Life History Strategies of the Spider Mite, *Tetranychus ludeni* Zacher, in Response to Environmental Conditions

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Abstract

Tetranychus ludeni Zacher (Acari: Tetranychidae) is an invasive pest of many horticultural crops and now occurs in all continents except Antarctic. Its invasive nature and pest status have stimulated the need for understanding the plasticity of its life history strategies under different environmental conditions. So far, little is known about effects of environmental factors on the life history traits in *T. ludeni*. In this thesis, I investigated its life history strategies in response to temperature, food deprivation, and predator threat. I show that life history traits of *T. ludeni* were highly flexible and adaptive to dynamic thermal environment. Higher temperature accelerated development but reduced adult body size and longevity. Increasing temperature elevated the intrinsic rate of increase (r_m) but shortened generation time (T) and the time to double the population size (Dt). The net population growth rate (R_0) was higher at 20 and 25ºC compared to lower and higher temperatures. Thermotolerance in *T. ludeni* was stage-dependent with eggs being the most vulnerable and adults the most tolerant. No eggs hatched after exposed to 45°C for ≥ 15 hours, 48°C for ≥ 12 hours, or 51°C for three hours; no adults survived 51°C or 54°C for ≥12 hours or 57°C for ≥ six hours, and heat tolerance of other life stages fell in between. Intermittent fasting (IF) had sex-specific and mating status-specific effects on the lifespan of *T. ludeni*. It extended the lifespan of females regardless of mating status but shortened that of mated males and had no effect on virgin males. IF reduced fecundity and egg size and extended offspring developmental time. My study indicates that predator-induced fears significantly lowered the fitness of *T. ludeni*. Cues from predators and injured prey shortened the longevity by 23–25% and oviposition period by 35–40% and reduced the fecundity by 31–37% in *T. ludeni* females. These cues significantly reduced intrinsic rate of increase (r_m) and net population growth rate (R_0), and extended time to double the population size (Dt). Findings from this study contribute to our understanding of the effects of environmental stressors on life history traits in *T. ludeni*, providing knowledge for pest risk analysis and development of pest management programs.

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I would like to dedicate this work to my beloved parents.

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Chapter 1 Introduction

1.1 General introduction

There are about 1,200 species in the mite family Tetranychidae (Bolland *et al*. 1998), many of which are important pests in agriculture and horticulture worldwide (Resh & Cardé 2003; Migeon *et al*. 2010; Alston & Reading 2011). Several major crop pests have developed resistance to synthetic pesticides, resulting in pest outbreaks (Ho 2000; Van Leeuwen *et al*. 2010). For example, the European red mite *Panonychus ulmi* (Koch) is resistant to azocyclotin (Bowie *et al*. 1988), and two-spotted spider mite *Tetranychus urticae* Koch to propargite (Bowie *et al*. 1988; Kabir *et al*. 1991). Their development of pesticide resistance and subsequent population outbreaks may be accelerated by the nature of their short life cycles, high fecundity and arrhenotokous reproduction (Helle 1965; Van Leeuwen *et al*. 2010). Under optimal conditions spider mite populations can increase rapidly and cause extensive plant damage (Warabieda 2015). For instance, *T. urticae* attack on soybean plants can reduce the yield by 40–60% (Cullen & Schramm 2009).

New Zealand has 10 *Tetranychus* species (Migeon *et al*. 2010), of which *T. ludeni* Zacher is an invasive pest that has been recorded in Europe, Asia, Africa, America, and Australasia (Migeon *et al*. 2010; CABI/EPPO 2011). It attacks more than 250 plant species (Gotoh *et al*. 2015) including economically important crops such as beans, eggplants, pumpkins, hibiscus, and other cucurbitaceous plants in warm regions and greenhouse plants in temperate areas (Adango *et al*. 2006; Fathipour & Maleknia 2016). For example, when feeding on velvet bean leaf, *T. ludeni* may cause a heavy loss of chlorophyll a and b (79.26 ± 4.3% and 74.09 ± 4.07%, respectively) and water from plants, leading to death (Kaimal & Ramani 2011a). *T. ludeni* has become a serious problem in India since early 1980's (Singh & Raghuraman 2011). In Australia, it is an important pest in cotton (Herron *et al*. 1998).

Pest monitoring and forecasting are essential components of integrated pest management (IPM) programs. To achieve successful pest forecasting, we need to understand biotic and abiotic factors that affect pest-host interactions (Singh *et al*. 2004) and pest life history strategies that vary in response to environmental conditions (Nylin 2001). The expression and evolution of life history traits are affected not only by genetic variation and constraints but also by the environment (Fabian & Flatt 2012). So far, little is known about the effects of environmental conditions on the life

history strategies in *T. ludeni*, making it difficult to evaluate its potential to invade and to become a major pest in different regions or conditions. Furthermore, understanding how *T. ludeni* responds to environmental stress is important for development of novel control measures for this pest.

In this chapter, I outline the relevance of my research and the aim and objectives of this thesis. I then carry out literature reviews on the current knowledge about the biology and ecology of spider mites and factors affecting their life history strategies.

1.2 Relevance of research

The world has been facing climate change, particularly the increase of temperature (Karl & Trenberth 2003; Loarie *at al*. 2009; Schneider 2001; Houghton 2005; Coumou & Rahmstorf 2012; Cohen *et al*. 2014), to which all organisms need to respond (Wilson & Martin 2012). Temperature is one of the most important factors affecting life history traits such as developmental time, survival, fecundity, body size, and longevity (Howe 1967; Bounfour & Taniguchi 2001; Harrison & Klok 2013; Ciota *et al*. 2014; Schulte 2015). In spider mites, each species, even each strain of the same species, may respond to temperature differently (Gotoh *et al*. 2010, 2015). Climate change also produces frequent extreme weathers including heat waves (Hayhoe *et al*. 2010; Trenberth & Fasullo 2012; Schär 2016), which may affect populations and individuals adversely by putting them outside of their physiological tolerance (Parmesan *et al*. 2000). Therefore, understanding how *T. ludeni* responds to temperature may provide knowledge for prediction of its potential expansion range and pest outbreaks and development of disinfestation measures.

Many studies report that increasing temperature creates phenological asynchrony between herbivores and their host plants, resulting in starvation (Schoonhoven *et al*. 2005; Singer & Parmesan 2010; DeLucia *et al*. 2012). Starvation may also occur when herbivores are carried by transportation or when their host plant reaches senescence. Many species including spider mites have developed strategies to adapt to temporary or intermittent fasting. For instance, starved *T. urticae* prefer to move to uppermost part of the host plant and spend more time for resting (Le Goff *et al*. 2012) and intermittently fasted *T. urticae* reduce their fecundity and extend their life span (Li & Zhang 2019a). Understanding how *T. ludeni* responds to food stress may provide knowledge for evaluation of its population dynamics in food depleted environment.

Predation risk almost always occurs in nature, to which prey may adaptively respond for their survival. For instance, *T. urticae* may avoid host plants with predator cues or tend to be more active and aggregate more tightly in the presence of these cues (Dittmann & Schausberger 2017; Zhang, van Wieringen *et al*. 2019). *T. kanzawai* Kishida disperse farther and avoid low quality host plant after exposure to a predator (Murase & Fujita 2018). *T. evansi* lay fewer eggs on a leaf with predator cues (Dias *et al*. 2016). These responses may lead to slower growth, lower reproductive outputs, shorter longevity, and longer development as they spend more time and energy on locomotion, and less time on feeding (Pallini *et al*. 1999; Hackl & Schausberger 2014). The non-consumptive effects by predator cues may be as strong as direct consumption (Preisser *et al*. 2005) and significantly alter prey life history (Kats & Dill 1998; Lehto & Tinghitella 2019; Tariel *et al*. 2020). Therefore, knowledge of the non-consumptive effects on *T. ludeni* helps understand their contributions to biological control using predators and paves the way for the development of novel control methods.

1.3 Aim and objectives

The present study aimed to explore life history strategies of *T. ludeni* in response to environmental conditions with four objectives:

1. To investigate growth, development, survival, and reproduction of *T. ludeni* under different constant and fluctuating temperatures;
2. To examine the effect of heat shock on survival and reproductive performance of *T. ludeni*;
3. To determine the effect of food stress on growth, development, survival, and reproduction of *T. ludeni*, and
4. To identify the effect of predation risk on the performance of *T. ludeni.*

1.4 Literature review

1.4.1 Taxonomy of *Tetranychus ludeni*

Mites are the most diverse group within the class Arachnida and have a large range of feeding habits including predators, scavengers, parasites, and herbivores. So far, more than 6,000 species of herbivorous mites have been described worldwide (Nesser & Craemer 2014). *T. ludeni* is

identified by Prof. Zhi-Qiang Zhang, University of Auckland, New Zealand and its classification position is as follows:

Class: Arachnida

Subclass: Acari

Order: Prostigmata

Family: Tetranychidae

Genus: *Tetranychus*

Species: *ludeni* Zacher

Adult females of *T. ludeni* are morphologically similar to other *Tetranychus* species, especially *T. cinnabarinus* (Boisduval). However, its males can be distinguished by the absence of the posterior angulation of the knob in aedeagus (Zhang 2002).

1.4.2 General biology of *Tetranychus ludeni*

The complete life cycle of *T. ludeni* includes eggs, larvae, quiescent larvae, protonymphs, quiescent protonymphs, deutonymphs, quiescent deutonymphs, and adults (Zhang 2003). Eggs laid by mated females are transparent while those by virgin females are reddish (Figure 1.1A). When closer to the hatching time, the part of gnathosoma, eye spots and legs of larvae can be seen from the egg case. Larvae that newly hatch from eggs can be identified by their small size and three pairs of legs (Kaimal & Ramani 2011b), and they actively feed on leaves for about one day before entering the first quiescence (Figure 1.1B). The protonymphs and deutonymphs have four pairs of legs and greenish spots on dorsolateral region (Figure 1.1C-E). The adult males are smaller than females and spindle in shape and pale yellow in colour while the females are cylindrical in shape and reddish in colour (Figure 1.1F; Kaimal & Ramani 2011b).

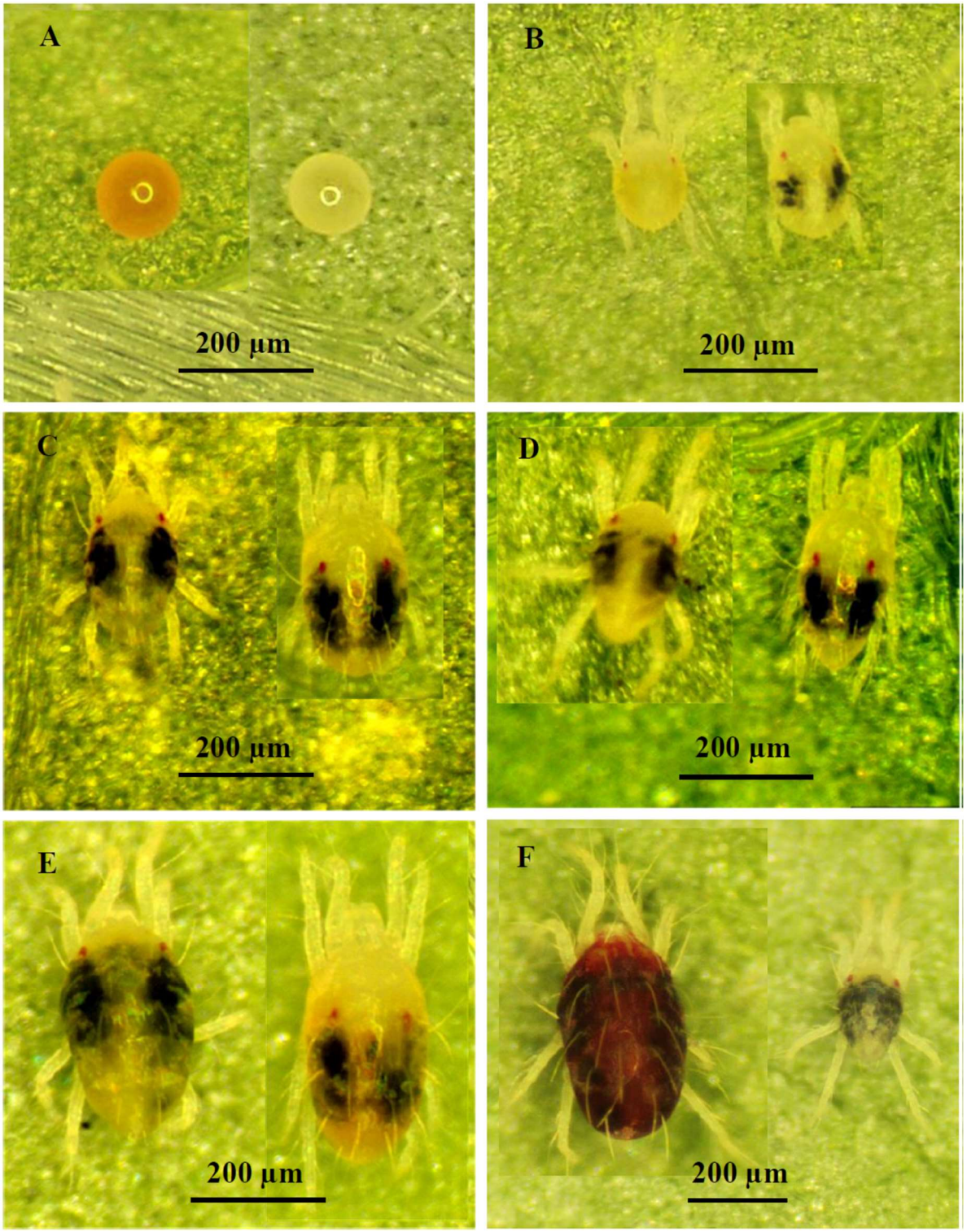

Figure 1.1 Life cycle and morphology of *T. ludeni*: (**A**) reddish (left) and translucent (right) eggs, (**B**) active (left) and quiescent (right) larvae, (**C**) active (left) and quiescent (right) protonymphs, (**D**) active (left) and quiescent (right) male deutonymphs, (**E**) active (left) and quiescent (right) female deutonymphs, and (**F**) female (left) and male (right) adults.

1.4.3 Reproduction of spider mites

Spider mites are haplodiploid arthropods, where mated females may lay unfertilised haploid eggs giving rise to sons and fertilised diploid eggs leading to daughters while virgin females can only produce haploid sons (Oliver 1971; De la Filia *et al*. 2015). Mated females of arrhenotokous species may produce males by avoiding some eggs from fertilisation (Tehri 2014). However, fertilised eggs are usually larger than the unfertilised ones (Macke *et al*. 2011).

Adult females can mate several times but normally only the first males to mate dominate paternity while the adult males can mate with more than one female and may combat against other males to enhance their reproductive success (Oku 2009, 2014). Mating behaviour of *T. ludeni* is similar to that of other *Tetranychus* species: mating occurs immediately after female emergence; the male will slip underneath the female from behind and arch the end of his hysterosomal abdomen upward to attain coupling, and during mating, the female is held by male with his two anterior pairs of legs for about one to three minutes (Puttaswamy & Channabasavanna 1979; Jose & Shah 1989).

Mating modifies female resource allocation pattern. Mated females generally have a shorter life span and concentrate their reproduction early in life, while virgin females tend to spread their reproductive investment in longer period to increase the possibility of finding a sexual partner (Macke *et al*. 2012). Despite both mated and virgin females can produce male offspring, they produce eggs of different size, i.e., male eggs produced by virgin females are bigger than those produced by mated females (Macke *et al*. 2011). Similarly, Zhou *et al*. (2018) find that eggs laid by virgin females are larger, which develop to sons with higher quality that can produce more daughters. Therefore, females may adjust their life history strategies depending on their mating status.

1.4.4 Life history

The life history is characterized by many traits, such as the number and size of offspring, survival rate, sexual maturity, and senescence (Roff 1992; Nettle & Frankenhuis 2020). Life history traits are affected by stressful environmental conditions, which organisms may need to adapt to or escape from (Shefferson 2010). The evolutionary response of organisms to these external forces of selection is also directed and limited by internal factors through trade-offs and constraints

(Braendle *et al*. 2011). Trade-offs can be described as the fitness cost paid when a beneficial change in one trait causes a detrimental change in the other, while constraints are often used to describe absolute limits upon trait expression and combination (Stearns 1989; Roff & Fairbairn 2007; Braendle *et al*. 2011).

Life history theory attempts to explain how natural selection and other evolutionary forces shape organisms in their survival and reproduction amid environmental challenges (Stearns 2000; Fabian & Flatt 2012). A complex pattern of life history traits that evolve in response to a particular environment is considered as a life history strategy (Rochet 2000). In nature, organisms show enormous amount of variation in life history strategies within and between species (Roff 1992). Like other ectothermic organisms, spider mites express variations in their life history traits to reflect adaptations to environmental pressure. In this section, I review some external factors that affect the life history traits of ectotherms.

1.4.4.1 Effect of temperature

Ectothermic organisms are significantly affected by their environmental temperature since their body temperature fluctuates as the environmental temperature changes (Zuo *et al*. 2012). They often grow slower and mature with larger body size under lower temperatures (Ray 1960; Angilletta *et al*. 2004; Walters & Hassall 2006; Plesnar-Bielak *et al*. 2013). For instance, the developmental time decreases with increasing temperatures in *Tetranychus* species (Gotoh *et al*. 2010, 2015). However, the temperature-body size rule may vary within species depending on the local habitats. For example, Pequeno *et al*. (2018) reveal that the body mass of soil mite *Rostrozetes ovulum* (Berlese) decreases by 9.72% in non-riparian forests but by 38.85% in riparian forests for each 1°C increase in maximum temperature. They argue that the temperature-size rule changes with other environmental factors, particularly oxygen and resource levels. Furthermore, constant and fluctuating temperature regimes may affect life history traits differently (Ruel & Ayres 1999; Ragland & Kingsolver 2008; Tougeron *et al*. 2021; Wu *et al*. 2015) and should be examined separately.

Ectothermic organisms may be vulnerable to thermal stress since their basic physiology is limited by ambient temperature. The predicted increase of global temperature will push many ectothermic organisms towards their physiological upper limits that may affect their reproduction

and survival (Schou *et al*. 2014). For instance, at 34°C *T. pacificus* McGregor, *Eotetranychus willamettei* McGregor, and *Galendromus occidentalis* Nesbitt reduce their fecundity, intrinsic rate of natural increase, and longevity (Stavrinides & Mills 2011). Organisms may be able to cope with adverse temperature conditions to some extent (Rodrigues & Beldade 2020). For example, a brief exposure to sublethal temperature may cause physiological acclimation which improves thermal tolerance (Angilletta & Angilletta 2009; Kingsolver *et al*. 2016; Lü & Liu 2017; Noh *et al*. 2017; Zhang *et al*. 2018). However, exposure to temperature outside their thermal tolerance may be lethal (Käfer *et al*. 2020).

1.4.4.2 Effect of food

Food depletion can induce behavioural changes and/or physiological adaptation (Zhang, Xiao *et al*. 2019). For instance, *T. urticae* on exhausted plants gather at the plant apex to form a collective silk-ball for aerial dispersal, or they do ambulatory dispersal in a local patch to find a new food resource (Jung 2005; Clotuche *et al*. 2013). The availability of food resources may determine the trade-offs between life history traits (Li & Zhang 2019a). For example, under food stress condition organisms tend to switch their resource allocation away from reproduction to their somatic maintenance (Shanley & Kirkwood 2000; Regan *et al*. 2020; Walzer & Schausberger 2015). In *T. urticae*, moderate level of intermittent fasting reduces fecundity but prolongs longevity in females (Li & Zhang 2019a).

Under limited food resources, females of spider mites may also adjust their reproductive investment by reducing their egg size (Walzer & Schausberger 2015). Egg size determines offspring initial size/resource (Fox *et al*. 1997) and offspring from larger eggs tend to have larger body size at hatching and higher survivorship than those from smaller eggs (Marsh 1986; Fleming & Gross 1990; Fox *et al*. 1994; Macke *et al*. 2011). Therefore, reduced investment per egg may result in lower offspring fitness (Warner & Lovern 2014). Furthermore, parental food regime may have transgenerational effects on the development rate (Johnson *et al*. 2014) and disease resistance (Dinh *et al*. 2021) of their offspring.

1.4.4.3 Effect of predation risk

Like other animals, spider mites face predation risk during their lifespan, which may be a prime selective force shaping their behaviours and life history traits (Lima & Dill 1990; Kats & Dill 1998). For instance, *T. evansi* and *T. kanzawai* use their dense webbing as a shelter or refuge (Oku *et al*. 2003a; Otsuki & Yano 2014; Lemos *et al*. 2015), *T. urticae* avoid host plants with cues of their predators (Pallini *et al*. 1999; Zhang, van Wieringen *et al*. 2019), and *T. kanzawai* females change their oviposition site from leaf surface to their web (Murase *et al*. 2017). However, the benefit of these antagonistic behaviours may cost the time and energy available for other fitness-enhancing activities (Lima 1998; Hackl & Schausberger 2014). For example, predator-induced stress reduces time for feeding and thus energy gain, resulting in smaller body size (Oku *et al*. 2003a; Bowler *et al*. 2013; Otsuki & Yano 2014; Lemos *et al*. 2015), lower fecundity (Choh *et al*. 2010; Bowler *et al*. 2013; Dias *et al*. 2016) and shorter longevity (McCauley *et al*. 2011; Sitvarin *et al*. 2015; Elliott *et al*. 2016).

