates through suppression of development and activity (Chown and Nicolson, 2004; Bale, 2010). Insects can therefore deal with winter conditions by either behavioral or physiological compensation arrested development e.g diapause (Delinger and Lee 2010). Knowledge on overwintering biology especially physiological responses is important especially in assessing insect population dynamics (Bale 2010). Furthermore, it can inform the overwintering insect stage, and potential numbers thus may help targeted control, and optimization of pest management. *Bactrocera dorsalis* was detected in all the districts in which the traps were placed. However, there were variations in abundance, with some districts such as Chobe, Ngamiland, North-East and Central showing activity all year round with seasonal fluctuations in abundance. These areas showed an increase in abundance from November and a decline in April/May (months associated with winter) (Fig. 4E), this trend corresponds to the microclimatic data revealing that temperatures increases from the onset of October/November and decrease rapidly from March/April (Fig. 4A-H). The large quantities of fruits left in orchards and irregular control methods may have contributed to increased population growth observed during those months. This indicated that *B. dorsalis* was thriving in these new invasion areas, as suggested by consistent detections all year round. This also suggests year round breeding since the temperatures recorded were favorable enough, even during winter, to maintain continuous fruit fly development, abeit slowly. These areas also showed a positive correlation between the observed population (fruit flies caught per trap/month) and average minimum and maximum temperature. The impact of abiotic factors on population dynamics was also noted by Mahomood *et al* (2002), who indicated that mean maximum and minimum temperatures have positive and highly significant correlation with the number of fruit flies per trap. Similar findings were also noted on the population dynamics of fruit flies of cucurbits (Ganie *et al*. 2013)., suggesting temperature may be the main determinant for *B. dorsalis* abundance in agroecosystems. Insects are ectotherms and they experience non-constant temperatures in nature (Chen *et al*. 2014). As such, their physiology and ecology are strongly dependent on ambient temperature, critically affecting their population dynamics (Allen 2011). Every insect requires a consistent amount of heat accumulation (heat units) to complete development of a certain life stage (Murray 2008) such as egg hatch, larva-pupa, pupa-adult and also for activity e.g adult flight. Using *Ceratitis* fruit fly species, Nyamukondiwa *et al* (2013) reported that populations increased after sufficient degree days have been accumulated (Early spring) and by contrast populations collapsed with increasing frequency of low temperatures that fell below the critical minimum temperature of activity. This finding is indeed in keeping with the current study. The growth and development of insects are dependent on temperature as temperature varies during varying seasons, therefore pest pressure and the need for control will vary (Murray 2008). Increase in temperature increases developmental time until temperatures become high enough to reach negative effects, on the other hand the cooler the temperature the slower the rate of growth and development (Drake 1994; Chen *et al*. 2015). At low temperatures the time for maturity (rate of growth and development) requires more time, while at higher temperatures, development time is shortened (Hartley *et al*. 2010). Since insect development is temperature dependent monitoring degree day accumulation is a valuable tool in predicting insect activity, accurate insect development and emergence is essential in successful pest management (Pedigo and Rice 2006). Moreover when integrated pest management tactics are timed properly with key insect developmental stages it can minimize the need for insecticide treatment (Wilby and Thomas 2002, Herms 2004).

There were sporadic populations in other districts South of the country (Kgatleng, Kweneng, Southern and South-East) suggesting repeated introductions of parent propagules and no consistent breeding population. *Bactrocera dorsalis* was first detected in the Chobe district (northern part of the country) in 2010 following its survey in 2007, and two other districts (North-East and Central) in 2013 (Ministry of Agriculture, year Pers Comm). The detection of *B. dorsalis* down South of the country may also suggest the spread and establishment of the fly from the North to the South of the country. The shift may be due to the current continued invasion by the fly species and also the appropriateness of the respective environments with regards to both environmental conditions and hosts availability supporting *B. dorsalis* survival. The results however revealed low abundance in the areas south of the country and this may be due to low vegetable and fruit production in these areas. The population dynamics of insects can be influenced by climate, vegetation and their interactions (Wolda 1978; Marinoni and Ganho 2003; Kittelson 2004; Torres and Madi-Ravazzi 2006). Food resources differ in space and time thereby affecting insects' population dynamics (Morais *et al.* 1999; Kittelson 2004; Bispo *et al.* 2006; Bispo and Oliveira 2007; Goldsmith 2007). The high abundance of *B. dorsalis* in the north region of the country may be due to the observed high fruit production in these areas.

Bactrocera dorsalis was not found in the Kgalagadi districts since this area is a too dry, desert-like and associated with low rainfall, high temperatures as well as low vegetation. Because of this, this area is generally not suitable for agricultural production, hence limiting the cultivated hosts for the fly species. For insects, extreme temperatures may be a limiting factor in population dynamics, (reviewed in Terblanche *et al*. 2011, 2015), hence an understanding of when and where these constraints may persist requires knowledge on the species thermal thresholds (Bowler and Terblanche, 2008).

The fruit sampling results revealed that four different fruit fly species were recorded in the sampled districts associated with the mango, orange, and guava fruits, during the (Nov-March 2015-2017) fruiting seasons. The recorded fruit flies include the *C. capitata*, *C. cosyra*, *C. rosa* and the Asian fruitfly *B. dorsalis*. It is important to also note that in these districts the dominance of these flies differed in relation to the location, time and the type of fruit. For example, *B. dorsalis* was the most dominant and abundant fruit fly infesting the mango in comparison to other species in each area(s), although the infestation levels were low in other areas south of the country. Abiotic environmental factors such as temperature also seem to have a contributory factor to the occurrence and abundance of these fruit flies, as it appears to be a connection between fruiting seasons of mango, oranges and guava (Nov-March) and the onset of summer temperatures (October/November). This relationship further signifies the importance of temperature to insect phenology (Wolda 1978; Mausse and Bandeira 2007). The results from fruit sampling reveal that mango was mostly dominated by *B. dorsalis* in terms of percentage infestation in all the districts sampled (Table. 1), followed by *C. cosyra* e.g in Chobe district having a 76.5% *B. dorsalis* and 17.7% *C. cosyra*. The emergence of these flies from the same fruit (mango) may signify some interspecific competition (exploitative or interference) between the two species. The competition between *B. dorsalis* and *C. cosyra* has been reported in a Tanzania and Benin (Vayssieres *et al*. 2005; Mwatawala *et al*. 2006). Related competition has also been reported in Kenya. Before the detection of *B. dorsalis*, *C. cosyra* was the dominant insect pest of mango but since 2004, a shift in dominance favoring *B. dorsalis* has been observed (Rwomushana *et al*. 2009). My results also revealed that apart from mangoes and oranges (cultivated fruits) *B. dorsalis* was also recorded on wild fruits such as *C. tomentosa* (Fig. 5), suggesting that the fly may switch hosts during times when cultivated fruits are off season. The shift of hosts ensures continuity of insect life-cycle ultimately survival of that particular insect and establishment (Forbes *et al*. 2017)

In conclusion, the present results reveal that following first invasion in 2010, *B. dorsalis* has spread to almost all but two districts South of Botswana, and has become established in the districts south of the country. Furthermore, repeated introductions of parent propagules into new areas still persist, and account for the inconsistent fly population numbers further south of the country. The relationship between population abundance and temperature showed that temperature may be the main factor limiting *B. dorsalis* abundance and distribution. Nevertheless availability of host plants (fruits) also has a significant contribution. Future work should look into the optimization of monitoring and control techniques for efficacious *B. dorsalis* management in Botswana. Furthermore, release of parasitoids may also assist in controlling this pest, especially in the 'wild' and non-commercial agricultural environments. This data may inform future modelling studies for *B. dorsalis* invasion potential and population abundance.