Predation risk may also have transgenerational effects on prey (Freinschlag & Schausberger 2016). For example, when spider mite females lay eggs on their webs in response to predation risk, their young cannot feed immediately after hatching, causing higher mortality (Murase *et al*. 2017). Maternal experience of predation threat may prolong the developmental period of the offspring (Freinschlag & Schausberger 2016; Elliott *et al*. 2016). The negative non-consumptive effects on prey by predation threat may be as strong as those by direct consumptions (Preisser *et al*. 2005; Buchanan *et al*. 2017; Gehr *et al*. 2018), leading to less damage to plants (Bowler *et al*. 2013). This information is important for evaluation of predation threat in biological control effectiveness using predators and development of novel pest control measures.

Chapter 2 Response to Thermal Environment in *Tetranychus ludeni* (Acari: Tetranychidae)

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Abstract

Tetranychus ludeni Zacher is a spider mite that has invaded all continents except Antarctica and become an economically important pest around the world. Understanding the plasticity of its life history traits as a response to temperatures provides critical information for its risk analysis and management. Here I tested its response to temperatures ranging from 15 to 30ºC over two generations. I found that there was no difference in the egg hatch rate and immature survival rate across temperatures in the first generation. However, the egg hatch rate was lower and immature survival rate was higher at 30ºC in the second generation. The sex ratio (proportion of females) of resultant adults was consistent under all test temperatures in both generations except for 30ºC in the second generation which was lower. Higher temperature accelerated development in both generations but the development at the lower temperatures was faster in the second generation. Adult body size in both generations generally decreased with the increase of temperature, with females being more likely than males to adjust body size in response to temperature changes they first experienced. Temperature-dependent body size was not translated into fecundity, but larger adults lived longer. The thermal threshold was lower and degree days (DD) were greater in the second generation than in the first generation. My findings indicate that life history traits of *T. ludeni* are highly flexible and adaptive to dynamic thermal environment in successive generations. Furthermore, increasing temperature elevated the intrinsic rate of increase (r_m) but shortened the generation time (T) and the time to double the population size (Dt). The net population growth rate (R_0) was higher at 20 and 25ºC as compared to lower and higher temperatures.

2.1 Introduction

Tetranychus ludeni Zacher is an invasive mite pest originating from Europe and now present in Asia, Africa, America, and Australasia (Migeon *et al*. 2010; CABI/EPPO 2011; Zhou *et al.* 2021). It attacks more than 250 plant species (Gotoh *et al*. 2015) including economically important crops such as carrots, beans, eggplants, pumpkins and other cucurbitaceous plants in warm regions and greenhouses of temperate areas (Zhang 2002; Adango *et al*. 2006; Fathipour & Maleknia 2016). Because temperature is a key factor influencing physiological processes in insects and mites (Ullah *et al*. 2011; Gotoh *et al*. 2015; Zou *et al*. 2018) and determining their distribution and abundance (Bale *et al*. 2002; Roy *et al*. 2002), global warming may further increase the distributional range of *T. ludeni* (Gotoh *et al*. 2015) and favor the spread of other invasive mites (Ghazy *et al.* 2019). Therefore, investigation into the influence of temperature on life history traits provides important information for pest risk analysis and management.

Evidence shows that egg hatch rate, immature survival, developmental time, adult longevity, and life table parameters vary substantially in different mite species and even different strains of the same species in response to test temperatures in the laboratory (e.g., Zhang *et al*. 1999; Da Silva 2002; Roy *et al*. 2003; Gotoh *et al*. 2010, 2015; Ullah *et al*. 2011; Riahi *et al.* 2013; Bazgir *et al*. 2015; Li *et al.* 2015; Liu & Zhang 2016; Bayu *et al*. 2017; Zou *et al*. 2018; Hasanvand *et al*. 2019; Ristyadi *et al*. 2019). This may be attributed to a diverse range of physiological limits in different species (Nguyen *et al.* 2014) and adaptation to temperature changes in different strains (Gotoh *et al*. 2010). However, most studies on the effect of temperature on mite life history traits analyze their data from only one generation, making it difficult to evaluate mites' potential adaptations to variations of temperature over generations. Hence, examination of life history traits in response to different temperatures for more than one generation can provide information on how mites may adapt to dynamic thermal environment for prediction of their potential of invasions and crop damage in the world.

Temperature is also an important factor regulating sex ratio in spider mites (e.g., De Moraes & McMurtry 1987; Margolies & Wrensch 1996; Roy *et al*. 2003; Gotoh *et al.* 2015). Roy *et al*. (2003) suggest that females have better capacities than males to disperse and survive under harsh conditions in some spider mite species, such as *T. mcdanieli* McGregor, and consequently, their sex ratio should be increasingly female-biased at extremely low or high temperatures indicating

deteriorating habitats. Yet, it is largely unknown whether this notion applies to other spider mites. Furthermore, body size of adults developing from immatures at different temperatures may vary substantially (Klok & Harrison 2013). For example, body size of geographic strains of a species in warmer regions should be smaller than in cooler regions (Walters & Hassall 2006; Plesnar-Bielak *et al*. 2013; Pequeno *et al.* 2018). However, it is not clear whether such temperature-dependent body size is translated into fecundity in spider mites.

In the present study, I aimed to investigate the life history strategies of *T. ludeni* in response to dynamic thermal conditions in two successive generations. Based on theoretic framework and empirical findings outlined above, I postulate that (1) higher temperature accelerates development and shortens adult longevity; (2) immature mortality is higher and sex ratio is more female-biased at low and high temperatures; (3) adults that develop from lower temperatures are larger and more fecund, and (4) mites can adapt to thermal changes over generations. To test these hypotheses, I carried out a series of experiments and compared various life history traits in response to dynamic temperatures within and between generations. I also created a life table using data collected.

2.2 Materials and methods

2.2.1 Breeding colony and test temperatures

I established a breeding colony of *T. ludeni* from field collected adults on *Passiflora mollissima* (Kunth) (Malpighiales: Passifloraceae) in Palmerston North, New Zealand. I maintained the colony on 20 potted kidney bean plants [*Phaseolus vulgaris* L. (Fabales: Fabaceae)] at 25 ± 1°C temperature and 50~70% RH with a photoperiod of 16L:8D hours. I replaced the 10 oldest plants fortnightly with new ones by cutting leaves of old plants with mites on and placing them on the top of new plants. I tested effects of four temperatures (15, 20, 25, and 30 ± 1°C) under the above RH and photoperiod conditions using four growth chambers (I-36VL, Percival Scientific Inc., Perry, Iowa, USA).

2.2.2 Effect of temperature on life history traits

To determine whether survival, development and reproduction of *T. ludeni* responded to the same temperature treatments differently in different generations, I exposed mites to the above test

temperatures for two generations and recorded egg hatch, immature survival and developmental time between oviposition and adult emergence, and sex ratio and body size of resultant adults. The first generation started from the eggs laid by mites from the breeding colony and the second generation commenced from the eggs produced by the first generation.

I performed 20 replicates per temperature treatment in each generation. For each replicate, I put 50 eggs on a bean leaf disc (3 cm × 3 cm) laid by mated females of ≤ 1-d-old at a test temperature. I placed the leaf disc (3 cm × 3 cm) upside down on a water saturated cotton pad in a Petri dish (5.5 cm diameter × 1.0 cm height) during the entire treatment period. I replaced leaf discs with fresh ones once every five days and examined each leaf disc daily until all individuals reached adult stage. I randomly selected two females and two males of resultant adults per replicate for body size measurement (area from top view) under a digital camera (Olympus SC30, Japan) connected to the stereomicroscope and a computer with Adequate Imaging software (CellSens® GS-ST-V1.7, Olympus, Germany) installed.

To determine fecundity and adult longevity, I randomly selected 20 newly emerged females and 20 newly emerged males from each of the above treatments in the first generation and individually paired them on a leaf disc (2 cm × 2 cm) in a Petri dish as mentioned above. Twenty-four hours after pairing, I individually transferred males onto clean leaf discs of the same size in Petri dishes and replaced leaf discs once every three days until males died. I allowed females to stay on the same leaf discs for three days, after which time, I individually transferred them onto new leaf discs of the same size in Petri dishes. I repeated this process until females died. I counted the number of eggs laid by each female and monitored adult longevity daily. Due to logistical reasons, I did not estimate the above parameters for the second generation.

I calculated the life table parameters (Jervis *et al*. 2005) for each temperature by using the above data collected in the first generation. The intrinsic rate of increase (r_m, daughters/female/day) was calculated by solving the Lotka-Euler equation, $\sum e^{-r_m x} l_x m_x = 1$, where x is the pivotal age of females, l_x is the proportion of females surviving to age x, and m_x is the number of daughters produced per female at age x. I also estimated other life table parameters, including the net reproductive rate ($R_0 = \sum l_x m_x$, daughters/female/generation), mean generation time [$T = \log_e(R_0)/r_m$, days], and doubling time [$Dt = \log_e(2)/r_m$, days]. For each treatment, a jackknife method (Caswell 2001) was used to estimate the life table parameters for each female.

2.2.3 Statistical analysis

I tested the distribution of data using a Shapiro-Wilk test (UNIVARIATE procedure) and used SAS 9.3 (SAS Institute 2011) to analyze all data. Data on the male and female adult body size, and ln(x)-transformed number of eggs laid and developmental period were normally distributed and thus analyzed using an mixed-factor analysis of variance (ANOVA, GLM procedure) followed by Tukey's Studentized range test for multiple comparisons. Data on egg hatch, immature survival, sex ratio (proportion of females) of resultant adults and life table parameters were not normally distributed even after transformation, and thus analyzed using a non-parametric mixed-factor ANOVA (GLM procedure). Data on adult survival were compared using a Wilcoxon test (LIFETEST procedure).

To determine the low temperature threshold (T_0) and degree-days (DD) required to start and complete development of immature stages, I fitted the developmental rates (y = 1/developmental time) over temperatures (T) using a linear regression (GLM procedure): $y = a + b$T, where a and b are estimates of the y intercept and slope, respectively. I then calculated $T_0 = -a/b$, and DD = $1/b$. According to Campbell *et al*. (1974), the standard errors of T_0 and DD were calculated as $(\bar{y}/b)\sqrt{s^2/(N\bar{y}^2)}+(SE_b/b)^2$ and SE_b/b^2, respectively, where s^2 is the residual mean sum of square of y, $\bar{y}$ the sample mean, SE_b the standard error of slope b, and N the total number of samples.

2.3 Results

I show that proportions of eggs that hatched and immatures that survived to adults and sex ratio of resultant adults were largely similar across test temperatures in both generations (Figure 2.1). The only difference occurred at 30ºC in the second generation where egg hatch rate was significantly lower than at 25 and 30ºC in the first generation ($F_{7,149} = 3.21$, $P = 0.0034$) (Figure 2.1A), immature survival rate was significantly higher than at 15ºC in the first generation ($F_{7,148} = 2.59$, $P = 0.0125$) (Figure 2.1B), and the proportion of resulting females was significantly lower than all other treatments in both generations ($F_{7,141} = 8.68$, $P < 0.0001$) (Figure 2.1C).

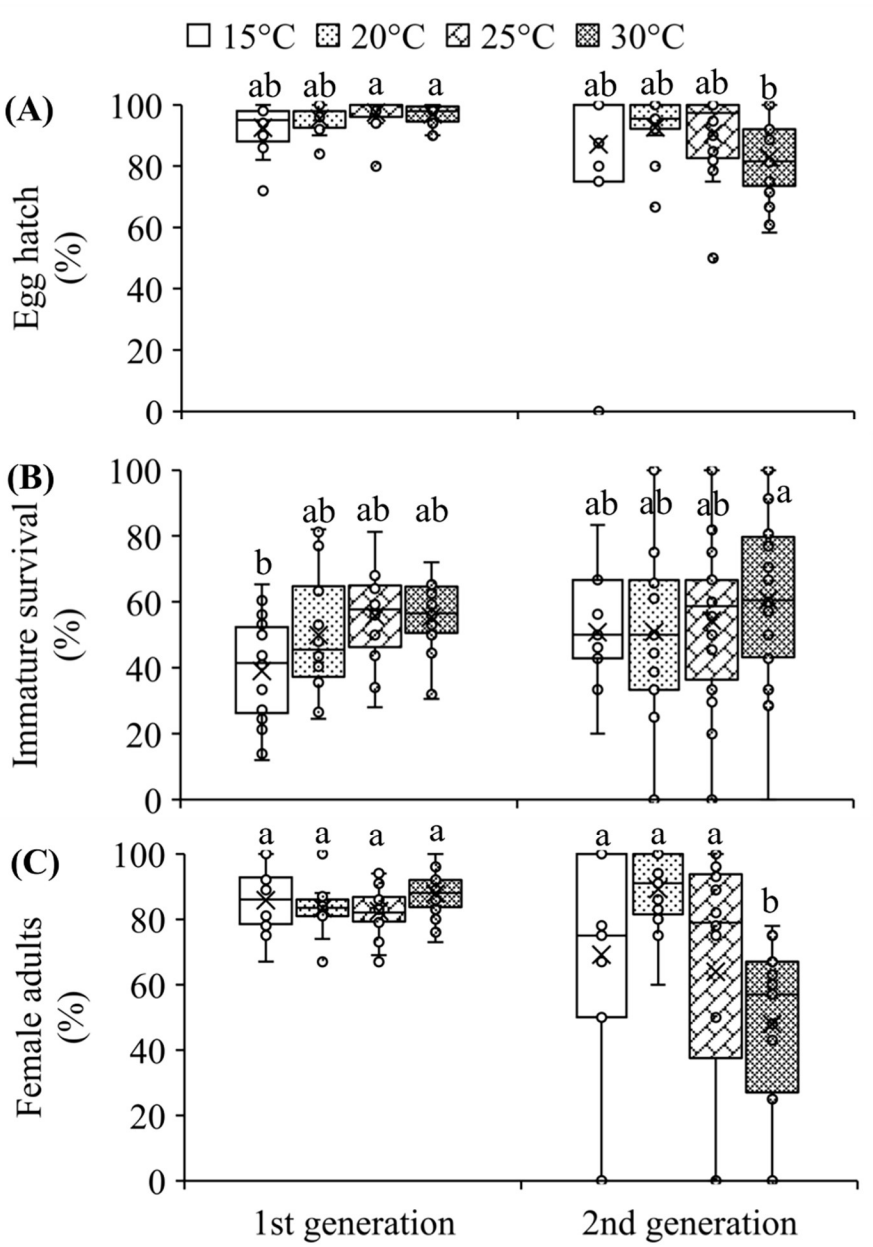

Figure 2.1 Mean (± SE) percentage of egg hatch (**A**), immature survival (**B**) and resulting female adults (**C**) at different temperatures in the first and second generations in *T. ludeni*. Columns with the same letters are not significantly different ($P > 0.05$). For each box plot, the upper and lower box lines represent 75% and 25% of scores falling beyond the upper and lower quartiles, respectively; the '×' and line in a box indicate the mean and median score, respectively; the '⊤' and '⊥' are the upper and lower whiskers showing scores outside the 50% middle; the circles are the outliers of scores.

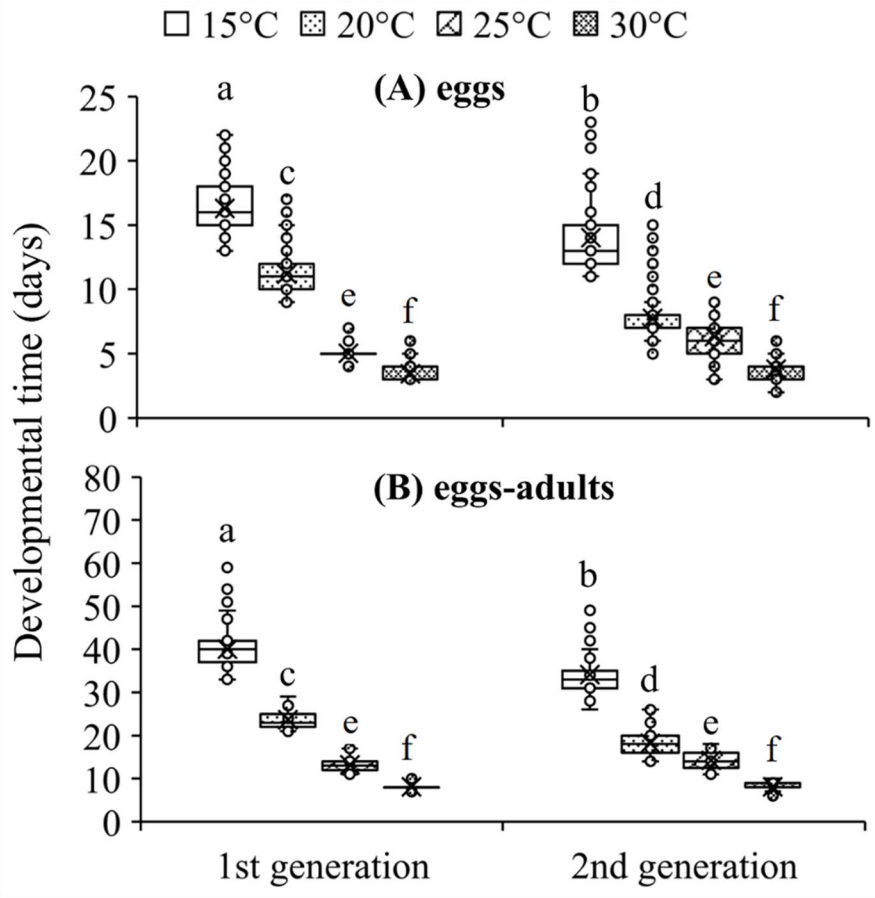

Figure 2.2 Mean (± SE) developmental time of eggs (**A**) and from eggs to adults (**B**) at different temperatures in the first and second generations in *T. ludeni*. Columns with the same letters are not significantly different ($P > 0.05$). For each box plot, the upper and lower box lines represent 75% and 25% of scores falling beyond the upper and lower quartiles, respectively; the '×' and line in a box indicate the mean and median score, respectively; the '⊤' and '⊥' are the upper and lower whiskers showing scores outside the 50% middle; the circles are the outliers of scores.

Tetranychus ludeni significantly accelerated their development with the increase of temperature in both generations, with the second generation developing significantly faster at 15ºC and 20ºC than the first generation ($F_{7,4770} = 7341.52$, $P < 0.0001$ for eggs, and $F_{7,2443} = 9463.52$, $P < 0.0001$ for egg-adult) (Figure 2.2). Adult body size of both sexes generally decreased significantly with the increase of temperature from 20ºC in both generations with it being the largest at 20ºC and smallest at 30ºC ($F_{7,263} = 30.30$, $P < 0.0001$ for females, and $F_{7,219} = 13.34$, $P < 0.0001$ for males) (Figure 2.3). Although the developmental rate and temperature were

significantly positively correlated in both generations, the low temperature threshold (T_0) was lower and degree days (DD) were greater in the second generation than in the first generation (Table 2.1).

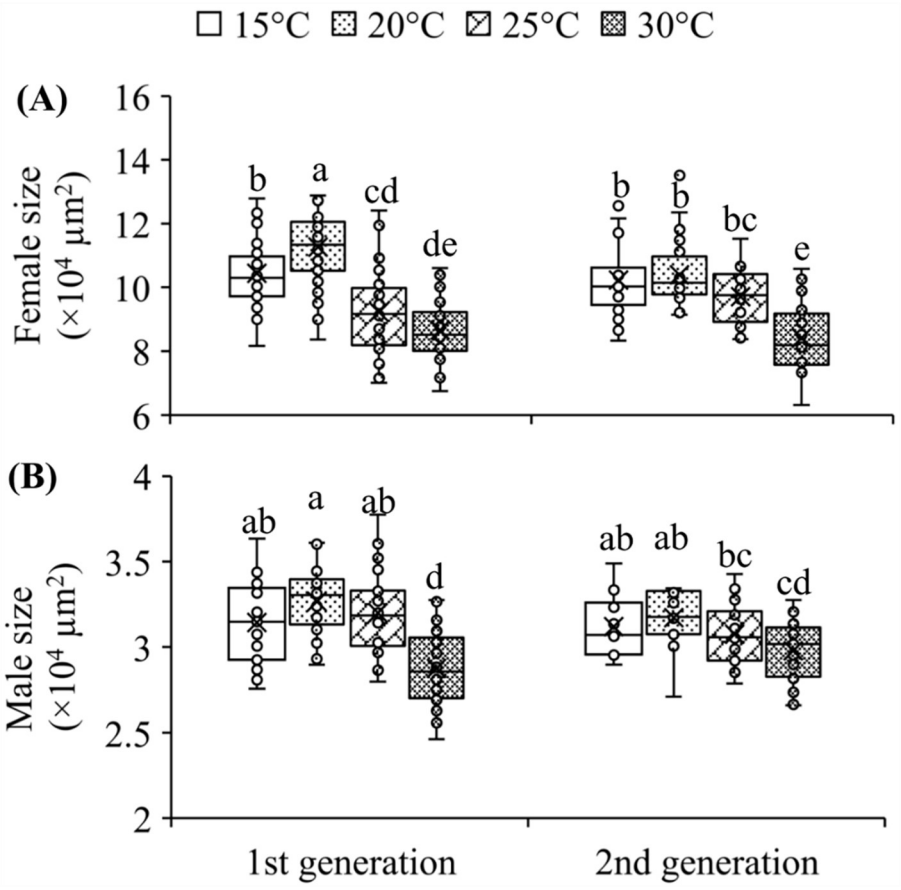

Figure 2.3 Mean (± SE) body size of female (**A**) and male adults (**B**) in *T. ludeni* at different temperatures. Columns with the same letters are not significantly different ($P > 0.05$). For each box plot, the upper and lower box lines represent 75% and 25% of scores falling beyond the upper and lower quartiles, respectively; the '×' and line in a box indicate the mean and median score, respectively; the '⊤' and '⊥' are the upper and lower whiskers showing scores outside the 50% middle; the circles are the outliers of scores.

Table 2.1 Relationship between developmental rate (1/d, y) and temperature (T, ºC), and the estimated mean (± SE) low temperature threshold (T_0, ºC) and degree day (DD) required to start and complete development of eggs and from egg to adult stage in *T. ludeni.*

Stage	Equation	R^2	$F_{(df)}$	P	T_0	DD
1st generation						
Egg	y = -0.2064+0.0164T	0.8807	$28221.50_{(1,2695)}$	< 0.0001	12.56±0.03	60.85±0.36
Egg-adult	y = -0.0864+0.0068T	0.9291	$25085.60_{(1,1913)}$	< 0.0001	12.72±0.03	147.15±0.93
2nd generation						
Egg	y = -0.1315+0.0132T	0.4043	$1466.21_{(1,960)}$	< 0.0001	10.23±0.13	75.62±1.98
Egg-adult	y = -0.0749+0.0064T	0.7896	$2003.54_{(1,534)}$	< 0.0001	11.70±0.11	156.28±3.49

In the first generation, lifetime fecundity of females that developed from 15ºC was significantly lower than that from other treatments ($F_{3,76}$ = 4.34, P = 0.0071) where fecundity was similar (Figure 2.4). Adult males had similar longevity at 15 and 20ºC but their longevity significantly decreased with the increase of temperature from 20 to 30ºC (x_3^2 = 52.88; P < 0.0001) (Figure 2.5A). Adult females survived significantly longer at 20ºC than at 15 and 25ºC, and their longevity was the shortest at 30ºC (x_3^2 = 83.29; P < 0.0001) (Figure 2.5B). As shown in Table 2.2, increasing temperature significantly elevated the intrinsic rate of increase (r_m) but significantly shortened the generation time (T) and the time to double the population size (Dt). The net population growth rate (R_0) was significantly higher at 20 and 25ºC as compared to lower and higher temperatures.

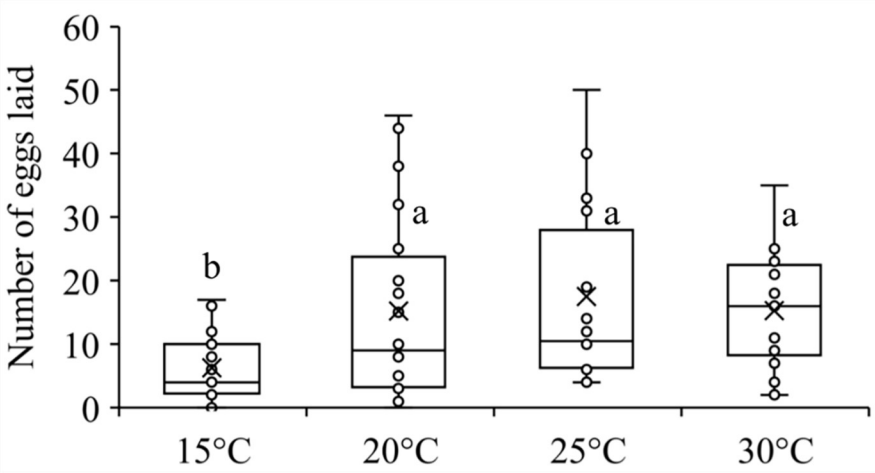

Figure 2.4 Mean (± SE) number of eggs laid by females during their lifetime at different temperatures in *T. ludeni*. Columns with the same letters are not significantly different ($P > 0.05$). For each box plot, the upper and lower box lines represent 75% and 25% of scores falling beyond the upper and lower quartiles, respectively; the '×' and line in a box indicate the mean and median score, respectively; the '⊤' and '⊥' are the upper and lower whiskers showing scores outside the 50% middle; the circles are the outliers of scores.

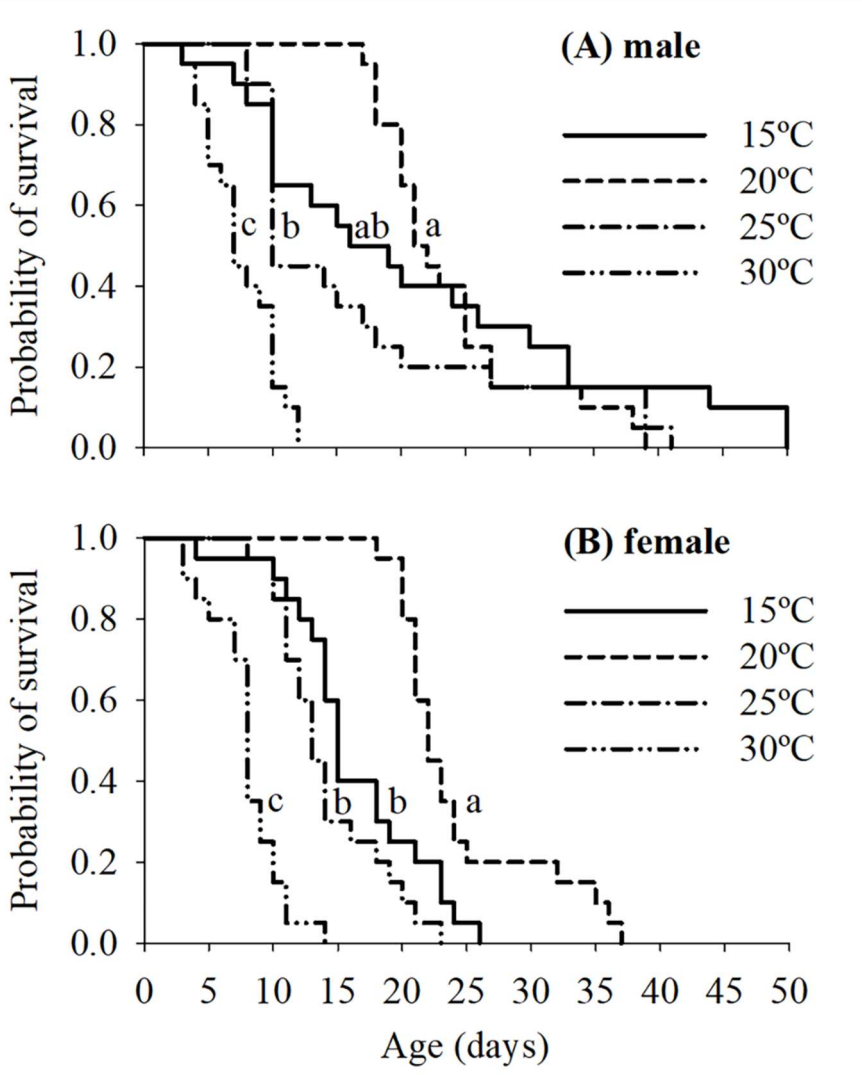

Figure 2.5 Survival of male (**A**) and female adults (**B**) at different temperatures in *T. ludeni*. Lines with the same letters are not significantly different ($P > 0.05$).

Table 2.2 Mean (± SE) life table parameters of *T. ludeni* at different temperatures.

Temperature (ºC)	r_m	R_0	T	Dt
15	0.0219±0.0001 d	1.95±0.02 d	30.35±0.39 a	31.64±0.22 a
20	0.0722±0.0001 c	6.85±0.09 a	26.61±0.16 b	9.60±0.02 b
25	0.1009±0.0005 b	6.55±0.10 b	18.60±0.11 c	6.87±0.04 c
30	0.1281±0.0010 a	4.00±0.05 c	10.81±0.05 d	5.42±0.04 d
$F_{(df)}$	$389.44_{(3,76)}$	$171.55_{(3,76)}$	$302.75_{(3,76)}$	$389.44_{(3,76)}$
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means followed by different letters in columns are significantly different ($P < 0.05$).

2.4 Discussion

I show that the egg hatch rate in the New Zealand strain of *T. ludeni* was > 95% without significant difference between test temperatures from 15–30ºC in the first generation (Figure 2.1A). My finding agrees to that for the Japanese strain exposed to similar temperature range for one generation (Gotoh *et al*. 2015). However, the hatch rate was significantly lower at 30ºC in the second generation than at 25ºC and 30ºC in the first generation (Figure 2.1A), indicating that exposure to 30ºC for more than one generation would reduce egg survival in *T. ludeni*. Immature survival remained similar across test temperatures in the first generation but was higher at 30ºC in the second generation (Figure 2.1B). This could be an adaptive strategy to compensate lower hatch rate at 30ºC in the second generation.

Like other haplodiploid species including spider mites (Roy *et al*. 2003; Macke *et al*. 2011; Tamura & Ito 2017), *T. ludeni* generally had a female-biased sex ratio across our test temperatures (Figure 2.1C). Based on the data from one generation, the sex ratio (proportion of female adults) is higher at 30ºC than at lower temperatures in the Japanese strain of *T. ludeni* (Gotoh *et al.* 2015) and it is more female-biased in *T. mcdanieli* at 15ºC and 34ºC as compared to temperatures between 20ºC and 32ºC (Roy *et al.* 2003). Roy *et al*. (2003) suggest that the increased female-biased sex ratio at extreme temperatures may be an evolutionary response to deteriorating habitats because tetranychid females appear to have better abilities than males to disperse and survive under harsh conditions. However, my findings show that the sex ratio was consistent across all test

temperatures in both generations except for 30ºC in the second generation which was significantly lower (Figure 2.1C). The higher immature survival (Figure 2.1B) and lower sex ratio (Figure 2.1C) at 30ºC in the second generation may be an adaptive response to higher temperature over generations in *T. ludeni*.

Similar to reports on *T. ludeni*'s Japanese strain (Gotoh *et al*. 2015) and other mite species (Margolies & Wrensch 1996; Da Silva 2002; Bazgir *et al*. 2015; Li *et al*. 2015; Zou *et al*. 2018), I found that the New Zealand strain of *T. ludeni* developed significantly faster with the increase of temperature from 15 to 30ºC in both generations (Figure 2.2), supporting the notion that increasing temperature elevates metabolic rate and thus shortens developmental time (Woods & Hill 2004; Potter *et al*. 2009; Schulte 2015). However, compared to the first generation, the second generation developed faster at 15ºC and 20ºC (Figure 2.2). These findings suggest that *T. ludeni* can adjust its developmental period based on its experience in the previous generation. Adaptation to thermal environment over successive generations is also reported in the predatory mite *Amblydromalus limonicus* Garman and McGregor (Acari: Phytoseiidae) (Walzer *et al*. 2020). I propose that *T. ludeni* accelerates its development at lower temperatures to promote an early start of reproduction after experiencing longer development and delayed reproduction in the first generation at these lower temperatures.

I demonstrate that *T. ludeni* adult body size in both generations (Figure 2.3) followed the so-called temperature-size rule where body size decreases with the increase of environmental temperature, as reported in many ectotherms (Atkinson 1994; Walters & Hassall 2006; Klok & Harrison 2013; Pequeno *et al*. 2018). However, the degrees and patterns of size changes in response to temperature differed between sexes as well as between generations. For example, the degree of size variation was greater in females than in males in the first generation while size changes of both sexes were smaller in the second generation (Figure 2.3). These findings have two implications: (1) females are more likely than males to adjust body size in response to temperatures they first experience, and (2) both sexes can adapt to temperatures they have experienced in the previous generation.

I found that temperature-dependent female body size did not translate into fecundity in *T. ludeni* because females that developed from 15–20ºC were larger than those from higher temperatures (Figure 2.3A) but females from 15ºC laid fewer eggs than those from higher

temperatures where there was no difference (Figure 2.4). These findings differ from those for the Japanese strain where highest and lowest fecundity occurs at 20ºC and 30ºC, respectively (Gotoh *et al*. 2015) and for the Brazilian strain where females lay greatest number of eggs at 30ºC (Da Silva 2002). In *T. urticae*, Riahi *et al*. (2013) and Zou *et al*. (2018) report the highest fecundity at 25ºC and 28ºC, respectively. My results indicate that temperature-dependent adult body size was associated with longevity and such association was different between sexes (Figures 2.3 and 2.5). In the first generation, larger male body size at 15–20ºC (Figure 2.3B) translated into longer longevity at the same temperature range (Figure 2.5A) while females had largest body size (Figure 2.3A) and longest longevity (Figure 2.5B) at 20ºC. In several other mite species (Riahi *et al*. 2013; Sugawara *et al.* 2017) and the Japanese strain of *T. ludeni* (Gotoh *et al.* 2015), longevity decreases with the increase of temperature.

In this study, the lower thermal threshold (T_0) and degree days (DD) for *T. ludeni* development from eggs to adults (Table 2.1) fell within the range from 7.8 to 13.8ºC and from 110 to 156 DD, respectively, reported for the genus *Tetranychus* for one generation (Sakunwarin *et al*. 2003; Gotoh *et al*. 2010, 2015; Ullah *et al.* 2011; Karami-Jamour & Shishehbor 2012; Riahi *et al*. 2013; Bayu *et al*. 2017; Hasanvand *et al*. 2019). The varying thermal requirements for development between species or strains of the same species probably reflect their adaptations to the local climate (Gotoh *et al*. 2015). Although the developmental rate and temperature were significantly positively correlated in both generations, T_0 was lower and degree day (DD) was greater in the second generation than in the first generation (Table 2.1). These results also suggest that *T. ludeni* can adapt to temperature changes by adjusting its developmental rate based on its experience in the previous generation. The life table shows that the intrinsic rate of increase (r_m) increased and doubling time (Dt) decreased with the increase of temperatures from 15 to 30ºC (Table 2.2). These may be attributed to the shorter developmental time (Figure 2.2) and more daughters produced during the females' early lifespan at higher temperatures (DR unpublished data). Because population size has a significant effect on reproduction and population growth in *T. ludeni* (Weerawansha *et al*. 2020), the population size changes in response to temperatures can further facilitate its invasion success.

In conclusion, life history traits of *T. ludeni* are highly flexible and adaptive to dynamic thermal environment over generations. I show that higher temperature accelerates its development in both generations, but the development at the lower temperatures is faster in the second than in

the first generation. This suggests that the mite shortens its developmental time at lower temperatures in the second generation to promote an early start of reproduction after experiencing longer development and delayed reproduction in the first generation at these temperatures. Adult body size in both generations decreases with the increase of temperature, with females being more likely than males to adjust body size in response to temperature changes they first experience. Larger body size results in greater longevity but not higher fecundity. Lower thermal threshold and greater degree days (DD) in the second generation than in the first generation suggest that *T. ludeni* can adapt to temperature changes by adjusting its developmental rate based on its experience in the previous generation.

Chapter 3 Dynamics of Life History Traits in *Tetranychus ludeni* Zacher in Response to Fluctuating Temperatures

This chapter was published in *Systematic and Applied Acarology* 24(11): 2272–2277 (2019).

Abstract

Tetranychus ludeni Zacher is an important pest attacking many crops around the world. Understanding its response to fluctuating temperature may provide useful information for anticipating its potential expansion and threat in different regions. Here I examined its life history traits under four fluctuating temperature treatments (15~21, 22~28, 29~35 and 36~42°C). I found that 100% of eggs hatched at 15~21 and 22~28ºC but there was no egg hatched at 36~42°C. The longest developmental period was recorded at 15~21°C, and highest percentage of hatched mites successfully developed to adult stage was recorded at 22~28°C. The proportion of female offspring was significantly higher at 29~35ºC but its body size was significantly smaller, and fecundity was significantly lower than other treatments. Adult longevity and temperature were inversely related in both sexes.

3.1 Introduction

Tetranychus ludeni Zacher (Acari: Tetranychidae) is an invasive spider mite that currently occurs in Europe, Asia, Africa, America, and Australasia (Migeon *et al*. 2010; CABI/EPPO 2011). It feeds on more than 300 plant species in 60 families and causes significant damage to a number of economically important crops such as eggplant, pepper, tomato, bean, pumpkin and other cucurbitaceous plants (Zhang 2002, 2003; Gotoh *et al*. 2015). In their study on effects of constant temperatures on several spider mite species, Gotoh *et al.* (2015) suggest that *T. ludeni* may be better adapted to warmer climate and predict that it could replace *T. urticae* to become a major pest of crops in the world. However, temperature in nature is fluctuating, typically higher during the daytime and lower during the nighttime, which could have different effects on organisms (Fischer *et al.* 2011; Paaijmans *et al.* 2013; Bowden *et al.* 2014; Gotoh *et al*. 2014; Zeh *et al.* 2014; Nachman & Gotoh 2015; Bayu *et al*. 2017). So far, how fluctuating thermal conditions affect life history traits of *T. ludeni* is unknown. In the present study, I investigated the plasticity of life

history traits in response to fluctuating temperatures in *T. ludeni* to provide information for predicting its potential to invade and to become a major pest in different regions or conditions.

3.2 Material and methods

3.2.1 Breeding colony

I maintained a breeding colony of *T. ludeni* on 20 potted kidney bean plants (*Phaseolus vulgaris* L.) in the Massey University Entomology and IPM Laboratory, Palmerston North, New Zealand, at 25 ± 1°C temperature and 50~70% RH with a photoperiod of 16L:8D hours.

3.2.2 Effect of fluctuating temperature

I performed four fluctuating temperature treatments (15~21, 22~28, 29~35 and 36~42°C) with 20 replicates per treatment in growth chambers (I-36VL, Percival Scientific Inc., Perry, Iowa, USA). The temperature setting protocol is shown in Figure 3.1. For each replicate, I randomly collected 20 female and 4 male adults from the colony, introduced them onto a bean leaf disc (3 cm × 3 cm) placed upside down on a water saturated cotton pad in a Petri dish (5.5 cm diameter × 1.0 cm height), and allowed them to stay on the leaf disc for 24 hours at 25°C. I kept 50 eggs on the leaf disc, removed the redundant eggs using a soft paintbrush, and then placed the Petri dish with 50 eggs into a growth chamber at a test temperature. I replaced the leaf disc with a fresh one once every five days. I took each Petri dish from the test temperature once a day for a few minutes and recorded egg hatch, immature stage mortality and adult emergence under a stereomicroscope (Leica MZ12, Germany) at 25°C. During the few minute's observation each day, I also sexed and removed emerged adults and recorded development period (from egg to adulthood) of each sex.

To determine the effect of treatments on adult body size, I randomly selected two females and two males of the resultant adults per replicate and measured their size under a digital camera (Olympus SC30, Japan) connected to the stereomicroscope and a computer with adequate imaging software (CellSens® GS-ST-V1.7, Olympus, Japan) installed. To obtain information on the effect of treatments on reproductive outputs and adult longevity, I randomly selected 20 newly emerged females and 20 newly emerged males (< 1 d old) from each of the above treatments and individually paired them on a leaf disc (2 cm × 2 cm) in a Petri dish as mentioned above. I

maintained all these adults under the same treatment conditions. Twenty-four hours later, I transferred the male to a clean leaf disc of the same size in another Petri dish and recorded its longevity. I allowed the female to lay eggs for 72 hours on the same leaf disc and then transferred her to a new leaf disc of the same size in another Petri dish. I repeated this process until her death, and recorded fecundity and egg hatch rate of all eggs laid by each female. Mite transfer, and fecundity and egg hatch recording were performed at 25°C for a few minutes.