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CHAPTER 3

Thermal biology of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae): Implications on population dynamics

3.1 Introduction

The survival of many organisms is determined by varying abiotic factors such as temperature and relative humidity. When these conditions exceed certain thresholds, the organism's fitness is impaired (Parsons 1979; Marchand and McNeil 2000; Huey and Berrigan 2001). Temperature moreover plays a key role in the life of insects through affecting their reaction rate and stability of molecules (Angilleta 2009). Consequently, this affects the insect's fitness traits, impacting directly on growth, development, reproduction, mortality and thus survival (Chown and Nicolson, 2004; Angilletta, 2009). Extreme temperatures degrade and unstabilize many of the insect's molecular components such as carbohydrates, proteins and membranes therefore insects cannot tolerate an infinite range of temperatures (Angilletta 2009). As a result it is imperative that insects remain in environments with suitable temperature otherwise their bodies might exceed the lower and upper threshold limits (Tattersall *et al.* 2011). Temperature varies in space and time (Feder *et al*. 2000; Gibbs *et al.* 2003; Huey & Pascual 2009; reviewed in Chown and Terblanche 2007) and insects deploy different responses to changes in temperature by altering their morphology, behavior and physiology (e.g. Kührt *et al*. 2006; Huey and Pascual, 2009). Behavioral adjustments act as a first line of defense and are dependent on the opportunities available on the insects' habitat (Kührt *et al*. 2006). If unfavorable temperatures persist, physiological responses are employed as critical mechanisms to ensure survival (Angilleta 2009). Temperatures which are lethal to insects are a function of both duration and severity of exposure to that particular temperature (Chown and Nicolson, 2004; Angilletta, 2009; Denlinger and Lee, 2010) Over long periods temperature affects seasonal phenology and evolutionary responses (Wallner, 1987; Selvaraj *et al*. 2013). Nevertheless, temperature at short-time scales also play an important role as it is a driver of insects population dynamics and consequently species abundance and geographic distribution over longer time scales (reviewed in Bale, 2002; Chown and Terblanche, 2007; Lee and Denlinger, 2010). Insects however can adjust the time-temperature phase space through phenotypic plasticity of thermal tolerance, and this can be achieved through pre-exposure to sub-lethal temperatures (discussed in Chown and Terblanche, 2007). A short term pre-exposure to sublethal temperatures is termed hardening (Bowler 2005; Lagerspetz 2006) while chronic longer term exposures is termed acclimation in the laboratory or acclimatization in the field (Huey and Berrigan 2001; Hoffmann *et al*. 2003). Hardening will result in reversible physiological responses while acclimation and acclimatization may result in both reversible and irreversible physiological responses (Hoffmann *et al*. 2003; Lagerspetz 2006; Chown and Terblanche 2007). There are many mechanisms that insects deploy to survive temperature extremes, for example at high temperatures insects produce heat shock proteins (HSPs) which act as molecular chaperones protecting essential proteins and key enzymes (McMillan *et al*. 2005). Heat shock proteins have also been implicated at low temperatures tolerance (e.g. Rinehart *et al*. 2007), signifying some degree of cross tolerance to multiple environmental stressors. Thus, phenotypic plasticity may alter performance of an organism through compensatory modifications of physiological function and tolerance as a result of changes in environmental conditions (Nyamukondiwa and Terblanche 2010).

At low temperatures many diapausing insects achieve increased cold tolerance through freeze avoidance using a combination of microhabitat selection and physiological alterations (Zachariassen, 1985; Duman, 2001) including the synthesis of polyols and sugars which act as cryoprotectants to lower their supercooling points (SCP), a point at which their internal body water freezes (Zachariassen, 1985; Duman, 2001). Therefore insects that are exposed to long periods of low temperatures during the commencement of overwintering or diapausing may increase the synthesis of certain polyols or sugars to lower the chances of freezing (Minder *et al* 1984; Khani *et al*. 2007). Indeed, the ability to overcome cold thermal stress is important for the success of insect population hence leading to evolutionary fitness in the wild (Loeschcke & Hoffmann 2007; Sørensen *et al*. 2009). Likewise the establishment success abundance and distribution of an invasive species is determined by the species relationship to climate variables (Sobero´n 2007; Sobero´n and Nakamura 2009). The determination of thermal tolerance is therefore a significant step in understanding the ways in which environmental variation may affect the population dynamics and fitness of an insect species (Nyamukondiwa *et al.* 2011; Ma *et al*. 2014).

The invasive Asian fruit fly *Bactocera dorsalis* Hendel (Diptera: Tephritidae) is a destructive quarantine insect pest to the fruit industry (Lux *et al*. 2003). It has showed a remarkable wide range rapid invasion throughout Africa (Drew *et al*. 2005), hence raising questions on its environmental niche breadth (De Villiers *et al*. 2015) and possible climate change responses (Hill *et al*. 2015). When fruit flies are accidentally introduced in novel environments different fitness traits such as reproduction, survival and longevity under different temperature variations are important factors to consider because they most likely form a critical part of the invasion process (Pieterse *et al*. 2016). Understanding the invasion potential, that is, where an insect pest is most likely to invade, and knowledge on its taxonomy are related questions that are vital to effective pest management (Kriticos *et al*. 2013). Here, I investigate the thermal tolerance *vis* Critical Thermal Limits (CTLs [CT_{min} and CT_{max}]), lethal temperature assays (Lower lethal temperature [LLT] and Upper lethal temperature [ULT]) and Super cooling points (SCPs) of *B. dorsalis* and assess the effects of developmental stage on thermal biology. These thermal tolerance traits are an indicator of field fitness and likely explain insect invasion potential, abundance and have direct implications on biogeographic patterns. To date, most research on *B. dorsalis* has focused on cold tolerance/pretreatment for postharvest disinfestation for export produce (Grout *et al*. 2011). Nevertheless, knowledge of thermal biology is highly critical in explaining the observed and the distribution of invasive insect species and development of phytosanitary measures and may be vital in integrated pest management.

The objective of this chapter was to determine the thermal tolerance of *B. dorsalis* in order to come up with thermal functional ranges for the fruit fly species. Specifically, I assessed the performance of *B. dorsalis* at low and high temperature CT_{min} and CT_{max}, and how these affect the *B. dorsalis* fitness. Second, I also determined the time-temperature interaction combinations which may be lethal (ULT and LLT) to the survival of *B. dorsalis* for three developmental stages (larvae, pupae and adults) as a way of determining how these affect the fitness and ultimately population dynamics. Last, I also assessed SCPs as a fitness trait that may likely explain freeze strategy across different *B. dorsalis* developmental stages. Results of this study may help explain the effect of temperature on *B. dorsalis* activity and population dynamics and may help give an insight on the role of temperature tolerance on *B. dorsalis* invasion potential.

3.2 Materials and methods

3.2.1 Insect culture

The initial colony of *B. dorsalis* was obtained from the International Centre for Insect Physiology and Ecology (ICIPE), in Kenya then cultured in the laboratory using on artificial diet as described by Gunnelo *et al* (1981). These were regularly supplemented with wild flies to prevent loss of fitness likely caused by laboratory adaptation. For adult eclosion, the pupae were reared in bugdorm cages ($240mm^3$; Bugdorm-BD43030F, Megaview Science Co., Ltd, Taiwan) in the laboratory under (12:12 L: D photoperiod; $28\pm1^{O}C$ and 65% RH) in Memmert climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany). Upon adult eclosion, flies were fed on a sugar diet, water and yeast (for protein) *ad libtum.* Thermal tolerance experiments were done using 4-7 days old adults of mixed sex, since sex appears not to have a significant effect on thermal tolerance (Nyamukondiwa and Terblanche 2009).