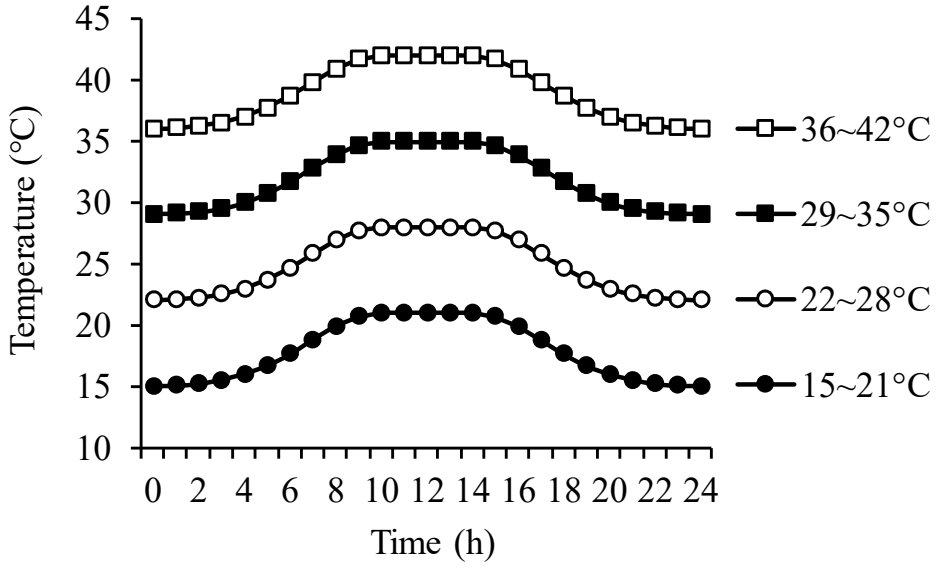

Figure 3.1 Illustration of the hourly temperature change following a modified Gaussian function (Schou *et al*. 2014): Temperature $_{(time)} = a \times e^{-[\frac{(time- \ )^2}{2c^2}]} + d$, where a (= 6) is the amplitude, b (= 10~14) is the time of the peak, c (= 3.16) is the width of the distribution, and d is the nighttime temperature. Time: 0~4 dark, 4~20 light, and 20~24 dark.

3.2.3 Statistical analysis

All data were analysed using SAS 9.4 with a rejection level set at $\alpha < 0.05$. A Shapiro-Wilk test (UNIVARIATE procedure) was used to test the distribution of data. Data on the immature survival rate, and male and female adult body size were normally distributed and thus analysed using an analysis of variance (ANOVA, GLM procedure) followed by Tukey's Studentized range test. Data on the number of eggs laid were ln(x) transformed to achieve normal distribution before ANOVA.

Data on egg hatch rate, and offspring sex ratio and developmental period were not normally distributed even after transformation, and thus analysed using a non-parametric ANOVA (GLM procedure) with a Tukey test for multiple comparisons. Data on adult survival were compared using a Wilcoxon test (LIFETEST procedure).

3.3 Results and discussion

My results show that no eggs hatched at the treatment of 36~42ºC, indicating that this temperature range is lethal to *T. ludeni* eggs. Similarly, Gotoh *et al.* (2015) report that *T. ludeni* and *T. urticae* eggs do not hatch at the constant temperature of 40ºC, and Roy *et al.* (2002) show that *T. mcdanieli* McGregor eggs fail to hatch at ≥ 36ºC. These findings suggest that many *Tetranychus* species may not be able to survive in regions with a period of late 30ºC to early 40ºC in a year, and that air temperature of ca. 40ºC can kill spider mite eggs for postharvest disinfestation.

I show that almost 100% of eggs hatched at 15~21 and 22~28ºC while only about 70% of eggs hatched at 29~35ºC ($F_{2,57}$ = 69.32, P < 0.0001) (Figure 3.2A). Among hatched mites, about 75% developed to adults under 22~28ºC compared to about 60% and 10% at 15~21ºC and 29~35ºC, respectively ($F_{2,57}$ = 224.32, P < 0.0001) (Figure 3.2B). These findings show that 22~28ºC is the optimal thermal condition for survival and development of *T. ludeni*. Based on the results, I propose that this mite may not be able to establish in regions or greenhouses with the daytime temperature ≥ 35ºC. The relatively low survival of immature stages at 15~21ºC may be attributed to significantly longer development period ($F_{2,1413}$ = 2328.85, P < 0.0001) (Figure 3.2C), which may pose higher risk of death (Murphy *et al*. 2018; Esbjerg & Sigsgaard 2019).

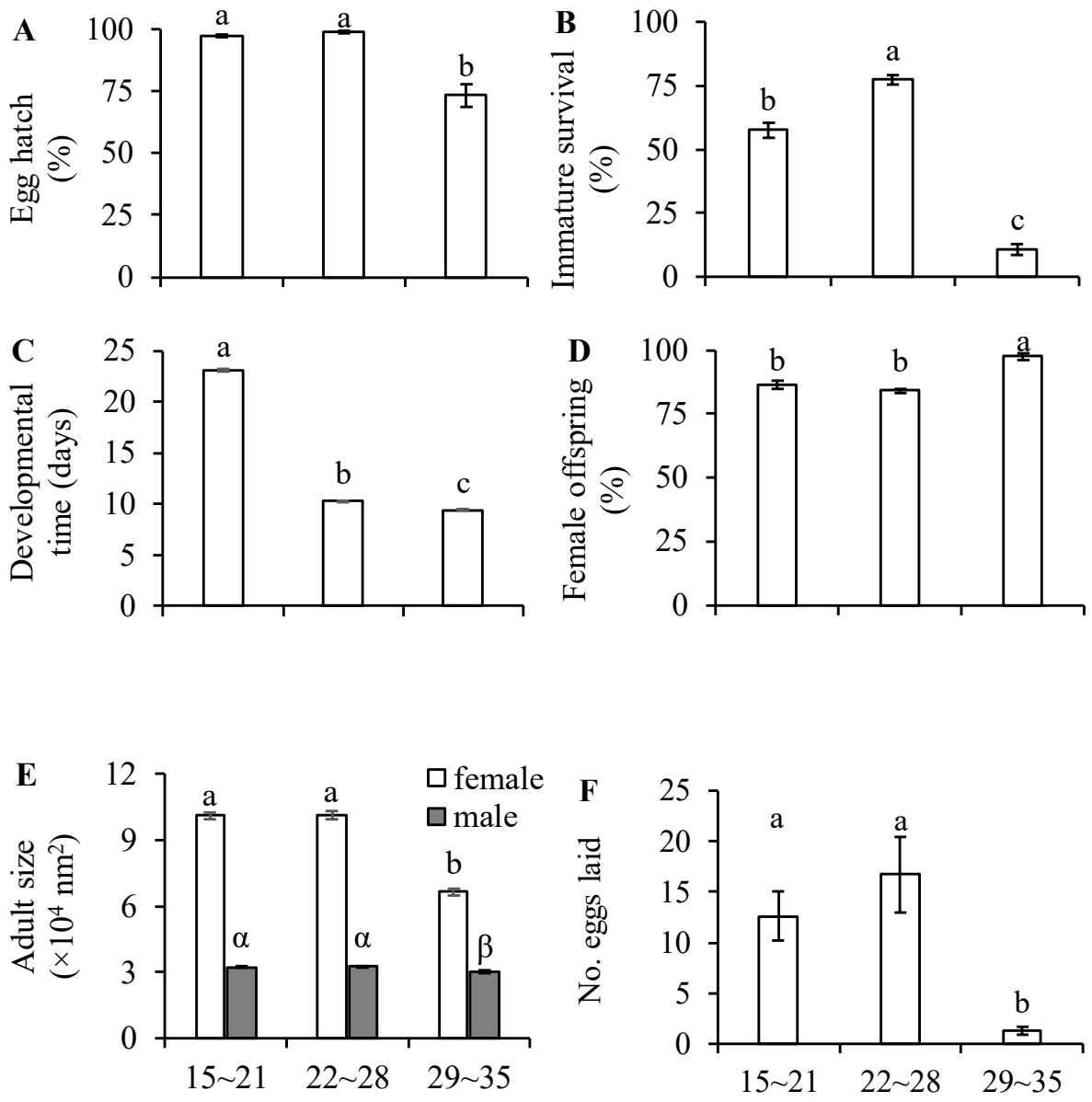

Figure 3.2 Mean (± SE) egg hatch rate (**A**), immature survival rate (**B**), developmental time (**C**), proportion of female offspring (**D**), adult size (**E**), and number of eggs laid (**F**) at different temperature ranges (ºC). For each category, columns with the same letters are not significantly different ($P > 0.05$).

Like many haplodiploid species (Roy *et al.* 2003; Macke *et al.* 2011; Tamura & Ito 2017; Zhou *et al*. 2018), *T. ludeni* generally had a female-biased sex ratio (Figure 3.2D). However, the proportion of female offspring was significantly higher at 29~35ºC than at 15~21 and 22~28ºC ($F_{2,52} = 22.70$, $P < 0.0001$) (Figure 3.2D). Roy *et al.* (2003) suggest that an increasing female-

biased sex ratio in *T. mcdanieli* at extremely high temperatures could be an evolutionary response of spider mites to deteriorating habitats because females have better capacities than males to disperse and survive under harsh conditions.

Adult *T. ludeni* developed from 29~35ºC were significantly smaller ($F_{2,78} = 3.6$, $P = 0.0320$ for male; $F_{2,106} = 149.54$, $P < 0.0001$ for female) (Figure 3.2E) and laid significantly fewer eggs ($F_{2,37} = 7.88$, $P = 0.0014$) (Figure 3.2F) than those from 15~21 and 22~28ºC. The present study supports both theoretical and empirical findings in many organisms, i.e., adult body size decreases with increasing temperature (Atkinson 1994; Walters & Hassall 2006; Klok & Harrison 2013) and female body size and fecundity are positively correlated (Honěk 1993). Furthermore, I demonstrate that adult longevity and temperature were inversely related in both sexes ($x_2^2 = 21.53$, $P < 0.0001$ for males; $x_2^2 = 12.00$, $P = 0.0025$ for females) with females being more tolerant than males at high temperatures ($x_1^2 = 26.76$, $P < 0.0001$ for 29~35ºC) but not at lower temperatures ($x_1^2 = 1.91$ and 0.01 for 15~21 and 22~28ºC, respectively; $P > 0.05$) (Figure 3.3).

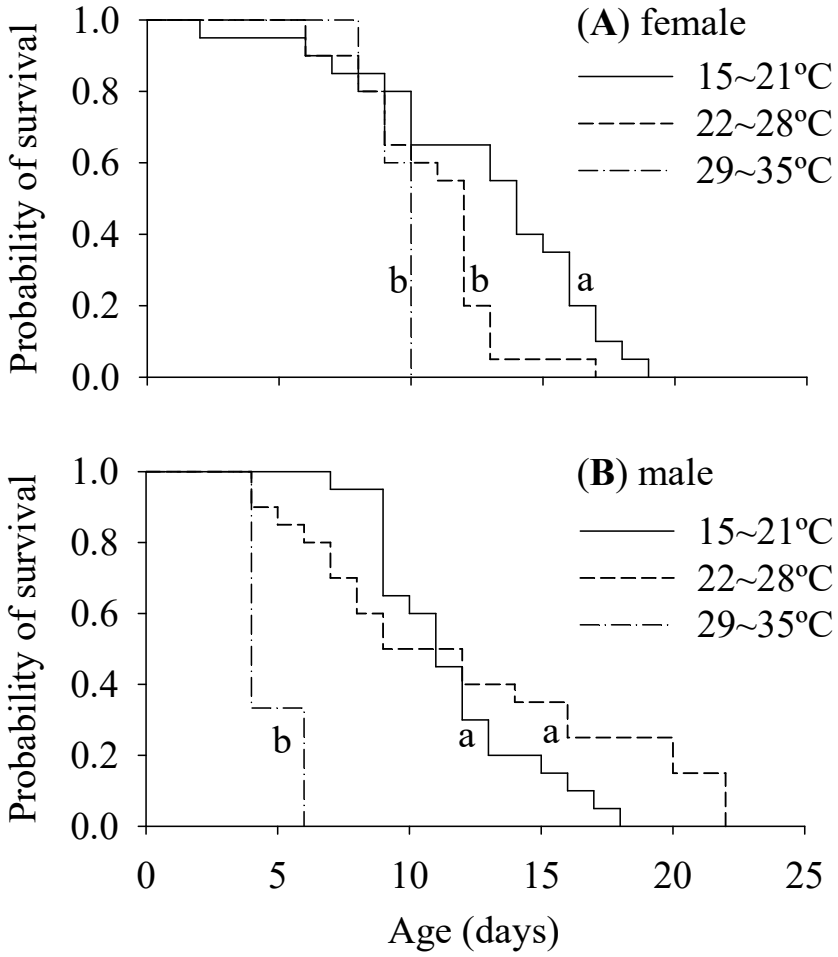

Figure 3.3 Survival of female (**A**) and male adults (**B**) at different fluctuating temperatures. Lines with the same letters are not significantly different ($P > 0.05$).

In summary, *T. ludeni* performs the best at 22~28ºC and the worst at 29~35ºC with no eggs surviving at 36~42ºC, suggesting that this mite is less likely to invade hot to very hot regions. The mite can reproduce well at 15~21ºC but at this temperature range, it develops twice slower and suffers 25% higher immature stage mortality than at 22~28ºC, implying that this mite has some difficulty to establish in cool regions and even if it survives in these regions, it is less likely to become a major pest. My results also suggest that *T. ludeni* can establish in the regions with mild to warm climate after invasion and become an important pest. New Zealand has a mild climate with the South Island being cooler. As a result, the mite population size may be larger in the North Island than in the South Island. Furthermore, it may not be able to survive in the regions with hot climate and greenhouses with a daytime temperature over 30ºC during the summer.

Chapter 4 Thermotolerance in a Spider Mite: Implications in Disinfestation Treatment

This chapter was published in *Systematic and Applied Acarology* 27(3): 473–481 (2022)

Abstract

Tetranychus ludeni Zacher is a European spider mite species and an important invasive pest in horticulture. I investigated the effects of hot air on its survival and reproduction, providing knowledge for development of disinfestation programs using heat. I tested how each life stage responded to heat treatments of five air temperatures (45 to 57°C) and five exposure durations (three to 15 hours). I showed that no eggs hatched after exposed to 45°C for ≥ 15 hours, 48°C for ≥ 12 hours, or 51°C for three hours; no adults survived 51°C or 54°C for ≥12 hours or 57°C for ≥ six hours, and heat tolerance of other life stages fell in between. Higher temperature and longer exposure time also reduced developmental success and fecundity. These findings suggest that we may be able to eradicate the mites of all stages using one hot air treatment at 57°C for six hours or two treatments at 51°C for three hours at a 10-day interval to kill all eggs in the first treatment and those laid by survived adults in the second. The eradication strategy using hot air of 51–57°C may be more suitable for treating plant residues on exported/imported machinery, farm equipment and containers because it may have negative impact on fresh postharvest products. With the knowledge that exposure to 45°C substantially reduced the mites' fecundity, particularly when the younger stages were treated, I suggest that heat treatment of fresh postharvest products with 45°C could still greatly reduce the quarantine risk of this pest.

4.1 Introduction

Heat treatment has been widely used as a non-chemical disinfestation measure for postharvest crops (e.g., Cowley *et al.* 1992; Waddell *et al.* 1993; Jessup *et al.* 1998; Jacobi *et al*. 2001; Macana & Baik 2018). In invertebrates, particularly insects and mites, response to high temperature and exposure duration varies among species (Bertelsmeier *et al*. 2015; Gray 2017; Kingsolver *et al*. 2021) as well as among life stages within species (Heather *et al*. 2002; Kingsolver *et al*. 2011; Gotoh *et al*. 2013; Chiu *et al*. 2014; Hsu *et al*. 2018; Yao *et al*. 2019). Therefore, the effectiveness

of heat disinfestation treatment should be determined by temperature, treatment duration (Dentener *et al*. 1997; Lurie *et al*. 1998; Finkelman *et al*. 2006; Hara 2013) and life stages treated (Heard *et al*. 1992; Heather *et al*. 2002; Gotoh *et al*. 2013; Hsu *et al*. 2018). However, heat tolerance benchmarks have not yet been established for most pest species.

Tetranychus ludeni Zacher (Acari: Tetranychidae) is a European spider mite species but has invaded many regions, including Africa, America, Asia, and Oceania (CABI/EPPO 2011; Zhou *et al*. 2021), and become an important pest of many crop species globally (Zhang 2003; Kaimal & Ramani 2011b). However, some countries still require disinfestation treatment of postharvest products for *T. ludeni*. For example, Korea bans *T. ludeni*-infested fruit and vegetables (MPI 2019). Several workers have tested the effectiveness of heat treatment to disinfest the spider mite *T. urticae* Koch on postharvest products, showing promising outcome (Waddell & Birtles 1992; Waddell *et al*. 1993; Gotoh *et al*. 2013). However, thermotolerance has yet to be established for *T. ludeni*. It is also unknown how high temperature could affect its reproduction.

In the present study, I investigated how each life stage responded to high temperature exposure in *T. ludeni*. I exposed all life stages to five temperatures for five durations and recorded their mortality rates, and developmental success and reproductive fitness of survived individuals. Information presented here is essential for development of heat disinfestation programs for this important pest. It may also provide knowledge for future evaluation of its invasion potential in relation to heat waves caused by climate change.

4.2 Materials and methods

4.2.1 Experimental mite preparation

I collected *T. ludeni* adults from *Passiflora mollissima* (Kunth) (Malpighiales: Passifloraceae) in Palmerston North, New Zealand, in 2017. A breeding colony from these adults was established and maintained on 20 potted kidney bean plants *Phaseolus vulgaris* L. (Fabales: Fabaceae) in the Entomology and IPM Laboratory of Massey University, New Zealand. I replaced ten oldest plants with new ones every two weeks by cutting the infested leaves of old plants and placing them on the top of new ones. The mite colony was maintained, and experimental mites prepared at 25 ± 1°C and 50~70% RH with a photoperiod of 16L:8D hours.

I randomly collected 20 adult females and four adult males from the colony and transferred them onto a bean leaf disc (3 cm × 3 cm) positioned upside down on a water-saturated cotton pad in a Petri dish (5.5 cm diameter × 1.0 cm height). The adults were allowed to stay on the leaf disc for 24 hours and then removed. The larvae were transferred to a new leaf disc of the same size immediately after the eggs laid by these adults hatched. I reared them at 25°C for 1, 4, 6 and 8 days to obtain larvae, protonymphs, deutonymphs and female adults, respectively, for experiment. In total, I set up 800 such leaf discs. To obtain adult males, I randomly collected 20 female deutonymphs from the colony, placed them on a leaf disc as above and allowed the newly emerged virgin females to lay eggs for 24 hours. The newly hatched larvae were then transferred to a new leaf disc and allowed to develop to adult males for the experiments.

4.2.2 Heat-dependent mortality rate in each life stage

To determine heat-dependent mortality rate of mites of each life stage, I treated eggs, larvae, protonymphs, deutonymphs, virgin adult males and females with five temperatures (45, 48, 51, 54 and 57°C) for five heat durations (3, 6, 9, 12 and 15 hours), resulting in a total of 150 treatments (6 life stages × 5 temperatures × 5 durations). There were 20 replicates for each treatment. For each replicate, 10 individuals were introduced onto a bean leaf disc (3 cm × 3 cm) on a water-saturated cotton pad in a Petri dish (5.5 cm diameter × 1.0 cm height) and then the dish was transferred into an incubator (Series Five, Contherm Scientific Company, New Zealand) with a treatment temperature. Immediately after treatment, I moved the Petri dishes to 25°C, and transferred all life stages except eggs onto new leaf discs in Petri dishes as above. Eggs that did not hatch in 10 days and individuals of other life stages that had no sign of movement 48 hours after treatment were considered as dead.

4.2.3 Effect of heat treatment on immature development to adult stage

To determine the probability of immature mites surviving heat-treatment to develop to adulthood, I randomly took up to 20 individuals that survived from each treatment of the above experiment and reared them individually at 25ºC on leaf discs as above. The leaf discs were replaced with new ones every 4 days and the total number of adults that emerged from these juveniles was recorded. However, if no juveniles survived in some treatments, I did not follow their development.

4.2.4 Effect of heat treatment on reproduction

To determine how heat treatment affected reproduction, I individually transferred up to 20 females that were heat-treated during the adult stage and 20 newly emerged females that developed from each heat-treated immature stage in the above experiment onto leaf discs and reared them at 25 on leaf discs as above. The mortality of females was monitored daily and leaf discs were replaced with new ones once every 4 days if they were still alive. The total number of eggs laid by each female was recorded. However, if no individuals survived in some treatments, I did not follow their reproduction in those treatments.

4.2.5 Statistical analysis

All data analyses were performed using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC). I analyzed the mortality rate (%) of different life stages using a generalized linear mixed model (GLIMMIX procedure) with temperature, exposure duration and their interactions as the fixed factors and replicate as a random effect followed by a Binomial distribution and a Logit link function for the model. A Tukey-Kramer test was used to compare the difference in the mortality rate between temperatures of a given duration and between durations of a given temperature within each life stage. The same method was used to compare the difference in overall mortality rate between life stages. The proportions of individuals that developed to adult stage after being treated at different temperatures for various durations were analyzed using a likelihood ratio test in a logistic regression model (GENMOD procedure) with a Binomial distribution and a Logit function used to the model and the CONTRAST statement for multiple comparisons. The generalized linear mixed model (GLIMMIX procedure) with a Poisson distribution and a Log link function was applied to determine the combined effect of temperature and duration on the number of eggs laid, followed by a Tukey-Kramer test for multiple comparisons.

4.3 Results

4.3.1 Heat-dependent mortality rate in each life stage

In each life stage, temperature and exposure duration significantly interacted ($F_{16,456}$ = 4.26~14.10, $P < 0.0001$), with higher temperature ($F_{4,76}$ = 8.26~136.64, $P < 0.0001$) and longer exposure ($F_{4,76}$ = 26.96~162.94, $P < 0.0001$) causing significantly higher mortality (Table 4.1). At any treatment duration no eggs hatched when temperature was 51°C or higher, and all life stages died at 57°C of any treatment duration except about 10% of adult females and 1% of deutonymphs which survived 57°C for 3 hours (Table 4.1). Our results also show that younger life stages, particularly eggs, were significantly more susceptible to heat treatment [overall mean (± SE) mortality rate (%) = 93.1 ± 0.9 for egg, 83.1 ± 0.9 for larva, 75.9 ± 1.1 for protonymph, 75.4 ± 1.1 for deutonymph, 71.8 ± 1.5 for adult male and 62.7 ± 1.7 for adult female; $F_{5,2975}$ = 529.33, $P < 0.0001$] (Table 4.1).

Table 4.1 Mean (± SE) mortality rates (%) of different life stages of *T. ludeni* after treatment at different temperatures for various durations

Life stage	Duration	Temperature (ºC)					$F_{4,76}$	P
	(hours)	45	48	51	54	57		
Adult female	3	11.5 (±3.2) D d	14.0 (±2.7) D d	25.0 (±5.6) D c	42.5 (±5.2) C b	89.0 (±3.5) B a	123.90	< 0.0001
	6	13.0 (±3.2) D d	14.0 (±3.1) D d	47.0 (±4.8) C c	85.5 (±4.3) B b	100 A a	162.94	< 0.0001
	9	21.5 (±3.4) C c	23.5 (±4.0) C c	54.5 (±4.4) B b	100 A a	100 A a	143.12	< 0.0001
	12	34.0 (±3.7) B c	61.5 (±6.3) B b	100 A a	100 A a	100 A a	129.14	< 0.0001
	15	47.0 (±3.8) A c	85.5 (±4.2) A b	100 A a	100 A a	100 A a	98.55	< 0.0001
	$F_{4,76}$	42.08	136.64	114.95	109.68	12.99		
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
Adult male	3	15.5 (±2.8) D e	29.5 (±3.7) D d	68.0 (±6.0) C c	86.5 (±5.3) C b	100 A a	147.17	< 0.0001
	6	15.0 (±3.9) D e	36.5 (±4.9) C d	81.0 (±3.2) B c	90.0 (±4.2) C b	100 A a	156.14	< 0.0001
	9	27.5 (±3.4) C e	37.5 (±3.2) C d	85.0 (±2.9) B c	94.5 (±3.1) B b	100 A a	145.79	< 0.0001
	12	44.5 (±4.4) B b	49.0 (±6.2) B b	100 A a	100 A a	100 A a	118.12	< 0.0001
	15	54.5 (±6.1) A c	79.5 (±4.3) A b	100 A a	100 A a	100 A a	80.03	< 0.0001
	$F_{4,76}$	52.45	54.25	40.01	13.91	0		
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	1		
Deutonymph	3	40.5 (±4.0) D c	43.5 (±5.8) D c	56.5 (±2.0) C b	59.0 (±3.6) B b	99.0 (±0.9) A a	54.92	< 0.0001
	6	52.0 (±4.8) C c	53.0 (±3.9) C bc	59.5 (±2.3) C b	64.5 (±4.9) B b	100 A a	40.46	< 0.0001
	9	54.0 (±4.1) BC d	67.0 (±3.3) B c	85.0 (±4.4) B b	96.5 (±1.8) A a	100 A a	66.22	< 0.0001
	12	59.0 (±3.0) B d	72.5 (±4.1) ABc	88.5 (±2.7) B b	95.5 (±1.5) A a	100 A a	55.57	< 0.0001
	15	68.5 (±2.7) A d	76.5 (±4.1) A c	94.0 (±1.8) A b	100 A a	100 A a	47.20	< 0.0001
	$F_{4,76}$	16.60	29.66	53.40	72.59	0.16		
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.9572		
Protonymph	3	38.0 (±2.8) C c	40.5 (±4.3) Dbc	44.0 (±3.9) D bc	45.5 (±3.5) C b	100 A a	54.52	< 0.0001
	6	47.5 (±4.9) C e	60.5 (±3.9) C d	76.5 (±3.6) C c	82.0 (±3.4) B b	100 A a	57.21	< 0.0001
	9	54.5 (±4.7) B e	62.0 (±4.2) C d	78.0 (±3.8) BCc	91.0 (±2.8) A b	100 A a	56.79	< 0.0001
	12	61.5 (±4.0) B e	76.5 (±3.6) B d	82.5 (±2.9) B c	89.5 (±3.1) A b	100 A a	38.33	< 0.0001
	15	81.5 (±2.8) A b	86.5 (±3.1) A b	100 A a	100 A a	100 A a	26.96	< 0.0001
	$F_{4,76}$	38.60	46.20	61.98	78.47	0		
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	1		
Larva	3	51.5 (±6.1) C b	56.0 (±3.8) D b	58.0 (±4.2) D b	90.0 (±3.1) B a	100 A a	64.92	< 0.0001

	6	62.5 (±4.0) B c	64.0 (±3.7) C c	78.0 (±3.0) C b	93.0 (±2.2) B a	100	A a	47.34	< 0.0001
	9	64.5 (±3.9) B c	71.0 (±2.7) B c	89.5 (±2.6) B b	96.5 (±1.3) A a	100	A a	49.63	< 0.0001
	12	76.5 (±3.5) A b	76.5 (±3.3) B b	95.0 (±1.4) A a	100 A a	100	A a	37.46	< 0.0001
	15	75.5 (±2.9) A c	82.5 (±3.6) A b	98.5 (±0.8) A a	99.5 (±0.5) A a	100	A a	36.74	< 0.0001
	$F_{4,76}$	17.27	17.99	54.22	8.26	0			
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	1			
Egg	3	32.0 (±6.8) D c	67.0 (±4.8) B b	100 A a	100 A a	100	A a	128.52	< 0.0001
	6	60.0 (±7.4) C c	95.0 (±2.2) A b	100 A a	100 A a	100	A a	75.79	< 0.0001
	9	76.5 (±5.0) B b	98.0 (±0.9) A a	100 A a	100 A a	100	A a	38.31	< 0.0001
	12	99.0 (±1.0) A a	100 A a	100 A a	100 A a	100	A a	0.16	0.9572
	15	100 A a	100 A a	100 A a	100 A a	100	A a	0	1
	$F_{4,76}$	107.95	55.57	0	0	0			
	P	< 0.0001	< 0.0001	1	1	1			

Mortality rate with the same small letters in rows or the same capital letters in columns within each life stage are not significantly different ($P > 0.05$)

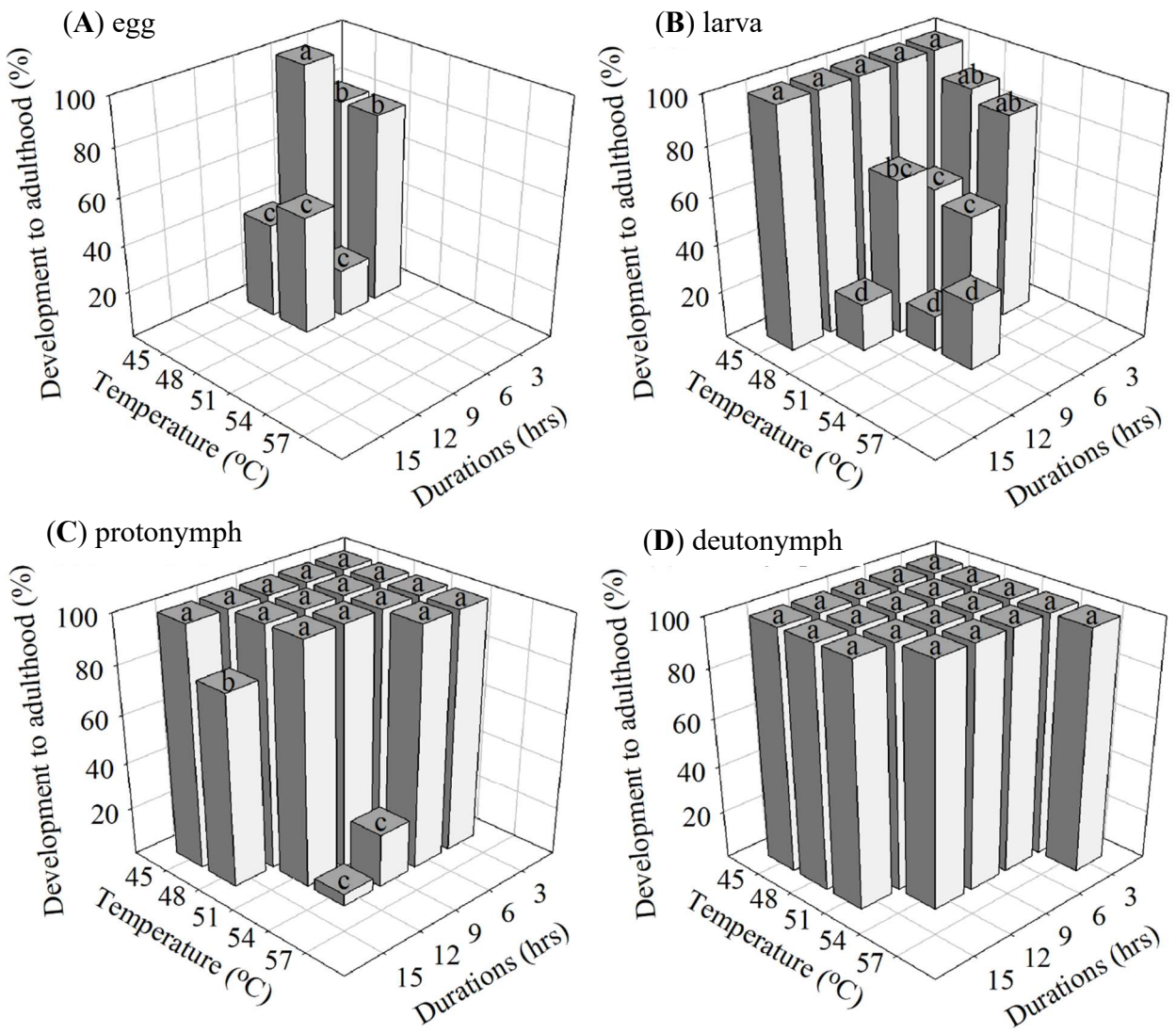

Figure 4.1 Proportion of immatures surviving heat treatment that developed to adult stage in *T. ludeni.* For each life stage, columns with the same letters are not significantly different ($P > 0.05$).

4.3.2 Effect of heat treatment on immature development to adult stage

I show that increasing treatment temperature and exposure duration significantly reduced the probability of immatures to develop to adulthood (x_5^2 = 34.71, $P < 0.0001$ for egg; x_{12}^2= 136.25, $P < 0.0001$ for larva; x_{17}^2 =180.34, $P < 0.0001$ for protonymph; x_{19}^2 = 0, $P = 1$ for deutonymph) (Figure 4.1). These findings also indicate that older immatures were significantly more likely to complete development across treatments (x_3^2 = 158.64, $P < 0.0001$) (Figure 4.1).

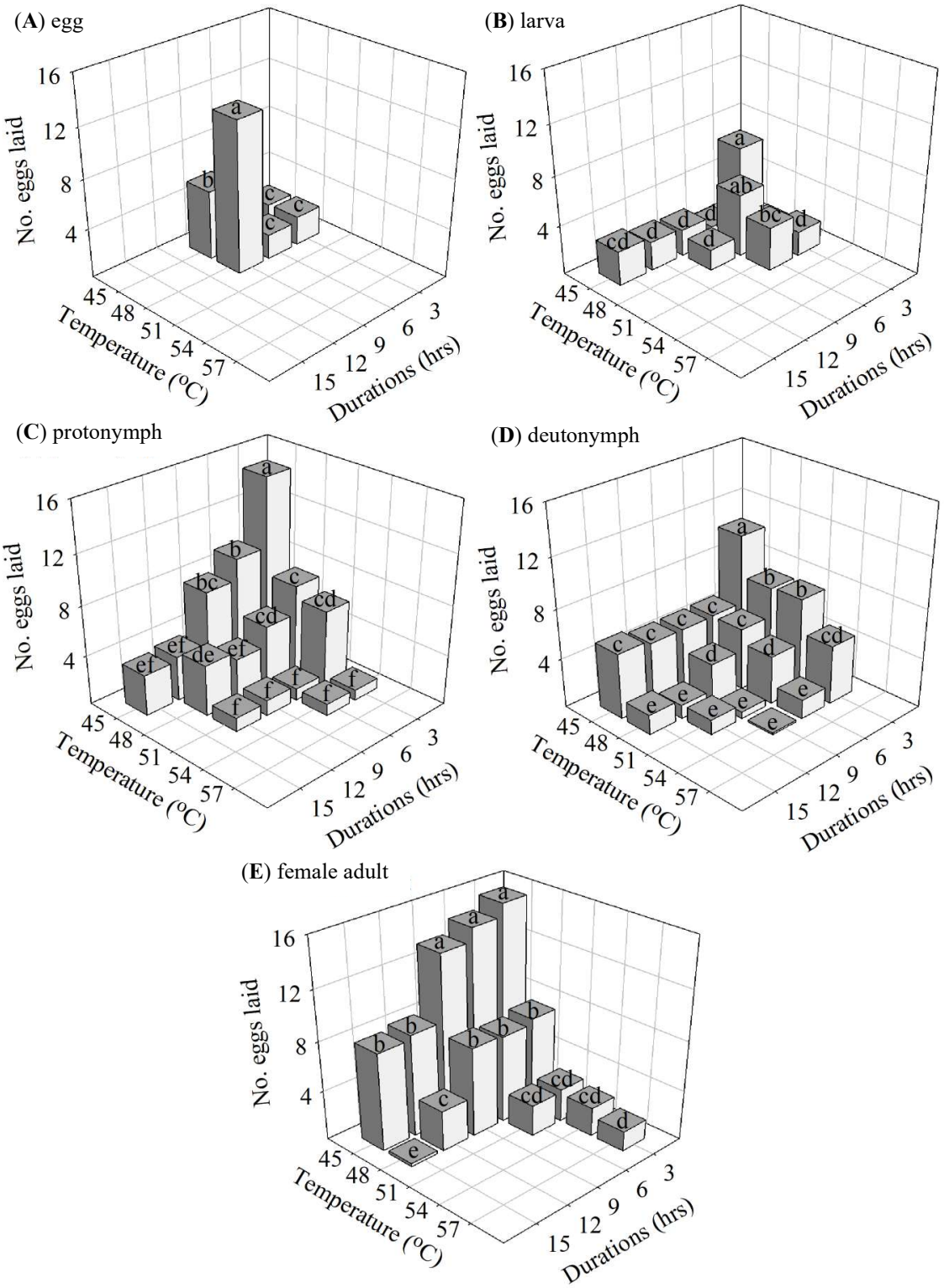

Figure 4.2 Mean number of eggs laid by *T. ludeni* females after their or their juvenile stages' exposure to different temperatures for various durations. For each life stage, columns with the same letters are not significantly different ($P > 0.05$).

4.3.3 Effect of heat treatment on reproduction

Females that resulted from eggs treated at > 48°C (Figure 4.2A) or larvae treated at > 51°C for any duration (Figure 4.2) did not lay any eggs. Those that developed from other treated life stages laid significantly fewer eggs with the increase of temperature and exposure duration ($F_{14,271} = 45.48$, $P < 0.0001$ for protonymph; $F_{16,310} = 26.68$, $P < 0.0001$ for deutonymph; $F_{13,266} = 54.32$, $P < 0.0001$ for adult female) (Figure 4.2C–E).

4.4 Discussion

Here I reported the responses of *T. ludeni* to air temperatures ranged from 45 to 57°C for three to 15 hours. I showed that higher temperature and longer exposure caused higher mortality to all life stages, but younger life stages were more sensitive to heat than older ones, with adults being most tolerant and eggs most susceptible (Table 4.1). All adults died after exposure to 51°C or 54°C for 12 hours or 57°C for ≥ six hours, while no eggs survived 45°C for ≥ 15 hours, 48°C for ≥ 12 hours, or ≥ 51°C for any duration. I also demonstrate that higher temperature and longer exposure time reduced the probability of heat-treated juveniles to successfully develop to adulthood, but older immatures were more likely to complete development across treatments (Figure 4.1). Females that developed from heat-treated eggs or larvae laid only a few eggs and those from other treated life stages laid fewer eggs with increasing temperature and exposure time (Figure 4.2).

Heat treatment has been used or trialed for killing insects (e.g., Cowley *et al*. 1992; Dentener *et al*. 1997; Jessup *et al*. 1998; Jacobi *et al*. 2001; Hara 2013; Macana & Baik 2018) and mites (Waddell & Birtles 1992; Waddell *et al*. 1993; Gotoh *et al*. 2013) on postharvest products. In *T. ludeni*, the egg stage was the least tolerant to air heat with all eggs killed at 51°C within three hours (Table 4.1). Because all life stages may be present at the same time due to short life cycle and overlapping generations (Adango *et al*. 2006; Ristyadi *et al*. 2019) and some individuals of life stages other than eggs may survive this temperature (Table 4.1), two treatments of 51°C for three hours at a 10-day interval can fully disinfest products with all eggs being killed in the first treatment and those laid by survived individuals destroyed in the second. Alternatively, we can eradicate the mites of all stages using one treatment of 57°C for about six hours.

However, the full disinfestation strategy using hot air of 51–57°C may be more suitable for treating plant residues on exported/imported machinery, farm equipment and containers because it may have negative impact on fresh postharvest products. With the knowledge that exposure to 45°C for a few hours substantially reduced the mites' fecundity, particularly when the younger stages were treated, I suggest that heat treatment of fresh postharvest products with 45°C could still considerably reduce the quarantine risk of this pest. Moreover, Auger *et al*. (2003) report that wettable sulphur can kill all stages of *T. urticae* at the air temperature of 35°C. I thus predict that air temperature much lower than 45°C for shorter than three hours can achieve complete disinfestation for *T. ludeni* if a chemical like wettable sulphur is also used. Further investigation into possible combinations of chemical and heat treatments for this pest would provide valuable information for exporters.

In conclusion, I demonstrate that heat shock tolerance is stage-dependent in *T. ludeni* with eggs being the most vulnerable and adults the most tolerant. Heat shock also reduces developmental success and fecundity. We may fully disinfest plant residues on machinery, equipment and containers using two treatments of 51°C for three hours at a 10-day interval or one treatment of 57°C for about six hours, and substantially reduce quarantine risk by treating fresh products with 45°C for a few hours. This study provides important knowledge for development of hot air disinfestation programs of this invasive pest. Information presented here may offer a reference for future evaluation of its invasion potential in relation to heat waves caused by climate change.

Chapter 5 Resource Allocation Strategies for Survival and Reproduction by an Invasive Pest, *Tetranychus ludeni*, in Response to Intermittent Fasting

This chapter was submitted to *Current Zoology* for publication.

Abstract

Intermittent fasting (IF) is a type of dietary restriction that involves fasting periods in intervals, which has been used as a strategy to improve health and extend longevity. Regular fasting is common during the process of biological invasions in nature. Yet, it is not clear how invasive animals adjust their resource allocations to survival and reproduction when periodical starvation occurs. Here, I used *Tetranychus ludeni*, a haplodiploid spider mite and an important invasive pest of horticultural crops around the world, to investigate the effects of IF on its life history strategies. I show that IF increased the longevity in females but not in males probably because of differences in resource storage, metabolic rate, and mating cost between sexes. In response to IF, females traded off fecundity and egg size but not the number of daughters for longevity gain, suggesting that *T. ludeni* females can adjust their life history strategies for population survival and growth during invasion process. Eggs produced by fasted females realized the same hatch rate and resultant young had the same survival rate as those by unfasted ones. In addition, IF had transgenerational maternal effects which prolonged offspring development period. I suggest that the longer immature developmental period can increase the body size of resulting adults, compensating egg size loss for offspring fitness. My findings provide insight into adaptive resource allocations as responses to fasting, knowledge of which can be used for evaluation of pest invasions and for management of animal survival and reproduction by dietary regulations.