3.2.2 Lethal temperature assays (LTA's)

LTA's were measured using standardized plunge protocols, with necessary modifications (as in e.g. Sinclair *et al*. 2006, Terblanche *et al.* 2008) using a programmable waterbath (Systronix, Scientific Engineering (Pty) Ltd, South Africa). ULT and LLTs were determined for a range of times (from 0.5 to 4hrs). A mixture of propylene glycol and water at a ratio of (1:1) was used to enable the waterbath to operate at subzero temperatures without freezing. Ten insects were put in three replicate 60ml polypropylene vials, then the vials were placed in a 33*22 ziplock bag, for each temperature/time treatment until a range of 0-100% mortality was recorded. In order to avoid desiccation related mortality, during ULT experiments, a piece of cotton wad was moistened with distilled water and suspended from the perforated lids of the vials. Temperatures in the vials were verified using digital thermometers (Fluke 53/54IIB, Fluke Cooperation, USA), before each treatment (as in e.g. Stotter and Terblanche 2009). Following ULT and LLT treatments, 'treatment vials' containing the assayed *B. dorsalis* were placed in an incubator at 28± 1°C; 65% relative humidity for 24 hours after which 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 (Chadawanyika and Terblanche 2010).

3.2.3 Critical thermal limits (CTL's)

CTLs were assayed as outlined by Nyamukondiwa and Terblanche, 2010. *Bactrocera dorsalis* (third instar larvae and 4-7 days old adults) were individually placed in the organ pipes connected to the water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany), then constantly exposed to different rates of cooling (for CT_{min}) or heating (for CT_{max}) (at ramping rates 0.12, 0.25 and 0.50 °C min^{-1}). CTLs 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 (see Nyamukondiwa and Terblanche 2010; Mitchell and Hoffman (2010). A mixture of propylene glycol and water at a ratio of (1:1) was used to enable the waterbath to operate at subzero temperatures. A thermocouple (type K, 36 SWG) digital thermometer (connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into the organ pipe control chamber to record temperature experienced by the flies. In the current study, CTLs were defined as the temperature at which *B. dorsalis* lost coordinated muscle function and also associated with the onset of muscle spasms (as in e.g. Nyamukondiwa and Terblanche, 2010).

3.2.4 Supercooling points (SCPs)

Supercooling points (SCP) points were measured in *B. dorsalis* for the three developmental stages (larvae, pupae and adult) as outlined by Nyamukondiwa *et al*. (2013). Fifteen individuals of *B. dorsalis* in each individual developmental stage were individually placed into 0.65ml of microcentrifudge tubes. Each insect was placed in contact with the tip of a type-T copper-constantan thermocouple (762-1121, Cambridge, UK), which was inserted through the tube lid and both the insect and thermocouple held in place using a cotton wool. Thermocouples were connected to one of two 8-channel Picotech TC-08 (Pico Technology, Cambridge, UK) thermocouple interfaces and temperatures were recorded at 1s intervals using PicoLog software for windows (Pico Technology, Cambridge, UK). Experiments began by holding individual insects at 15°C for 10 mins (to allow equilibration of insect body temperatures) before ramping down at 0.5°C min^{-1} until SCPs were recorded. SCPs were defined as the lowest temperature recorded before an increase/spike in temperature, indicative of the latent heat of crystallization (see e.g. Nyamukondiwa *et al*. 2013).

3.2.5 Statistical analyses

CTLs met the linear model assumptions of constant variance and normal errors, so the effects of ramping rate on CTLs were analysed using one-way ANOVA in STATISTICA version 13 (Tulsa, OK, USA) with the dependent variable being either CT_{max} or CT_{min} and categorical predictor was the ramping rate (0.12, 0.25, 0.50 ^{O}C/min). Tukey-Kramer's *post-hoc* tests were used to separate statistically heterogeneous groups. Lethal temperature assays (LLT and ULT) and SCPs were analyzed using a Generalized Linear Model (GLM) assuming Binomial for Lethal temperature assays and Gaussian distribution for Supercooling points and a logit link function in R statistical software. Temperature-time graphs were computed in Origin Pro 8.

3.3 Results

3.3.1 Effects of ramping rates on critical thermal limits

Ramping rate and developmental stage (adult vs larvae) had significant effects on CT_{max} (Table 1; Fig. 1A). Faster ramping rate (0.50°C/min) resulted in improved CT_{max} for both developmental stages, while slower ramping rates generally compromised CT_{max} (Fig 1A). Nevertheless, interaction between developmental stage and ramping rate was not significant for CT_{max}. Similarly, ramping rate and developmental stage had significant effects on CT_{min}, but the interaction between the two was not significant (Table 1; Fig. 1B). Nevertheless, there was no significant difference in ramping rate for *B. dorsalis* adult CT_{min}. However, for larvae, the slowest ramping rate rates improved CT_{min} with the fastest rate significantly different from the other two and having a compromised CT_{min} (Fig 1B). Furthermore, larvae showed a significantly enhanced low temperature tolerance, measured as CT_{min} (at all ramping rates) (Fig. 1B) relative to the adults.

Table 1: Summary results from One-way ANOVA showing the effects of ramping rate on Critical thermal maxima and minima (CT_{max} and CT_{min}) for *B. dorsalis* larvae and adult. d.f = Degrees of freedom, SS = Sum of Squares, MS = Mean Sum of Squares.

Effect	SS	d.f	M.S	*F-value*	*P-value*
CT_{max}					
Ramping rate	151.5	2	75.7	28.97	0.032669
Developmental stage	12.2	1	12.2	4.68	<0.0001*
Ramping rate * Developmental stage	15.5	2	7.8	2.97	0.055298
CT_{min}					
Ramping rate	25.235	2	12.618	6.817	<0.001*
Developmental stage	63.948	1	63.948	34.550	<0.000*
Ramping rate * Developmental stage	8.274	2	4.137	2.235	0.111630