5.1 Introduction

Intermittent fasting is a type of dietary restriction that involves fasting periods in intervals (Catterson *et al*. 2018; Chambers 2019; Gunnars 2020). It may reduce oxidative damage (de Azevedo *et al*. 2013), improve cardiovascular health (Abdellatif & Sedej 2020), increase hepatic

metabolism capacity (Barboza & Jorde 2002), and enhance glucose tolerance (Wei *et al*. 2019). As a result, it should benefit overall health and longevity of animals (Anson *et al*. 2003; Brandhorst *et al*. 2015; Lee & Longo 2016). Dietary restriction is probably the most reproducible and effective method to regulate aging and increase healthy lifespan in many species (Gribble *et al*. 2014; Lee & Longo 2016; Wang *et al*. 2017; Liang *et al*. 2018). However, its effect on longevity is not always consistent (Longo & Mattson 2014). For instance, it extends the lifespan of mice if starting at the younger age but not at older age (Goodrick *et al*. 1990), prolongs the female longevity in a spider mite when practiced during the adult stage (Li & Zhang 2019a), but shortens the female longevity in a predatory mite (Gotoh & Tsuchiya 2009).

The longevity discrepancy in response to fasting may be attributed to different reproductive modes (Li & Zhang 2019a) and trade-offs among reproduction, growth, and somatic maintenance (Merry 2002; Kirkwood 2005) in different taxa. Under limited pool of energy available, organisms may change their resource allocation strategies to maintain the balance between different fitness functions (Stearns 1992; Bancroft & Margolies 1996; Martin *et al*. 2007). However, previous work on the effect of intermittent fasting focuses more on lifespan and less on reproduction (Li & Zhang 2019a). Furthermore, most investigations use model animals as study material, such as fruit flies, worms, and mice (Nakagawa *et al*. 2012). It is thus important to understand how non-model organisms manage the trade-offs between reproduction and other life functions in response to dietary restriction (Gladyshev 2016; Li & Zhang 2019a). Haplodiploid animals could be ideal alternative models for investigating such trade-offs because both virgin and mated females can reproduce where the former give birth to haploid sons and the latter produce both diploid daughters and haploid sons (de la Filia *et al*. 2015; Lohse & Ross 2015).

Tetranychus ludeni Zacher (Acari: Tetranychidae) is a haplodiploid spider mite from Europe but has now invaded all continents except Antarctic (Migeon *et al*. 2010; CABI/EPPO 2011). It infests more than 300 plant species in 60 families, including many economically important crops such as bean, papaya, guava, eggplant, pumpkin, and apple (Bolland *et al*. 1998; Zhang 2003). Previous studies suggest that its invasion success may be facilitated by its flexible reproductive strategies (Zhou *et al*. 2020, 2021) and good adaptation to external environment (Zhang 2003; Gotoh *et al*. 2015; Ristyadi *et al*. 2019, 2021). Like many other invasive animals (Zhang *et al*. 2019; Papach *et al.* 2020), *T. ludeni* may encounter intermittent food shortages when senescence of their host plants occurs or when they are carried by wind or transportation to new

habitats. To date, little is known about how periodical food shortages affect life history traits in *T. ludeni* and whether this species has developed strategies for invasion success in response to periodical starvation.

In the present study, I investigated how intermittent fasting influenced both males and females in *T. ludeni*. I set up a series of intermittent fasting regimes during the adult stage and recorded their longevity, reproductive outputs, and offspring fitness. My findings provide new insights into how these mites adjust their resource allocation strategies under different intermittent fasting conditions. This work also generates important knowledge for evaluation of their population dynamics under food stress environment during different invasion stages and information for management of animal survival and reproduction by dietary regulations.

5.2 Material and methods

5.2.1 Breeding colony and experimental mite preparation

A breeding colony of *T. ludeni* was reared on about 20 potted kidney bean plants (*Phaseolus vulgaris* L.), in the Massey University Entomology and IPM Laboratory, Palmerston North, New Zealand. I replaced 10 oldest plants with new ones fortnightly and transferred mites from the old plants to the new ones by cutting the leaves of old plants and placing them on the top of new ones. The mite colony was maintained, and experiments carried out at 25 ± 1°C temperature and 50–70% RH with a photoperiod of 16L:8D hours.

I randomly collected female deutonymphs from the colony and introduced them onto 10 fresh bean leaf squares (3 cm × 3 cm, 30 individuals per leaf square), each being placed on a water-saturated cotton pad in a Petri dish (5.5 cm diameter × 1.0 cm height). I allowed them to develop to virgin adult females for experiment. To obtain virgin adult males for experiment, I randomly collected 20 newly emerged virgin females prepared above, placed them on a leaf square (3 cm × 3 cm), and allowed them to lay eggs for 24 hours. I prepared six such leaf squares. I allowed the eggs laid by virgin females on the leaf square (≈ 20–30 eggs) to develop to virgin male adults on the leaf square.

5.2.2 Effects of intermittent fasting on life history traits

To determine the effect of intermittent fasting on lifespan and reproduction in *T. ludeni*, I treated virgin and mated females and virgin and mated males with three intermittent fasting durations, i.e., 0, 24, and 48 hours, resulting in 12 treatments (4 life forms × 3 fasting durations). I set up maximum duration of fasting up to 48 hours as it has been recorded as the maximum duration of fasting that can be tolerated by *T. urticae* without causing extreme negative effects (Li & Zhang 2018). Each treatment had 25 replicates with one individual per replicate. Immediately after emergence I transferred a male and a female together (for mated mites) or an individual female or male (for virgin mites) onto a leaf square (2 cm × 2 cm) placed on a water-saturated cotton pad in a Petri dish (5.5 cm diameter × 1.0 cm height) using a soft paint brush. I allowed these mites to feed for 24 h and then transferred them (mated and virgin) individually once a day onto a new leaf square (0-h fasting treatment) or a green plastic sheet (2 cm × 2 cm) (Colourhide, China) placed on a water-saturated cotton pad in a Petri dish. I used the mites that stayed on plastic sheets for 24 and 48 h for treatments of 24-h and 48-h fasting, respectively. I treated the mites with three intermittent fasting periods from emergence to death using the following fasting sequence: 24-h feeding → 0-, 24- or 48-h fasting → 24-h feeding → 0-, 24- or 48-h fasting → 24-h feeding → 0-, 24- or 48-h fasting → continuous feeding until death (Figure 5.1). I set up the above fasting protocol because *T. ludeni* mate immediately after emergence (Puttaswamy & Channabasavanna 1979; Jose & Shah 1989), start laying eggs on the second day into adulthood and oviposition period lasts 7 to 12 days (Kaimal & Ramani 2011b; Rode *et al*. 2018).

I checked the mites daily and recorded the pre-oviposition period (from an adult female emergence to its first egg being laid), oviposition period (from the first egg being laid to the last egg being laid by an adult female), daily fecundity (the number of eggs produced by a female per day), lifetime fecundity (total number of eggs produced by a female), and adult lifespan (from emergence to death). I randomly selected two eggs laid by each female daily and measured the egg size under a digital camera (Olympus SC30, Japan) connected to the stereomicroscope and a computer with adequate imaging software (CellSens® GS-ST-V1.7, Olympus, Germany) installed, without removing these eggs from the original leaf square/plastic sheet. I allowed all eggs laid on each leaf square/plastic sheet to hatch. Upon hatching, I transferred neonate mites to a fresh leaf square and allowed them to feed on the leaf square for five days, and then transferred

them to a new leaf square once every five days until they developed to adults. I recorded the egg hatch rate, adult emergence rate, number of daughters produced, and developmental period (from eggs to adults).

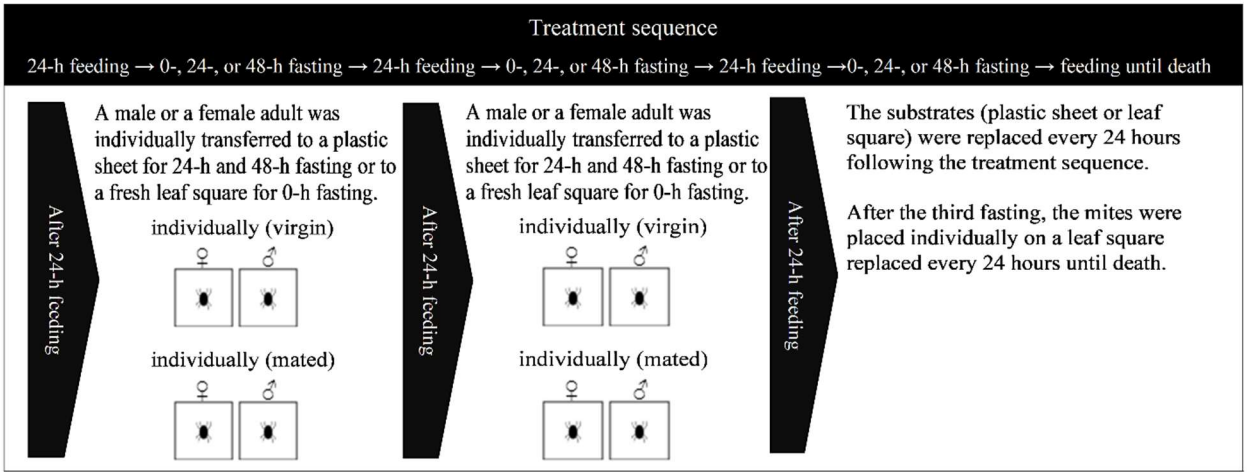

Figure 5.1 Diagram of intermittent fasting sequence.

5.2.3 Statistical analysis

I analysed all data using SAS 9.4 with a rejection level set at $\alpha < 0.05$. Data on adult survival were compared using a Wilcoxon test (LIFETEST procedure). Data on the oviposition period, emergence rate of virgin females' offspring, fecundity of virgin females, numbers of daughters produced by mated females, arsine-square-root transformed emergence rate of mated females' offspring and square-root transformed fecundity of mated females were normally distributed, and thus analysed using an analysis of variance (ANOVA, GLM procedure) followed by Tukey's Studentized range test for multiple comparisons. Data on preoviposition period, egg hatch rate, egg size, and developmental period of offspring were not normally distributed even after transformation, and thus analysed using a non-parametric ANOVA (GLM procedure) with a Bonferroni test for multiple comparisons.

5.3 Results

5.3.1 Effect of intermittent fasting on adult survival and preoviposition and oviposition periods

Intermittent fasting of 48 h significantly prolonged female longevity (virgin female: x_2^2 = 8.80, P = 0.0123; mated female: x_2^2 = 11.94, P = 0.0026) (Figure 5.2A–B). Fasting had no significant impact on virgin male survival (x_2^2 = 1.74, P = 0.4184) (Figure 5.2C) but intermittent fasting of 48 h significantly reduced survival probability in mated males (x_2^2 = 9.30, P = 0.0096) (Figure 5.2D). Fasting significantly prolonged the preoviposition period ($F_{2,48}$ = 101.42, P < 0.0001 for virgin females; $F_{2,61}$ = 4.26, P < 0.0186 for mated females) (Figure 5.3A) but had no significant effect on oviposition period ($F_{2,48}$ = 0.06, P = 0.9402 for virgin females; $F_{2,61}$ = 2.25, P = 0.1140 for mated females) (Figure 5.3B).

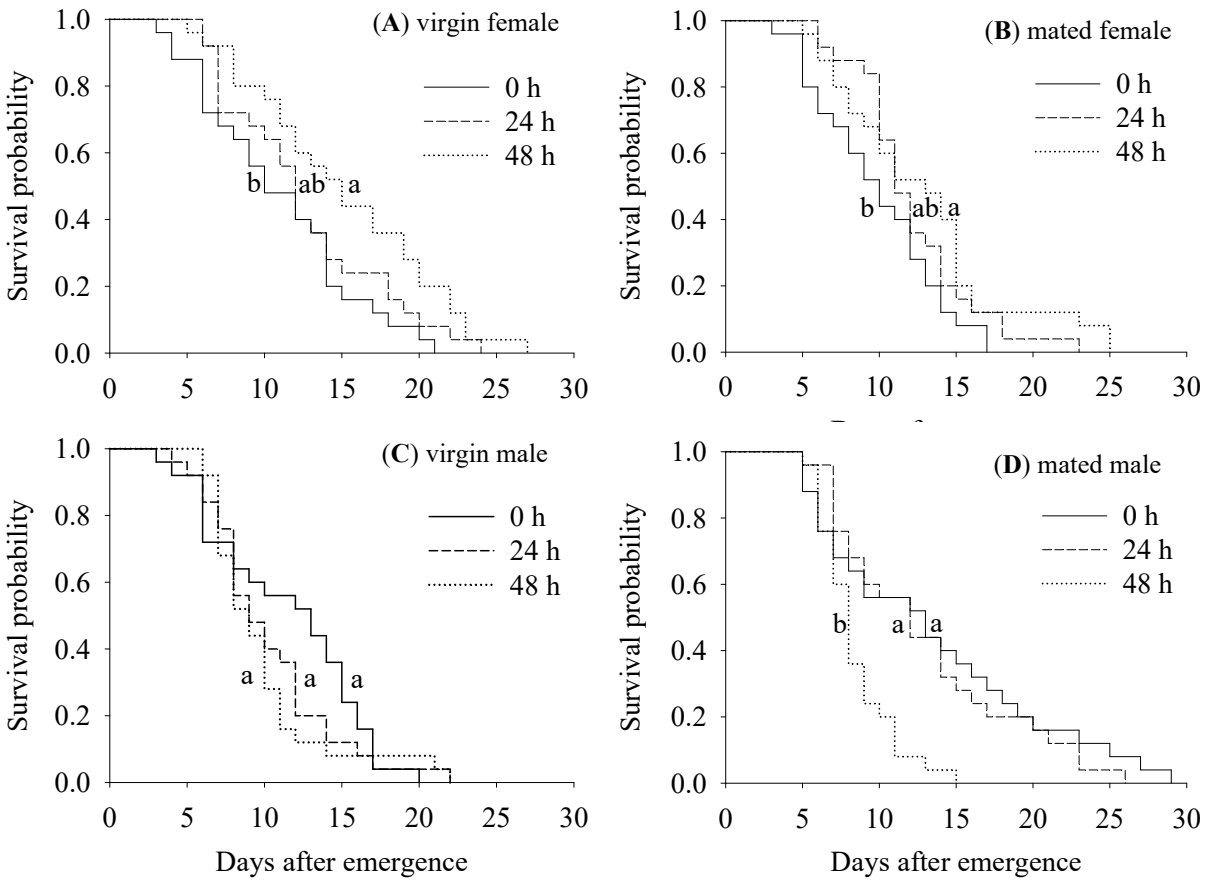

Figure 5.2 Survival probability of virgin females (**A**), mated females (**B**), virgin males (**C**) and mated males (**D**) fasted for different durations in *T. ludeni*. Lines with the same letters are not significantly different ($P > 0.05$).

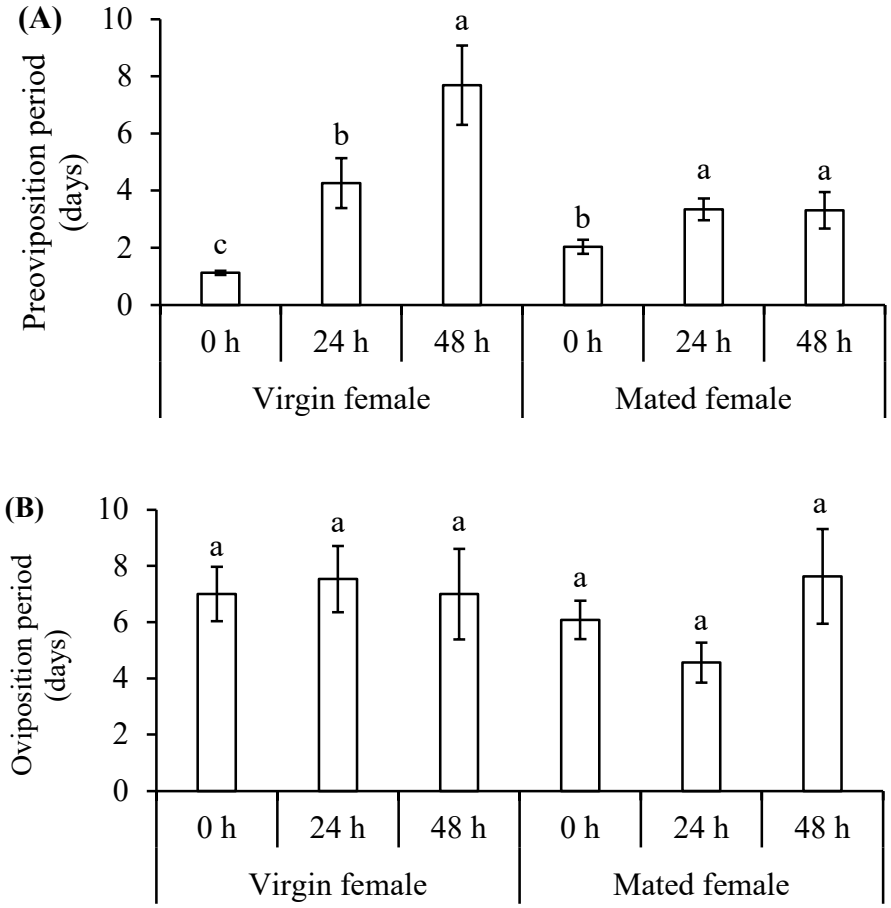

Figure 5.3 Mean (± SE) preoviposition (**A**) and oviposition period (**B**) of virgin and mated females fasted for different durations in *T. ludeni*. For virgin or mated females, columns with the same letters are not significantly different ($P > 0.05$).

5.3.2 Effect of intermittent fasting on lifetime fecundity, egg size and daughter production

Fasting had no significant impact on lifetime fecundity of virgin females ($F_{2,48} = 1.91$, $P = 0.1586$) but significantly reduced that of mated females ($F_{2,72} = 13.04$, $P < 0.0001$) (Figure 5.4A). Egg size significantly decreased when virgin females were fasted for 48 h ($F_{2,309} = 8.76$, $P = 0.0002$) and mated females fasted for 24 and 48 h ($F_{2,329} = 6.57$, $P = 0.0016$) (Figure 5.4B). The number of daughters produced by mated females was not significantly different between treatments ($F_{2,33} = 1.72$, $P = 0.1951$) (Figure 5.4C).

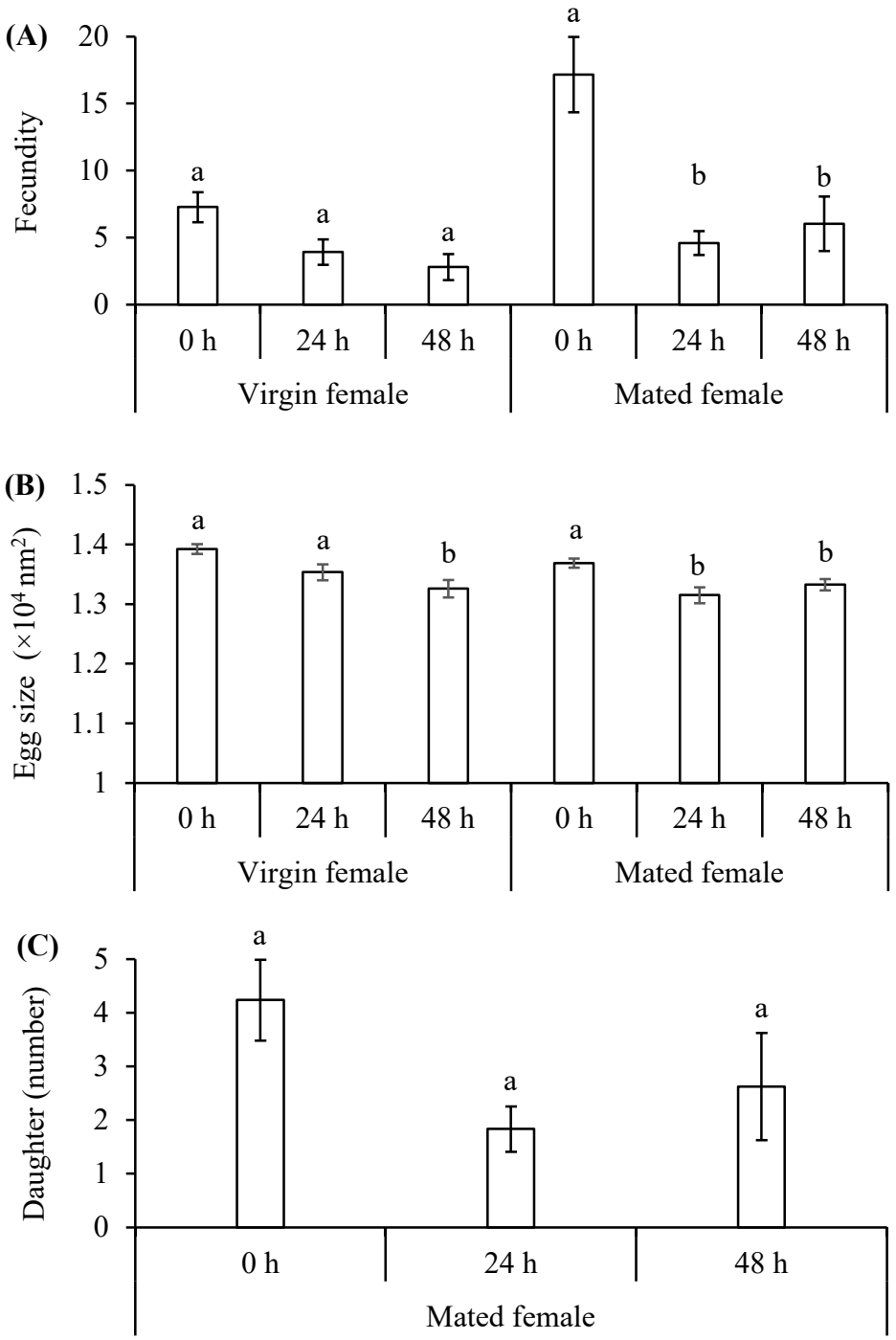

Figure 5.4 Mean (± SE) lifetime fecundity (**A**) and egg size (**B**) of virgin and mated females, and number of daughters (**C**) produced by mated females fasted for different durations in *T. ludeni*. For virgin or mated females, columns with the same letters are not significantly different ($P > 0.05$).

5.3.3 Effect of intermittent fasting on offspring performance

Fasting during the adult stage of mothers did not significantly affect egg hatch rate [mated females: 71.56 ± 6.45, 68.17 ± 6.96 and 65.98 ± 8.30% for 0, 24, and 48 h fasting, respectively ($F_{2,61}$ = 0.15, P = 0.8651); virgin females: 69.52 ± 5.32, 66.77 ± 6.04 and 46.28 ± 11.19% for 0, 24, and 48 h fasting, respectively ($F_{2,48}$ = 1.52, P = 0.2288)] and adult emergence rate [mated females: 55.67 ± 20.96, 24.04 ± 6.02 and 25.21 ± 8.31% for 0, 24, and 48 h fasting, respectively ($F_{2,54}$ = 1.90, P = 0.1597); virgin females: 51.20 ± 7.06, 48.83 ± 7.86 and 34.42 ± 10.25% for 0, 24, and 48 h fasting, respectively ($F_{2,42}$ = 0.93, P = 0.4011)]. Compared to the control, sons from virgin mothers needed significantly longer developmental time after their mothers experienced fasting ($F_{2,115}$ = 5.29, P = 0.0064) while fasting had no effect on developmental time of sons from mated mothers ($F_{2,19}$ = 0.09, P = 0.9177) (Figure 5.5). Fasting significantly prolonged developmental period of daughters produced by mated mothers ($F_{2,129}$ = 3.57, P = 0.0311) (Figure 5.5).

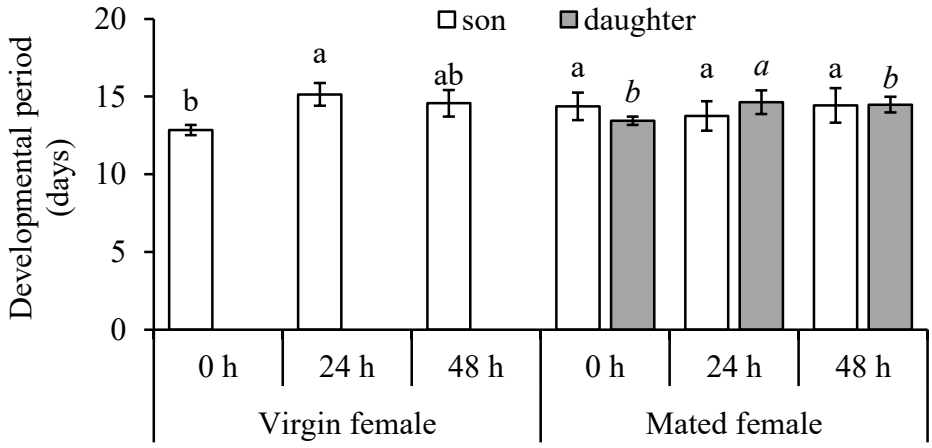

Figure 5.5 Mean (± SE) developmental time of offspring of virgin and mated females fasted for different durations in *T. ludeni*. For sons produced by virgin or mated females, columns with the same letters are not significantly different (P > 0.05); for daughters produced by females, columns with the same italic letters are not significantly different (P > 0.05).

5.4 Discussion

My results demonstrate that intermittent fasting (IF) during the first half of *T. ludeni* adult life increased female longevity by 15–39% regardless of mating status (Figure 5.2A–B). Several studies have explored the possible mechanisms behind the IF-induced longevity extension in invertebrates. For example, Honjoh *et al*. (2009) and Kogure *et al.* (2017) suggest that this could result from gene expression changes, downregulating insulin-like peptides in nematodes. In their study on fruit flies, Catterson *et al.* (2018) indicate that IF in early adult life can raise lipid content, improve gut health later in life, and increase longevity. Because regular starvation often occurs during invasion process (Zhang *et al*. 2019; Papach *et al*. 2020) and *T. ludeni* can reproduce through mother-son mating without inbreeding depression (Zhou *et al.* 2020, 2021), the increase of female longevity in response to IF may contribute to its invasion success.

However, the effect of IF on males was mating-status-specific, i.e., it shortened the longevity of mated males and had no impact on virgin males (Figure 5.2C–D). Several factors could explain the observed patterns. First, *T. ludeni* females are much larger than males (Kaimal & Ramani 2011b; Li & Zhang 2018) and should have more resources to lift lipid content in response to IF. Second, compared to females, males are more active (van Leeuwen *et al.* 2009; Li & Zhang 2018) with higher metabolic rates (Finiguerra *et al*. 2013), resulting in higher energy expenditure and reducing the longevity benefit (Shigenaga *et al*. 1994) from IF. Finally, mating is costly to males in terms of ejaculation (van Voorhies 1992; Shapiro *et al*. 1994; Olsson *et al*. 1997; Scharf *et al*. 2013), resulting in earlier death if mated males are also fasted. To date, it is unknown whether the haplodiploid nature of the spider mite also plays a role in the observed sex specific IF impact on longevity.

I show that IF prolonged preoviposition period in both virgin and mated *T. ludeni* females (Figure 5.3A). Because diet during adulthood determines ovarian development and egg maturation in many species (Adams 2000; Danks 2000; Hahn *et al*. 2008; Mirth *et al*. 2019), fasting may delay this process, leading to longer preoviposition period. The IF-induced delay in reproduction may also contribute to the increase of female longevity. However, IF had no effect on oviposition period regardless of mating status (Figure 5.3B), suggesting that females of this species strive to spread their egg laying for the same time frame to maximize the chance of population survival under different situations. Although IF reduced fecundity (Figure 5.4A) and egg size (Figure 5.4B), it

had no effect on the number of daughters produced (Figure 5.4C). These findings suggest that females trade off fecundity and egg size but not daughter numbers for somatic survival when diet is restricted in their early life. Due to lack of inbreeding depression in *T. ludeni* (Zhou *et al*. 2020, 2021), maintaining production of similar number of daughters under the diet-restricted environment may be an important strategy for population survival and growth, facilitating invasion success.

Looking into the effect of mothers' experience on their offspring performance, I found that IF did not alter egg hatch rate and immature survival to adulthood, suggesting that smaller egg size induced by IF in mothers does not reduce egg viability and offspring endurance in *T. ludeni*. This seems to contradict previous findings in some other species where egg size determines offspring fitness (Carlberg 1991; Stewart *et al*. 1991; Braby 1994; Fox 1994; Roff 2000; Macke *et al.* 2011; Koch & Meunier 2014; Warner & Lovern 2014). However, IF had transgenerational maternal effects on offspring developmental time, with sons from fasted virgin mothers and daughters from fasted mated mothers needing longer time to develop to adults (Figure 5.5). I suggest that *T. ludeni* can compensate egg size loss for offspring fitness by extending developmental period which results in the same adult size as those from large eggs (Fox 1993; Fox & Dingle 1994). This may yet be another strategy this mite has developed to cope with food shortage. Further studies on this aspect are warranted to confirm the transgenerational effects on offspring reproductive outputs in *T. ludeni*.

In conclusion, IF increases female but not male longevity probably due to differences in resource storage, metabolic rate, and mating cost between sexes. In response to IF, females trade off fecundity and egg size but not daughter numbers for longevity gain, suggesting that *T. ludeni* females can adjust their life history strategies for population survival and growth during invasion process where food shortage is common. Eggs produced by fasted females achieve the same hatch rate and resulting young have the same survival rate as those by unfasted ones. Furthermore, IF also has transgenerational maternal effects which prolong offspring development period. I suggest that the longer immature developmental period can increase the body size of resulting adults, compensating egg size loss for offspring fitness. These resource allocation strategies of *T. ludeni* coupled with its adaptation to wide ranges of hosts (Gotoh *et al*. 2015) and temperatures (Gotoh *et al*. 2015; Ristyadi *et al.* 2019, 2021) may have facilitated its invasion success. This work provides insight into adaptive resource allocations in response to fasting and useful knowledge for

evaluation of pest invasions and management of animal survival and reproduction by dietary regulations.

Chapter 6 Predator-Induced Fears Bear Significant Cost to an Invasive Spider Mite *Tetranychus ludeni*: Implications in Pest Management

This chapter was submitted to *Pest Management Science* for publication.

Abstract

The success of biological control using predators is normally assumed to be achieved through direct predation. Yet, it is largely unknown how the predator-induced stress to prey may contribute to biological control effectiveness. Here, I investigate variations in life history traits and offspring fitness of the spider mite *Tetranychus ludeni* in response to cues from the predatory mite *Phytoseiulus persimilis* and injured *T. ludeni,* providing knowledge for evaluation of the non-consumptive contribution to the biological control of *T. ludeni* and for future development of novel spider mite control measures using predator cues. My results show that cues from predators and injured prey shortened the longevity by 23–25% and oviposition period by 35–40% and reduced the fecundity by 31–37% in *T. ludeni* females. These cues significantly reduced intrinsic rate of increase (r_m) and net population growth rate (R_0), and extended time to double the population size (D_t). Predator cues significantly delayed lifetime production of daughters. Mothers exposed to predator cues laid significantly smaller eggs and their offspring developed significantly more slowly but these eggs had significantly higher hatch rate. These findings demonstrate that predator-induced fears significantly lowered the fitness of *T. ludeni,* suggesting that these non-consumptive effects can contribute to the effectiveness of biological control to a great extent. My study provides critical information for evaluation of biological control effectiveness using predators and paves the way for identification of chemical odors from the predator and killed prey and development of new materials and methods for the control of spider mite pests.

6.1 Introduction

Predator-prey interactions affect life history traits and behavioral strategies of species involved (Abrams 2000; Ferrari *et al*. 2010a; Moore & Biewener 2015; Otsuki & Yano 2019), resulting in demographic changes in these species (Delić & Fišer 2019). Plant feeding mites can detect predation threat through chemical cues (Grostal & Dicke 2000; Schoeppner & Relyea 2009; Hermann & Thaler 2014; Azandeme-Hounmalon *et al*. 2016; Jacobsen *et al.* 2016; Gyuris *et al.* 2017; Calvet *et al.* 2018) and respond accordingly (Eklöv 2000; Oku *et al*. 2003b; Fievet *et al*. 2008; Lucon-Xiccato *et al*. 2016; Gyuris *et al*. 2017; Gancedo & Ituarte 2018; Ameri *et al*. 2019). Buchanan *et al.* (2017) suggest that the threat cues affect both behavioral and physiological traits in prey but have stronger impacts on behavioral traits. For example, spider mites tend to stay away from the areas with predator cues (Grostal & Dicke 1999; Bowler *et al*. 2013), aggregate more tightly in such areas (Dittmann & Schausberger 2017), change locomotion activity (Gyuris *et al*. 2017), or shift their oviposition site from leaf surface to their webs (Lemos *et al*. 2010; Otsuki & Yano 2017).

Predation threat incurs trade-offs between risk avoidance and other life functions in prey (Ferrari *et al*. 2010b; Luong *et al*. 2017), altering their life history traits (Kats & Dill 1998; Eklöv 2000; Persons 2002; Nelson 2007; Lemos *et al*. 2010; Walzer & Schausberger 2011; Orrock *et al*. 2013; Lucon-Xiccato *et al*. 2016; Tarierl *et al*. 2020) and reducing their fitness and population size (Buchanan *et al*. 2017; Sheriff *et al*. 2020). These non-consumptive impacts on prey by predation threat may be transgenerational (Li & Zhang 2019b; Tariel *et al*. 2020; Des Roches *et al*. 2022), and can affect more individuals (Preisser 2009) and have influence at least as strong as direct predation on prey population dynamics (Preisser *et al*. 2005; Zanette *et al*. 2011; Macleod *et al*. 2014; Buchanan *et al*. 2017; Pessarrodona *et al*. 2019). For example, predation threat lowers spider mites' fecundity (Dias *et al*. 2016; Li & Zhang 2019b), shortens their adult lifespan (Li & Zhang 2019b) and prolongs their offspring development (Freinschlag & Schausberger 2016; Li & Zhang 2019b). Furthermore, shift of oviposition from leaf surface to webs in response to predation threat results in higher egg mortality due to wind and rain (Okada & Yano 2021).

Application of predatory mites for biological control is a well-established method against spider mite pests in the world, with *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) being the most used predator for the control of the serious invasive pest, *Tetranychus urticae* Koch

(Acari: Tetranychidae) (e.g., Opit *et al*. 2004; Chacon-Hernandez *et al*. 2019; Migeon *et al*. 2019; Yanar *et al*. 2019; Tiftikci *et al*. 2020). These reports mainly evaluate the effectiveness of biological control based on the relationship between the release of *P. persimilis* and population size changes of *T. urticae*, assuming that the spider mite population decline is caused by direct predation. However, non-consumptive effects of predators on prey fitness and forage may also contribute to pest biological control but have been rarely evaluated (Hermann & Landis 2017; Kahl *et al*. 2021). Two recent reports shed some light on this matter using an insect (Wen & Ueno 2021) and a mammal (Villalobos *et al*. 2022) predator-prey systems, respectively. The former indicates that predator cues can significantly reduce pest fitness and suppress pest population and the latter shows that these cues can repel the pest. To date, little is known about whether the predator-induced fears could enhance the effectiveness of spider mite biological control and help develop novel pest control measures for this important group of plant pests.

Tetranychus ludeni Zacher is also an important invasive spider mite pest which is native to Europe and now occurs in all continents except Antarctica (Bolland *et al*. 1998; CABI/EPPO 2011). It attacks over 300 hosts, including many economically important crops such as bean, eggplant, hibiscus, apple, pumpkin, and many other cucurbitaceous plants (Bolland *et al*. 1998; Zhang 2003). Yet, biological control of *T. ludeni* using predatory mites is still poorly understood and knowledge about the effect of predator-induced stress on its fitness is lacking. The limited studies on *T. ludeni* biological control so far mainly focus on the predators from the genus *Neoseiulus* (Acari: Phytotseiidae) (Reichert *et al*. 2016, 2017; Ballal *et al*. 2021). However, Escudero & Ferragut (2005) and Zhang (2002) show that *P. persimilis* performs similarly well on both *T. ludeni* and *T. urticae*, suggesting that this predatory mite can also control *T. ludeni* effectively.

In the present study, I aimed to evaluate how cues from *P. persimilis* and injured *T. ludeni* affected the life history traits and offspring performance of *T. ludeni* females. I exposed mated adult females of *T. ludeni* to these cues throughout their life and recorded their longevity, fecundity, daughter production, and offspring fitness. I then analyzed the changes in their life history traits and calculated life table parameters. This study generated critical information about non-consumptive effects by predation threat on prey population regulations, offering the first knowledge for evaluation of non-consumptive contribution to the biological control of *T. ludeni* and for development of novel spider mite control measures using predator cues.

6.2 Materials and methods

6.2.1 Environmental conditions

Mite colonies were maintained, and all experiments conducted at 25 ± 1°C and 50–70% RH with a photoperiod of 16L:8D hours.

6.2.2 *Tetranychus ludeni* colony and experimental mites

Tetranychus ludeni were reared on approximately 20 potted kidney bean plants (*Phaseolus vulgaris* L.) in an environmental room of the Massey University Entomology and IPM Laboratory in Palmerston North, New Zealand. Every fortnight I replaced 10 of the oldest plants with new ones. I cut the leaves of the old plants and placed them on the top of the new plants, allowing mites to move to the latter.

To obtain 1-day-old mated adult females for experiment, I randomly took deutonymph females from the colony and introduced them onto a bean leaf disc (3 cm × 3 cm, 30 individuals per leaf disc) placed on a water saturated cotton pad in a Petri dish (5.5 cm diameter × 1.0 cm height) until they developed to adult stage. Immediately after adult emergence I transferred 20 of those females and 5 males randomly taken from the colony onto a new leaf disc in a Petri dish and allowed them to stay together for 24 hours before used for experiment.

6.2.3 *Phytoseiulus persimilis* colony and experimental mites

I obtained the predatory mite *P. persimilis* from Bioforce Ltd, New Zealand, and reared them on four potted kidney bean plants heavily infested with *T. ludeni* in the laboratory. I replaced two oldest plants with new ones every three days and allowed mites to migrate as above. The predatory mite colony was maintained in a metal framed cage (120 cm length × 60 cm height × 60 cm width) with transparent mica plastic and fine woven wire mesh walls (0.25 × 0.25 mm aperture) in a separate environmental room.

6.2.4 Effect of predatory and injured conspecific cues on life history traits of *Tetranychus ludeni*

To assess how maternal stress induced by predators and injured conspecifics affected the life history traits of *T. ludeni*, I exposed 1-day-old mated adult females to three different types of cues: (1) bean leaf disc with trace of predators, (2) bean leaf disc with injured conspecifics, and (3) clean bean leaf disc (control with neither predator nor injured conspecific cues). Each treatment had 20 replicates. Predator trace consists of metabolic waste products, eggs and footprints left by the predators on the leaf surface (Walzer & Schausberger 2011). For each replicate in treatment (1), I randomly selected five adult female predators from the colony and transferred them onto a bean leaf disc (2 cm × 2 cm) placed on a water saturated cotton pad in a Petri dish (14 cm diameter × 1.5 cm height). Twenty-four hours later, I removed the predator adults and redundant eggs, ensuring two predator eggs remained on the leaf disc. If the number of eggs on the disc was fewer than two, I transferred predator egg(s) onto the disc from a separate leaf disc (3 cm × 3 cm). For treatment (2), the injured conspecifics were prepared by piercing four adult females of *T. ludeni* on a leaf disc with an insect pin.

In each replicate, I released a 1-day-old mated adult female of *T. ludeni* to the middle of a leaf disc, bearing either predator cues, injured conspecifics, or none of these, placed on a water saturated cotton pad in a Petri dish, and allowed her to stay on the leaf disc for 24 hours. I then transferred the mite to a new leaf disc with the same cues daily until death. I recorded oviposition period (from the first to last eggs laid), daily fecundity (the number of eggs produced per day), lifetime fecundity (total number of eggs produced), and adult lifespan (from emergence to death). I randomly selected two eggs laid by each female daily and measured their size on the leaf disc where they were laid using a digital camera (Olympus SC30, Japan) connected to the stereomicroscope and a computer with adequate imaging software (CellSens® GS-ST-V1.7, Olympus, Germany) installed. I reared all eggs laid by *T. ludeni* each day on their original leaf disc [predator eggs in treatment (1) were removed]. I allowed eggs laid on each leaf disc to hatch and then transferred mites to a fresh and clean leaf disc and replaced the leaf disc once every five days until they developed to adults. I checked all leaf discs daily and recorded the number of eggs hatched, number and sex of emerged adults, and developmental time from egg to adult stage. I

calculated the life table parameters (Jervis *et al*. 2005) for each treatment using the above data (see Statistical analysis below).

6.2.5 Statistical analysis

All data were analyzed using SAS 9.4 with a rejection level set at $\alpha < 0.05$. Data on adult survival were compared using a Wilcoxon test (LIFETEST procedure). Data on the ln(x)-transformed oviposition period, fecundity and adult emergence rate were normally distributed (Shapiro-Wilk test, UNIVARIATE procedure) and thus analyzed using ANOVA (GLM procedure) followed by a Tukey test for multiple comparisons. However, data on egg size, egg hatch rate, and developmental period from egg to adulthood were not normally distributed and thus analyzed using a non-parametric ANOVA (GLM procedure) with a Bonferroni test for multiple comparisons.

I modified an exponential functional model (Archontoulis & Miguez 2015) to fit the data on the cumulative proportion of daughters produced over female age (NLIN Procedure), i.e., cumulative proportion of daughters = $a \times \exp(b \times \text{age})$, where a is a constant, and b is the increase rate of cumulation. The difference in b was compared between socio-environmental cues according to Julious (2004): if the 95% CLs overlap, then there is no significant difference.

I calculated the intrinsic rate of increase (r_m, daughters/female/day) by solving the Lotka-Euler equation, $\sum e^{-\mathrm{r_m}x}\mathrm{l}_x\mathrm{m}_x = 1$, where x is the female pivotal age, l_x is the proportion of females surviving to age x, and m_x is the number of daughters produced per female at age x. I also calculated other life table parameters, including the net reproductive rate ($\mathrm{R}_0 = \sum \mathrm{l}_x\mathrm{m}_x$, daughters/female/generation), mean generation time [$\mathrm{T} = \log_e(\mathrm{R}_0)/\mathrm{r_m}$, days], and doubling time [$\mathrm{Dt} = \log_e(2)/\mathrm{r_m}$, days]. I used the bootstrap method (Huang & Chi 2012; Yu *et al*. 2013) with 50,000 bootstrap samples to calculate the pseudo-values of a given parameter and employed the paired-bootstrap test (Efron & Tibshirani 1993; Mou *et al*. 2015; Reddy & Chi 2015) for multiple comparisons between any two cues (TTEST Procedure). The significance was determined according to the 95% t-based confidence limits (95% CLs), i.e., if 0 is not within the 95% CLs, the mean difference between the two cue treatments is significantly different.