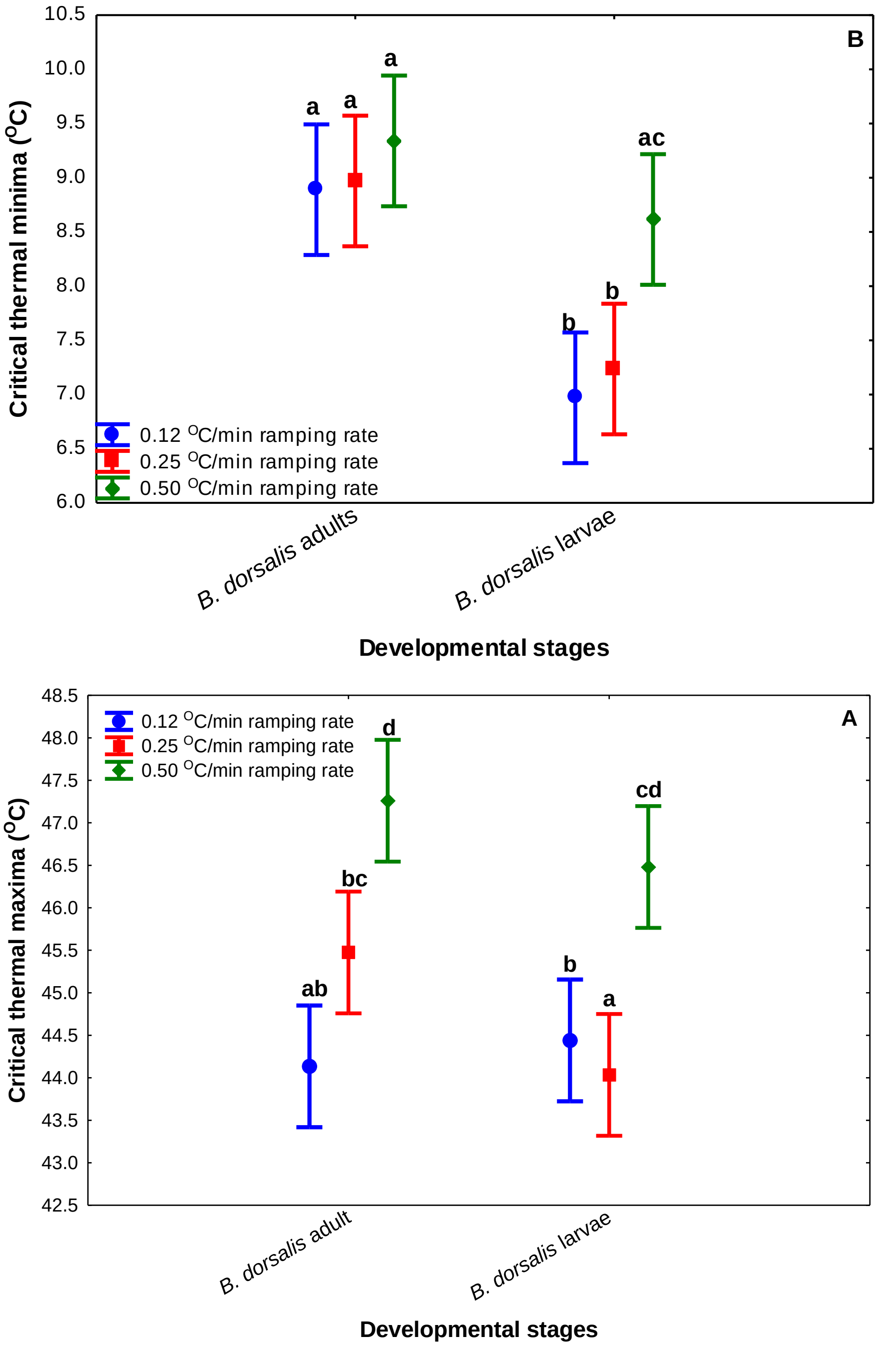


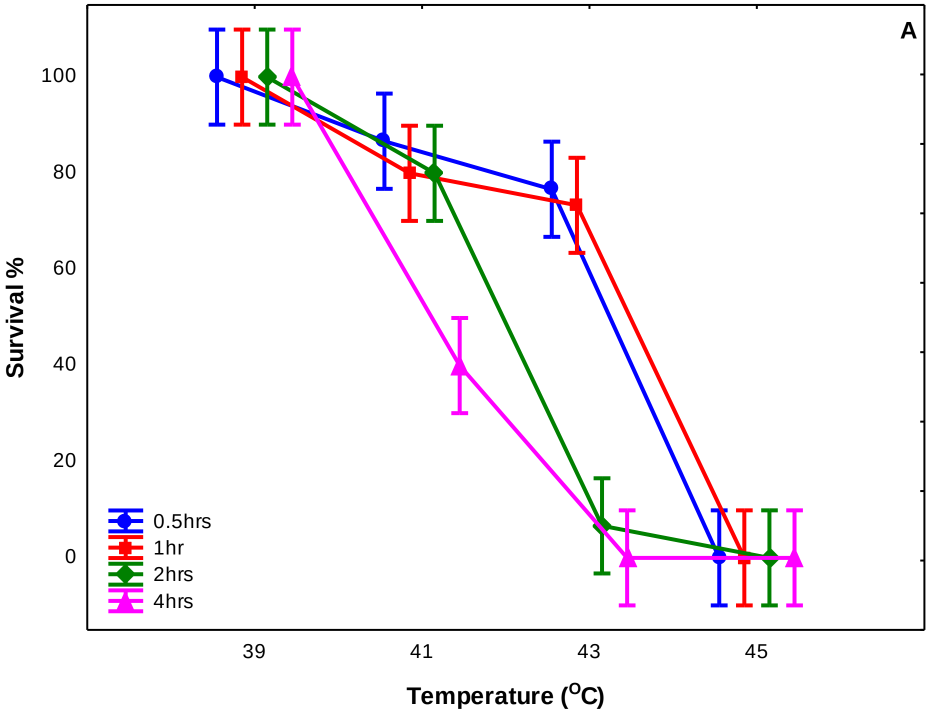
Figure 1: The effect of ramping rate (0.12, 0.25 and 0.5°C/min) and developmental stages on *B. dorsalis* (A) adult and Larvae CT_{max} and (B) Adult and larvae CT_{min}. Means with the same letter(s) are not statistically significant. Each point represents mean ±95% CL. Tukey-Kramer's *post-hoc* tests were used to separate statistically heterogeneous groups.

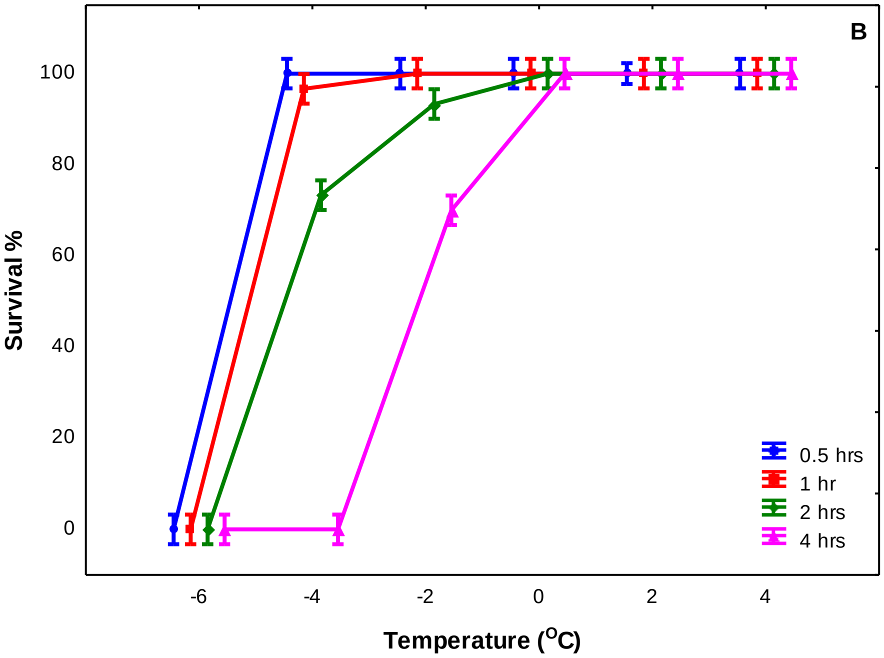
3.3.2 Lethal temperature assays

An increase in duration and severity at both high and low temperatures resulted in increased adult, pupae and larvae (Table 2; Fig 2) mortalities. For ULT 100% survival was observed at 39°C at all durations (0.5, 1hr, 2hr and 4hrs) for adults and pupae; 0% survival at 45°C for (0.5, 1 and 2hrs) but when the duration doubled (4hrs) at 43°C for adults 0% mortality was also observed. while for larvae 100% was observed at 38°C for (0.5,1,2 and 4hrs) and 0% at 41°C (0.5,1,2 and 4hrs) Time * temperature interaction effects were significant only for adults (see Table 2). Larvae had a narrower temperature range in comparison to other developmental stages. Larvae survival ranged from ULT_{100} (38°C) to ULT_0 (41°C) (Fig 2E) compared to adults ULT_{100} (39°C) to ULT_0 (45°C) (Fig 2A) and pupae ULT_{100} (39°C) to ULT_0 (45°C) (Fig 2C). Larvae were generally more vulnerable to high temperature mortality than other developmental stages (Fig. 2E). In LLT assays, time * temperature interactions were only significant for pupae ($P < 0.0001$) and not adults and larvae developmental stages. Pupae however had a broader temperature range compared to adults and larvae; pupae survival ranged from LLT_{100} (4°C) to LLT_0 (-8°C) (Fig 2D) while adults LLT_{100} (4°C) to LLT_0 (-6°C) and Larvae LLT_{100} (4°C) to LLT_0 (-4). Pupae had a generally high temperature tolerance with ULT (39°C) to LLT (-8°C) (Fig 2C, D) compared to adults ULT (39°C) to LLT (-6°C) (Fig 2A, B) and larvae ULT (38°C) to LLT (-4°C) (Fig 2E, F).

Table 2: Summary of the effects of temperature and duration of exposure on the survival of *B. dorsalis* adults, pupae and larvae. Analysis was done using Generalized Linear Models (GLZ) assuming binomial distribution with a logit link function in R-sofware 3.3.0. 3 replicates of 10 individuals were used for both high and low temperatures (ULT and LLT).

Developmental stage	**parameter**	χ^2	d.f	*P-value*
Upper lethal temperatures	Time	77.91	3	< 0.0001*
Adults	Temperature	396.14	3	< 0.0001*
	Time*temp	22.79	9	< 0.001*
Pupae	Time	8.28	3	< 0.000*
	Temperature	365.81	3	< 0.000*
	Time*temp	0.31	9	1
Larvae	Time	54.11	3	< 0.000*
	Temperature	371.72	3	< 0.000*
	Time*temp	2.33	9	< 0.9852*
Lower lethal temperatures	Time	104.44	3	< 0.0001*
Adults	Temperature	645.76	5	< 0.11*
	Time*temp	19.61	15	0.1873
Pupae	Time	11.57	3	< 0.000*
	Temperature	33.93	6	< 0.000*
	Time*temp	664.37	18	< 0.000*
Larvae	Time	104	3	< 0.0001*
	Temperature	392.84	3	< 0.0001*
	Time*temp	4.75	9	0.8555


A
Survival %
100
80
60
40
20
0
39
41
43
45
Temperature (OC)
0.5hrs
1hr
2hrs
4hrs


B
Survival %
100
80
60
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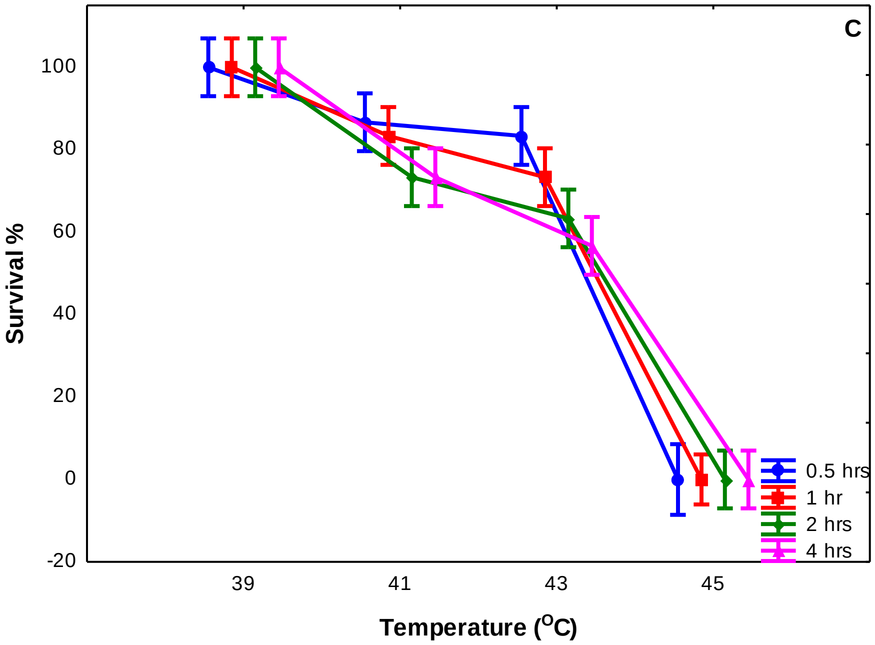
20
0
-6
-4
-2
0
2
4
Temperature (OC)
0.5 hrs
1 hr
2 hrs
4 hrs


C
Survival %
100
80
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20
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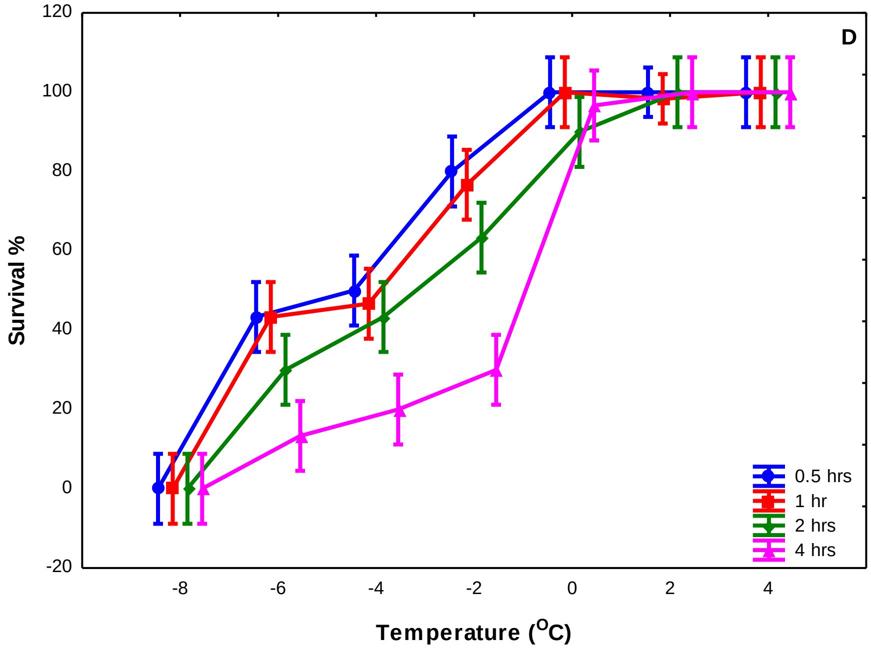
-20
39
41
43
45
Temperature (°C)
0.5 hrs
1 hr
2 hrs
4 hrs


D
Survival %
120
100
80
60
40
20
0
-20
-8
-6
-4
-2
0
2
4
Temperature (°C)
0.5 hrs
1 hr
2 hrs
4 hrs

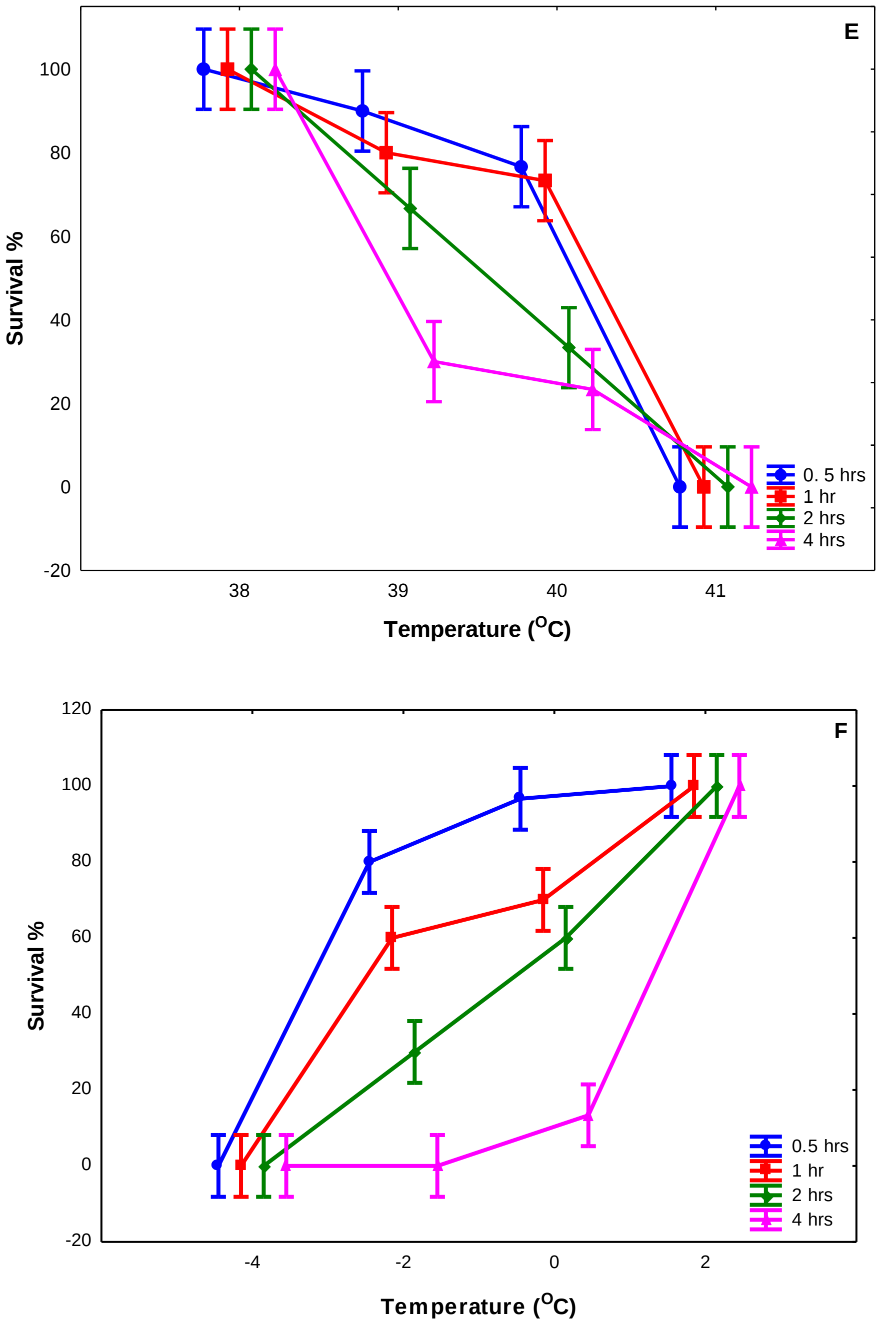


Figure 2: The effect of high and low temperatures on the survival of *B. dorsalis*, at different temperature x time (0.5 to 4hrs of exposure) interactions on Adults (A) Upper lethal temperature (B) lower lethal temperature, Pupae (C) Upper lethal temperature (D) Lower lethal temperature, Larvae (E) Upper lethal temperatures (F) Lower lethal temperature. With 3 replications, *N*=10 individuals per replicate/exposure. Error bars represent 95% CL.

3.3.3 Supercooling points

Supercooling points were significantly affected by developmental stages (Fig. 5). There was a significant difference between adults and larvae (χ^2 = 41.208, d.f = 2, P <0.0001) as well as larvae and pupae (χ^2 = 41.208, d.f = 2, P <0.0001). However, there was no significant difference between adults and pupae (χ^2 = 41.208, d.f = 2, P >0.005). The mean supercooling points for larvae, pupae and adults were -12.18, -16.6, -16.5 ^{O}C respectively.

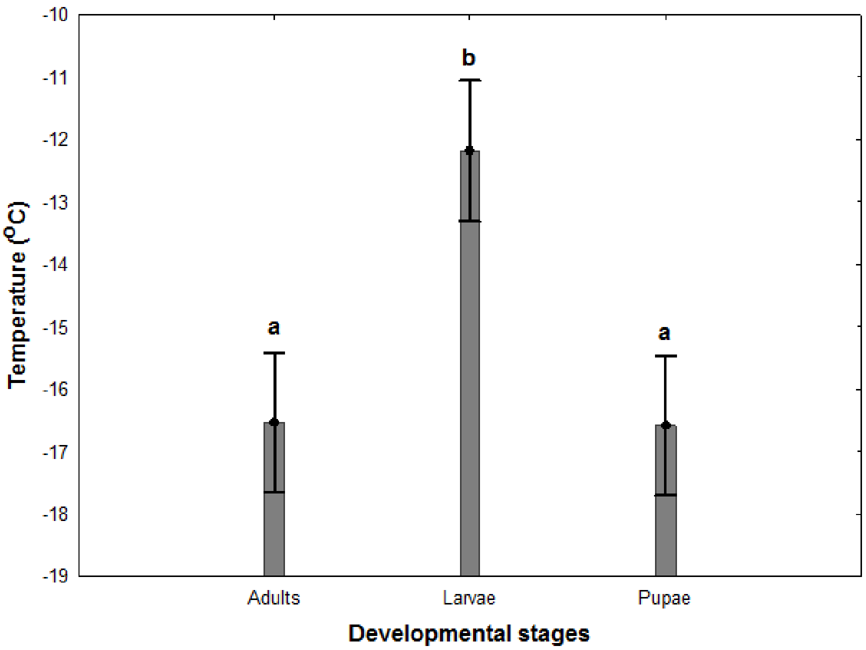


Figure 3: The effect of developmental stage on supercooling points of *B. dorsalis* adults, larvae and pupae with *N*=16 individuals per developmental stage. Error bars representing 95% CL, means with the same letters are statistically not significant.

3.4 Discussion

3.4.1 Effects of ramping rate on Critical thermal limits

The success of many invasive species in novel environments is believed to stem from their superior competitive abilities, resource pre-emption and abiotic factors including temperature (Juliano 1998; Bruno *et al*. 2005). The extent to which an invasive species can extend its range in response to climate change is largely related to climatic factors such as temperature (Andre-Wartha and Birch 1954). According to Terblanche *et al*. (2007), CTL's are significantly affected by rate of temperature change and start temperature as well as the duration of the experiment. Our CTLs results revealed improved CT_{max} following faster ramping rates for both developmental stages and generally improved CT_{min} for larvae following slower ramping rates. Furthermore, developmental stage did not have much effect across all ramping rates for $CT_{max.}$ However, there was marked differences between the two developmental stages and across the three ramping rates for CT_{min}. The results for CT_{max} symbolise that prolonged heating rates may affect the activity of *B. dorsalis* therefore compromising their fitness. This is in line with the notion that insects performance is a function of both duration and severity of exposure (Chown and Nicolson, 2004; Angilletta, 2009; Denlinger and Lee, 2010), suggesting that *B. dorsalis* may not be able to adjust its phenotypic plasticity at high temperatures at the short timescale. Faster cooling rates 0.5°C/min^{-1} resulted in a higher CT_{min} for both developmental stages (adults and larvae) while slower rates of cooling lowered/improved CT_{min} of *B. dorsalis* with larvae showing a significant difference between faster and slower ramping rates hence suggesting an enhanced phenotypic plasticity, at least for larvae only. Nevertheless, ramping rate did not significantly affect CT_{min} for *B. dorsalis* adults. This result may suggest intra-species ontogenetic differential phenotypic plasticity of low temperature tolerance, with larvae having inherently higher plasticity. Indeed, such is the case that less mobile stages have enhanced inherent basal and plastic thermal tolerance responses (Huey 1991), relative to adults. This may be because the later (adults) have more capacity to compensate for temperature tolerance through behavior, a mechanism likely constrained for more immobile stages. The effect of ramping rate on CT_{min} suggests larvae may have developed some heat protection during prolonged ramping duration. These results are in agreement with most studies (see discussions in Nyamukondiwa *et al.* 2010; Nyamukondiwa and Terblanche 2011), as it is typically known that slower cooling rates tend to lower the CT_{min} leading to improved acute low temperature tolerance (Kelty and Lee 1999, Powell and Bale 2004, Overgaard *et al*. 2006). In addition, slower rates provide sufficient time for hardening as a form of phenotypic plasticity that protects the cells from subsequent injury (Overgaard *et al* 2006). These results support RCH responses as has been reported in other Tephriteds e.g. *Bactrocera oleae* (Koveos 2001) and *Eurosta solidaginis* (Bale *et al.* 1989; Lee *et al*. 1993), and Diptera *Sarcophaga crassipalpis* (Rinehart *et al*. 2000), *Musca domestica* (Coulson and Bale 1990). Nevertheless, CT_{max} results suggest both developmental stages of *B.*

dorasalis tested here cannot adjust their thermal tolerance in the short term, otherwise termed rapid heat hardening (see e.g. Hoffmann *et al*. 2003).

3.4.2 Lethal temperature assays

Climate change can have a detriment effect on ectotherms. This is because change in climate is associated with extremes of temperatures and increased variability. Ectotherms always track environmental temperature conditions and without physiological compensation, their body temperatures are always in equilibrium with the environment. In consequence, they ought to compensate physiologically to cope with heterogeneous stressful environments. The impact can either be indirect through activity constraints or through direct effects including for example death due to cellular injury and sterility (Sinclair *et al*. 2012; Andrew and Terblanche 2013). This has a significant impact on insect population dynamics (Terblanche *et al.* 2011, 2015). Therefore knowledge on thermal tolerance is important in understanding the ability of insects to survive shifts in temperature in the rapid ongoing climate change. My LLT and ULT results show that both exposure (severity) and time of exposure (duration) play a significant role in the survival of *B. dorsalis*. The results show a reduction in survival at all developmental stages as the duration of exposure is increased (Chown and Terblanche 2007). The ULT's and LLT's for adults, larvae and pupae ranged from 39-45, -6– 4°C; 38-41, -4–2°C and 39-45, -8–4°C respectively at 0.5 to 4hrs treatments (see Fig. 2). The results reveal that pupae are more temperature tolerant in comparison with other mobile developmental stages (e.g. larvae and adults), which are able relatively more mobile and can compensate through behavior (Huey 1991). As expected behavioral regulation is straightforward for a highly mobile individual living in air than for a less active individual living in a thermally conductive medium such as soil (Huey 1991). However, temperature avoidance is limited when it comes to pupae. Duration of exposure to lethal and sub-lethal temperatures plays an important role ecologically as it determines traits such as activity, severity of injury and possibly time required for recovery following stressful thermal conditions (Chown and Nicolson, 2004).

Field microclimatic data reveal that the upper and lower temperature limits are frequently encountered in nature (see Fig. 4). This suggests that temperature associated mortality may be the chief factor dictating the establishment/ survival of *B. dorsalis* flies unless otherwise these flies develop some form of mechanisms of survival to counteract these conditions. Relationships between thermal tolerance traits tested here and ambient microclimate temperatures suggest high temperature may be a critical factor limiting *B. dorsalis* survival in summer. Nevertheless, my tests were done under closed environments, where the insects are limited for behavioral thermal compensation. Under natural conditions, insects may seek shade, and may not likely face prevailing temperatures. In related cases, the difference between ambient and insect body temperature may be >20˚C (Marsh 1985; Chown and Nicholson 2004), signifying the significance of behavioral modification for thermal fitness. Invasion success is highly dependent on a number of factors *vis a vis* environmental tolerance (Chown and Nicolson, 2004), availability of host plants (Long *et al*. 2004) and increased and repeated propagules pressure (Rouget and Richardson 2003).

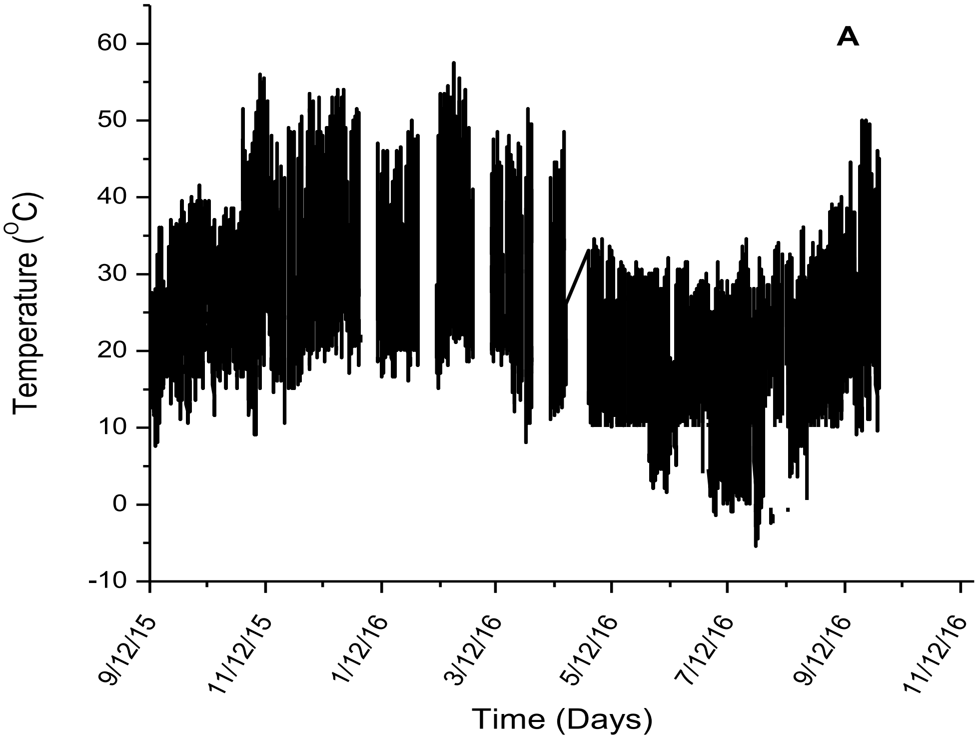


Figure 4: Microclimatic temperature data in austral summer (December 2015-April 2016) and winter (May-July 2016) in Kgatleng (S24.56449; E0.26.16940). Temperature was recorded using Thermocron iButtons (Dallas Semiconductors, Model DS1920) (0.5 °C accuracy; 1 h sampling frequency).

3.4.3 Supercooling points

Low temperature exposure functions as the main trigger of cold-hardiness to insects (Baust and Lee 1981). Temperatures below zero may adversely affect animals by direct lethal effects or damage through ice-formation (Ramlov 2000). Animals therefore overcome low temperature injury through different freeze strategies/ mechanisms; e.g. (1) freeze avoiding animals avoid freezing through supercooling and succumb to internal tissue ice formation; (2) freeze tolerance, those that can survive intracellular ice formation and (3) cryoprotective dehydration, a phenomenon found mainly in soil dwelling animals, where animals depress body water content to levels ≤10%, so as to improve supercooling potential (Lee 2010). Freeze-tolerance however is the main cold-tolerance strategy amongst insects whilst for non-insects freeze avoidance is the dominant (Sinclair and Chown 2005). Our experiments showed SCPs were significantly affected by developmental stage. Pupae and adults had relatively depressed SCPs relative to larvae. The reason for this differential SCPs is unknown. Nevertheless, different factors affect supercooling points including water content, gut contents and the availability of ice nucleating agents (Carrillo *et al*. 2005). Larvae of *B. dorsalis* likely have more water content, and from the dietal feeding, is more likely to have internal gut contents which may act as sites of ice formation. This availability of ice nucleating agents in larvae, relative to the pupae and adults, may likely explain the compromised SCPs for larvae, relative to the other developmental stages. According to Sinclair (1999) an animal is considered as freeze-tolerant if its LLT is lower than its SCP. This therefore suggest *B. dorsalis* is chill susceptible, since in all cases, LLT experiments showed mortality temperature for the three developmental stages is higher than the respective SCPs. Our SCP results are consistent with like studies to date. For example, *Tenebrio malitor* Linnaeus larvae had compromised SCPs than the adults. This is also because larvae produce low levels of thermal hysteresis and antifreeze proteins in their hemolymph (Macmillan 2012), likely compromising supercooling ability. Some insects also produce nucleating material that ensures that freezing occurs at temperatures relatively higher than the subzero temperatures (Dunman 2001). In many studies (e.g. Salt 1936, 1953; Chauvin and Vannier 1997; Koch *et al*. 2004) feeding developmental stages have proved to have high SCPs as opposed to non-feeding stages. Knowledge on SCPs gathered here is important in predicting freeze strategy of *B. dorsalis*, and explaining insect mortality during periods of variable and extreme low temperatures temperature stress.

In conclusion results here show a wide thermal breath for *B.dorsalis*, and the ability, at least for limited developmental stages, to compensate physiologically to low and high temperature to phenotypic plasticity. Such attributes are critical for invasive species and likely shape their invasion potential under climate change. While our personal observations recorded *B. dorsalis* in all districts of Botswana (Chapter 2), in some of the areas, populations were not consistent. Nevertheless, if hosts are available, it seems temperature in Botswana may not likely offset *B. dorsalis* invasion and consequent establishment. I suggest wider range of trait conditions be tested before solid conclusions are drawn on the relationship between thermal biology and *B. doralis* invasion potential. Nevertheless, current results are significant in establishing pest risk analysis and in developing phytosanitary regulations and inform pest management strategies in a globally changing environment.

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CHAPTER 4

General Discussion

Tephritid fruitflies within the genus *Bactrocera* are recognized worldwide in the fruit industry as one of the most destructive pests of fruits and vegetables (White and Elson-Harris 1992; Clarke *et al*. 2005). They are generally endemic to Asia and the pacific, and comprise, amongst other genus *Bactrocera cucurbitae* (Coquillett), *B. dorsalis* (Hendel), *B. latifrons* (Hendel), *B. oleae* (Gmelin), and *B.zonata* (Saunders). Among Tephritidae (Diptera) Dacine fruit flies are documented invaders and rank high on the quarantine list worldwide (Clarke *et al*. 2005). One of such invaders was detected in 2003 in Kenya (Lux *et al*. 2003) as *Bactrocera invadens* (Drew Tsuruta and White) (Drew *et al*. 2008), however later synonymized with *B. dorsalis* (Hendel) (Schutze *et al*. 2015). *B. dorsalis* is a devastating pest that can severely affect sustainable agriculture and rural livelihoods as well as export markets (Follett and Neven 2006). It has become a major threat to fruit-growers in the continent, and negates economic transformation of African livelihoods through agriculture (Lux *et al*. 2003). The fly attacks a wide range of crops and wild host fruits including mainly citrus and mango (Rwomushana 2008). Although probably native to Sri-Lanka (Drew *et al*. 2008), *B. dorsalis* has since spread its geographical range to many other African countries hence raised questions surrounding its environmental niche breath (De villers *et al*. 2015) and climate change responses (Hill *et al*. 2016). In this thesis, I therefore conducted surveillance in order to determine the establishment and abundance of *B. dorsalis* in Botswana, following its detection in Chobe in 2010 (Chapter 2). Furthermore in order to understand how climate change may contribute to the observed distribution and abundance of *B. invadens* in Botswana, I conducted thermal tolerance experiments by measuring different standardized temperature activity traits *vis a vis* CTLs, LLAs and SCPs (Chapter 3). These traits likely shape *B. dorsalis* population establishment and thus invasion potential in both space and time.

Climate plays a significant role as a determining factor of insects' abundance and distribution. In response to climate change, natural populations are shifting their geographic distribution and abundance (Parmesan and Yohe 2003) in search of optimal conditions. Nevertheless climate alone is not a determining factor in insect population dynamics. There is a correlation between climate and quality and quantity of resources (Wolda 1978), therefore availability of resources will also affect insect's population dynamics. These together combined play a role in molding of insects' abundance and distribution.

During my survey (Chapter 2), I found a trend across time, microclimatic biotic and abiotic conditions and population phonologies. Districts with high vegetable and fruit production had consistent populations all year round varying seasonally, areas such as the Chobe, Ngamiland, North-East and the Central district. The consistent population of *B. dorsalis* in these areas is a sign that the environment is conducive for its establishment and can support adult populations all year round. Furthermore, fly catches all year round may point to the notion that temperatures are high enough in winter to facilitate development of flies all year round, abeit slowly. Moreso, it also symbolizes *B. dorsalis* overwinters as an adult, and that fly development does not stop but is rather slowed down during otherwise low temperatures in winter. However in the southern districts of the country, *vis* South-east, Southern, Kgatleng and Kweneng, my results indicate an intermittent population abundance, signifying inconsistent breeding. This therefore means, there may be repeated introductions of parent propagules, hence the inconsistent populations. Moreover, these areas have lesser agricultural activities compared to the north, and thus limited plant resources to support *B. dorsalis* continuous populations. My survey also reveals that there is a link between temperature fluctuations and insects abundance e.g. (Fig 4E) signifying that temperature may be a limiting factor in insects abundance. Population increase peak in summer associated with high temperatures and decreases in winter associated with low temperatures. Fruit sampling survey revealed that *B. dorsalis* had the highest percentage composition from all the sampled fruits and despite the competition for hosts (oranges, mangoes ad guavas) with other fruit flies such as *C. cosyra* and *C. capitata*. *B. dorsalis* was the dominant fruit fly infesting mango fruits in Botswana (Chapter 2, Table 1) followed by *C. cosyra.* My survey also revealed that apart from the cultivated fruits, *B. dorsalis* has an alternative hosts such as the wild e.g. *Capparis tomentosa* (Chapter 2, Fig 5) which may serve as a key hosts during seasons when cultivated fruits are not available. These alternative hosts may support all year round breeding of *B. doralis* populations, consistent with my current observed results for Chobe district.

My thermal tolerance results indicated that indeed insect fitness is a function of both severity and duration for both lethal LTAs and CTLs. CT_{max} improved at faster ramping rates, indicating the inability of *B. dosarlis* to shift its high temperature tolerance at short timescales, termed otherwise rapid heat hardening (Nyamukondiwa *et al.* 2010). On the other hand, CT_{min} improved at slower ramping rates, indicating the ability of the species to shift its thermal tolerance at short timescales, termed otherwise rapid cold hardening (see Kelty and Lee 2001; Nyamukondiwa *et* *al.* 2010). At short time-scales *B. dorsalis* will not be able to shift its phenotypic plasticity as a response to high temperature fluctuations. At low temperatures CT_{min} reveal that larvae has a higher phenotypic plasticity than adults and indeed this is the case in many insect species e.g. *Ceratitis* (Nyamukondiwa *et al.* 2010). This phenotypic plasticity recorded here, may facilitate the invasion potential of this species, as has been reported in other like species (see e.g. Nyamukondiwa *et al.* 2010). My LTA results indicate that as duration of exposure to stressful temperature is increased, the survival of *B. dorsalis* is reduced for all the developmental stages, consistent with reports by Chown and Nicolson (2004). In addition pupae showed a higher temperature tolerance relative to other developmental stages, consistent with other studies to date which have shown that immobile stages have a more inherent increased basal temperature tolerance relative to more mobile stages (see Zhao *et al*. 2017).

4.1 Conclusion

In conclusion temperature plays an important role in insect population dynamics and invasion potential of many biological organisms. Understanding the thermal tolerance of *B. dorsalis* gives an insight into the invasion potential of this fly, its thermal breath and thermal limits to activity. These parameters may help inform whether a population may establish, upon introduction to a novel thermal environment, and may help modeling potential distribution of invasive species. In this study, evidence on thermal tolerance reveals that indeed temperature fluctuations affect the physiology and thus population abundance, and distribution of *B. dorsalis*. Microclimatic temperatures recorded in the survey areas show that the temperatures fall within the thermal breath of *B. dorsalis* activity measured here, thus, support population establishment. Furthermore the synchrony between temperature, hosts and *B. dorsalis* population abundance shows climate plays a critical role in host plant abundance and directly on insect development and thus population numbers. These results are important for mechanistic models predicting the potential invasiveness and distribution of invasive species, may aid pest risk analysis and pest management.

4.2 References

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