6.3 Results

6.3.1 Effect of predation risks on adult survival, fecundity and daughter production

Females exposed to injured conspecific or predator cues died significantly earlier than the control (x_2^2= 11.06, P = 0.0040) (Figure 6.1). Exposure to injured conspecific or predator cues significantly shortened the oviposition period ($F_{2,55}$ = 5.60, P = 0.0061) (Figure 6.2A) and substantially reduced lifetime fecundity ($F_{2,55}$ = 3.03, P = 0.0566) (Figure 6.2B). Predator cues also resulted in significantly slower increase of cumulative daughter production (Non-overlapping 95% CLs) (Figure 6.3, Table 6.1).

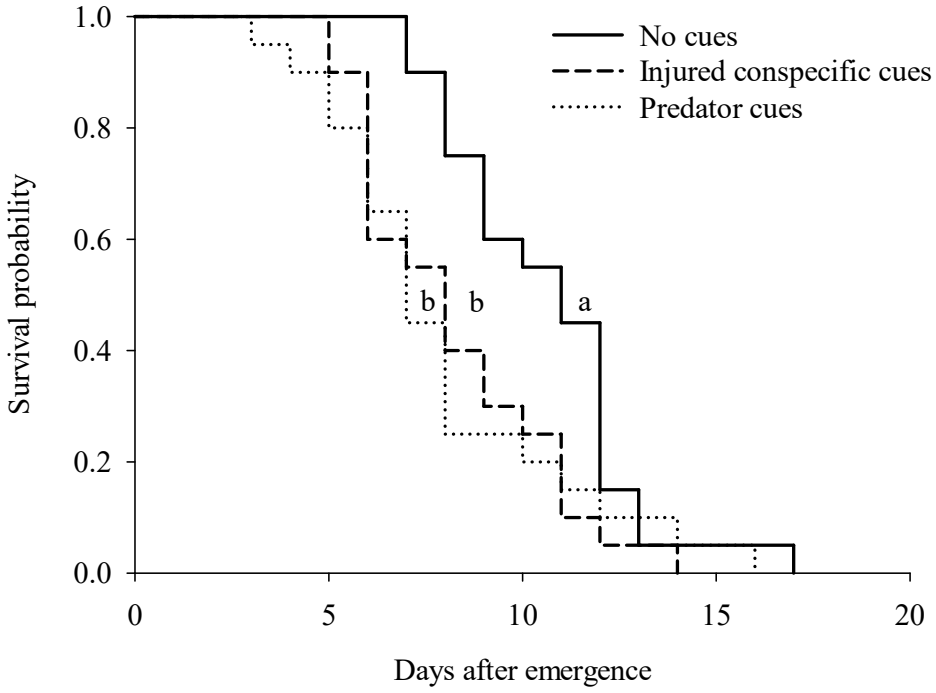

Figure 6.1 Survival probability of *T. ludeni* females exposed to different cues. Lines with the same letters are not significantly different (P > 0.05).

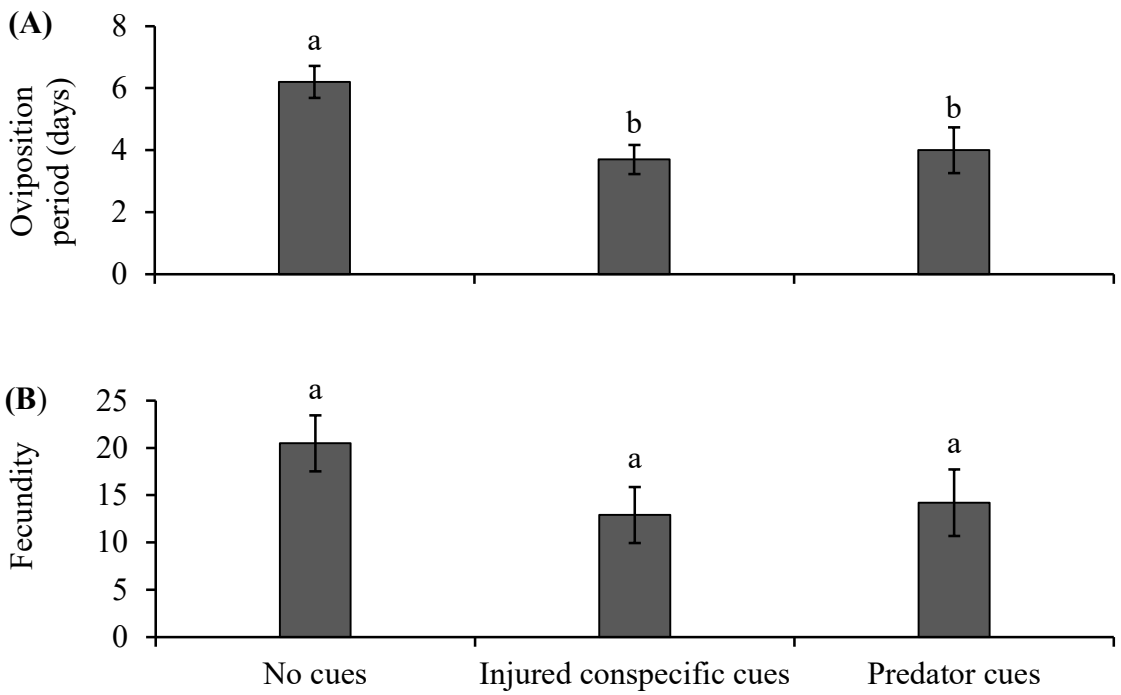

Figure 6.2 Mean (± SE) oviposition period (**A**) and fecundity (**B**) of *T. ludeni* females exposed to different cues. Columns with the same letters are not significantly different ($P > 0.05$).

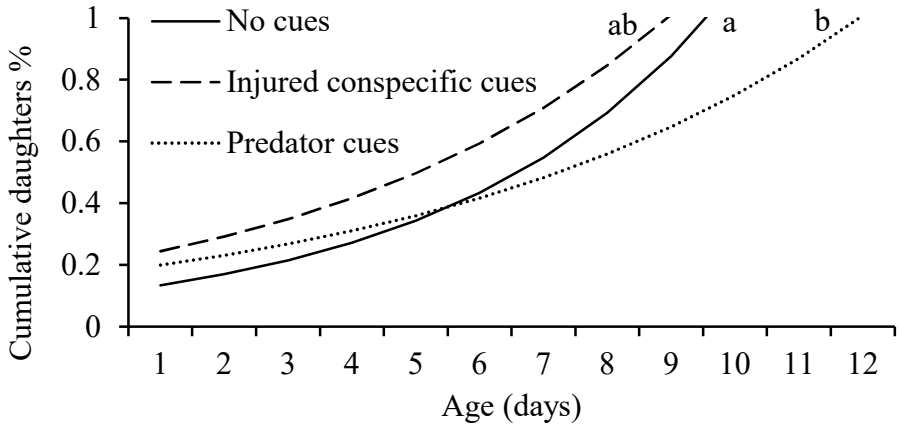

Figure 6.3 Cumulative proportion of daughters produced by *T. ludeni* females exposed to different cues. Lines with the same letters are not significantly different (Overlapping 95% CLs).

Table 6.1 Parameters of cumulative proportion of daughters.

Cue	*a*		*b*		R^2	$F_{(df)}$	*P*
	Estimate	95% CLs	Estimate	95% CLs			
No	0.1060 a	0.0686~0.1435	0.2346 a	0.1891~0.2801	0.6941	$158.82_{(2,140)}$	< 0.0001
Injured	0.2046 a	0.1253~0.2838	0.1775 ab	0.1225~0.2324	0.6856	$71.96_{(2,66)}$	< 0.0001
Predator	0.1723 a	0.1088~0.2358	0.1472 b	0.1070~0.1873	0.6233	$79.44_{(2,96)}$	< 0.0001

$y = a \times \exp(b \times \text{age})$. Estimated values in columns followed by different letters are significantly different (overlapping 95% CLs, $P < 0.05$).

6.3.2 Effect of predation risks on offspring fitness

Eggs laid by mothers exposed to predator cues were significantly smaller than those laid by control mothers ($F_{2,451} = 3.38$, $P = 0.0350$) (Figure 6.4A). After mothers were exposed to predator cues, the hatch rate of their eggs was significantly higher than that of other treatments ($F_{2,55} = 5.75$, $P = 0.0054$) (Figure 6.4B). Furthermore, the developmental time of immatures produced by mothers exposed to predator cues was significantly longer than that of other treatments ($F_{2,280} = 5.38$, $P = 0.0051$) (Figure 6.4C). However, treatment had no significant effect on offspring survival to adulthood ($F_{2,49} = 0.14$, $P = 0.8666$).

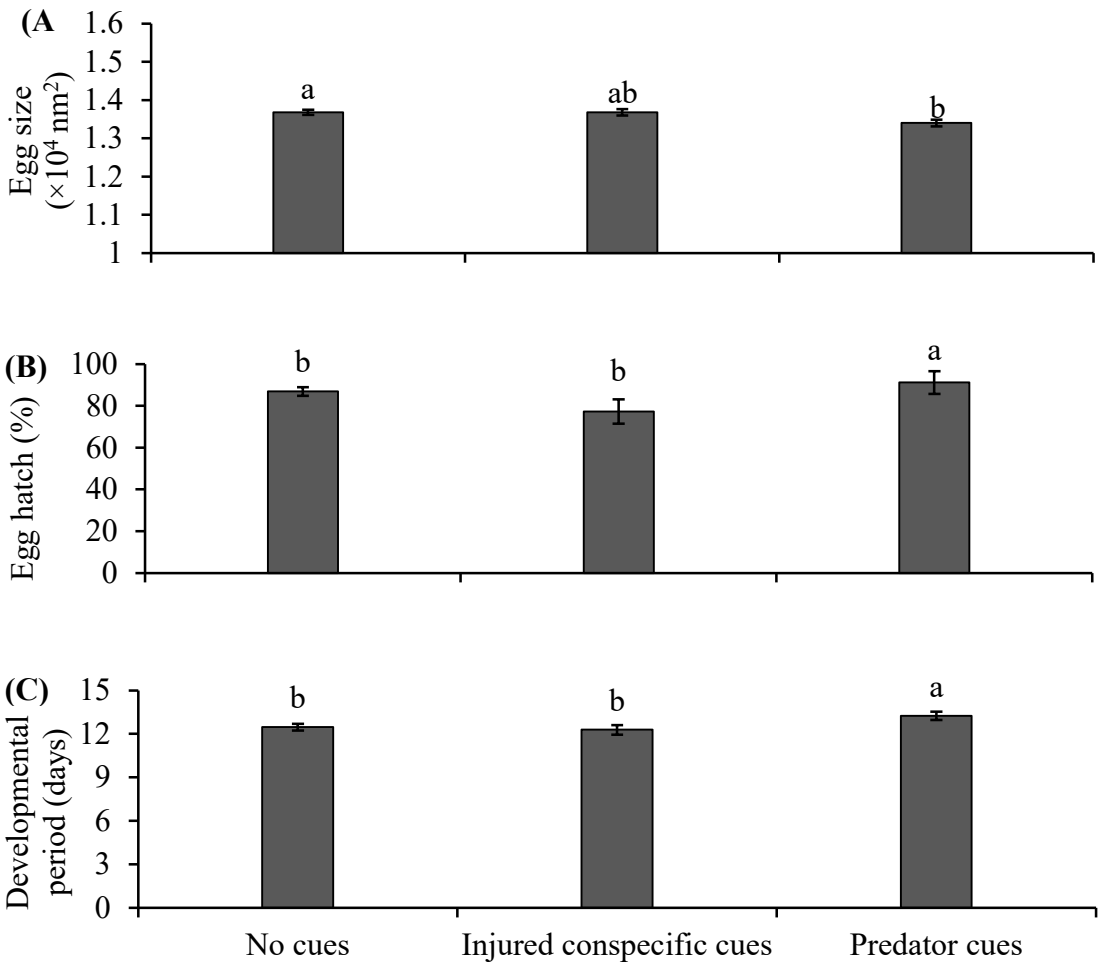

Figure 6.4 Effect of mothers' exposure to different cues on mean (± SE) egg size (**A**), egg hatch rate (**B**), and offspring developmental period (**C**) in *T. ludeni*. Columns with the same letters are not significantly different ($P > 0.05$).

6.3.3 Effect of predation risks on life table parameters

As shown in Table 6.2, females exposed to injured conspecific and predator cues had significantly lower intrinsic rate of increase (r_m) and net population growth rate (R_0) and required significantly longer time to double the population size (D_t). Females had significantly longer generation time (T) after exposed to predator cues and significantly shorter generation time after exposed to conspecific cues (Table 6.2).

Table 6.2 Mean (± SE) life table parameters of *T. ludeni* in response to different cues.

Cue	r_m	R_0	T	D_t
No cues (No)	0.1002 ± 0.0004 a	5.04 ± 0.04 a	16.15 ± 0.07 b	6.92 ± 0.03 b
Injured conspecific (Inj.)	0.0828 ± 0.0014 b	3.35 ± 0.06 c	14.58 ± 0.14 c	8.42 ± 0.15 a
Predator (Pred.)	0.0852 ± 0.0010 b	4.20 ± 0.06 b	16.84 ± 0.14 a	8.16 ± 0.10 a
95% CLs (No vs Inj.)	0.0144 ~ 0.0204	1.56 ~ 1.83	1.25 ~ 1.89	-1.81 ~ -1.19
95% CLs (No vs Pred.)	0.0128 ~ 0.0172	0.13 ~ 0.25	-0.10 ~ -0.39	-1.44 ~ -1.04
95% CLs (Inj. vs Pred.)	-0.0059 ~ 0.0011	0.19 ~ 0.37	-2.65 ~ -1.87	-0.09 ~ 0.62

Estimated values in columns followed by different letters are significantly different. For each parameter, 95% CLs > 0 or < 0 indicates a significant difference between treatments.

6.4 Discussion

In the present study, I demonstrate that cues from predatory mite *P. persimilis* and injured spider mite *T. ludeni* shortened the longevity of *T. ludeni* females by 25 and 23%, respectively (Figure 6.1). These findings suggest that in addition to direct predation, the non-consumptive mortality caused by both predator threat and injured prey can substantially reduce the spider mites' feeding time and thus damage to crops. The earlier prey death induced by predation threat could be attributed to various factors, such as the energic costs of natural enemy avoidance (Luong *et al*. 2017), decrease of foraging rate or food intake (Hermann & Thaler 2014; Wineland *et al*. 2015; Des Roches *et al*. 2022) and increase of oxidative damage (Janssens & Stoks 2013). Furthermore, predator cues may affect prey through physiological pathways by inducing stress hormones to divert its resource allocation to other physiological process that may translate into lower survivorship (Hawlena & Schmitz 2010; Sitvarin *et al*. 2015).

Clinchy *et al.* (2013) suggest that the presence of predators could induce sustained stress in prey and compromise their reproductive fitness. I show that predation threat lowered reproductive outputs and population growth in *T. ludeni.* For example, cues from predators and injured prey shortened oviposition period by 35–40% (Figure 6.2A) and reduced fecundity by 31–37% (Figure

6.2B). As shown in Figure 6.3 and Table 6.1, cues from predators but not injured *T. ludeni* slowed down lifetime daughter production. Furthermore, predation threat reduced intrinsic rate of increase (r_m) and net population growth rate (R_0), and extended time to double the population size (D_t) (Table 6.2). These results suggest that predator-induced stress can help suppress pest population growth by reducing their reproductive outputs and delaying production of daughters. Our results support notions that the non-consumptive impacts on prey may be as strong as direct consumption (Preisser *et al*. 2005). Similarly, in response to predator cues, both oviposition period (Li & Zhang 2019b; Moghadasi *et al*. 2019) and fecundity (Škaloudová *et al*. 2007; Ferrari & Schausberger 2013; Moghadasi *et al*. 2019; Venkanna *et al*. 2021) significantly decline in *T. urticae* and several other prey species.

The present study partially supports previous reports that non-consumptive impacts of predation threat on prey are transgenerational (Li & Zhang 2019b; Tarierl *et al*. 2020; Des Roches *et al*. 2022). I found that *T. ludeni* mothers exposed to predator cues laid significantly smaller eggs (Figure 6.4A) and their offspring had significantly longer developmental period (Figure 6.4C) as compared to other treatments. Smaller eggs may result in smaller adults which may have lower reproductive fitness (Honěk 1993; Fox 1994) but prolonged developmental time may help gain more body mass (Fox 1993; Fox & Dingle 1994). Moreover, eggs laid by mothers exposed to predator cues had significantly higher hatch rate than those in other treatments (Figure 6.4B) and treatment had no significant effect on offspring survival to adulthood. These results suggest that *T. ludeni* juveniles can somewhat compensate egg mass loss caused by their mothers' experience in predation threat. The present study did not find any evidence that mothers' experience of injured prey cues could influence their offspring fitness (Figure 6.4). Taken together, the impact of predator-induced stress on offspring fitness appears to be weaker than on their mothers' and its contribution to pest population suppression could be less significant. However, in the presence of predation threat *T. kanzawai* females shift their oviposition from leaf surface to webs, resulting in higher egg mortality due to wind and rain (Okada & Yano 2021). This could increase offspring mortality further in *T. ludeni*, which is worth testing in the future.

I suggest that the non-consumptive effects reported in this study may play a critical role in biological control using predators and should be considered for the evaluation of total *T. ludeni* population suppression by *P. persimilis*. After the release of predators in the field, cues from both predators and killed prey should co-exist. However, it is not yet known whether they have

synergistic or additive impacts on prey mortality and reproductive fitness because these two cues were tested separately in the present study. Further investigations into their combined effects on prey are thus warranted. In addition, recent reports show that chemical cues from predators can be used for insect (Wen & Ueno 2021) and rodent (Villalobos *et al*. 2022) pest management. These studies have laid new foundations for future research on novel pest control materials and methods for spider mite and other plant pests. For example, future studies can involve extraction of odors released by *P. persimilis* and injured prey, identification of key compounds in these odors, and tests of the effects of individual and combined compounds on prey behavior and fitness and plant damage.

In conclusion, *Tetranychus ludeni* females exposed to cues from predatory mite *P. persimilis* and injured *T. ludeni* have significantly higher mortality, lower reproductive fitness, and slower population growth. In addition to predation, these non-consumptive effects on the spider mite can have major contribution to the effectiveness of its biological control using predators. The transgenerational impact of the predation threat appears to be weaker and its contribution to pest population suppression could be less significant. Identification and tests of chemical odors from the predator and killed prey may have high potential for the development of novel materials and methods for the control of spider mite and other plant pests.

Chapter 7 General Discussion and Conclusion

7.1 Introduction

In this thesis, I have carried out a series of experiments to investigate life history strategies of an invasive horticultural pest, *T. ludeni*, in response to temperatures, food stress, and predation risk, and their implications in pest management. Knowledge generated is important for understanding the mechanisms behind its invasion success and pest outbreaks and developing effective pest management strategies. In this chapter, I summarise and discuss my main findings and their relevance to the invasion success and management of *T. ludeni* and recommend future research.

7.2 Responses to thermal conditions in *Tetranychus ludeni*

To generate critical information for evaluation of the invasion and outbreak potential and development of thermal disinfestation measures for *T. ludeni*, I have examined its response to fluctuating (Chapter 2) and constant temperatures (Chapter 3), and thermotolerance to high temperatures (Chapter 4). Different species of arthropods show various responses to temperature ranges whether they be constant or fluctuating (Wu *et al*. 2015). Bayu *et al.* (2017) suggest that spider mites are more adaptive to the fluctuating than constant temperatures. My results demonstrate that *T. ludeni* perform better at 20~30ºC regardless of the temperature type and all eggs died at 51ºC.

The lower thermal threshold (T_0) for development from eggs to adults is between 11.7 and 12.7ºC in *T. ludeni* (Table 2.1), falling into the range from 7.8 to 13.8ºC reported for the genus *Tetranychus* (Sakunwarin *et al*. 2003; Gotoh *et al*. 2010; Ullah *et al*. 2011; Karami-Jamour & Shishehbor 2012; Riahi *et al*. 2013). The varying thermal threshold between species and among populations or strains of the same species probably reflects their adaptations to the local climate (Gotoh *et al*. 2015). Compared to other *Tetranychus* species such as *T. urticae*, *T. piercei* and *T. phaselus* (Gotoh *et al.* 2015), *T. merganser* and *T. kanzawai* (Ullah *et al.* 2011), *T. turkestani* (Karami-Jamour & Shishehbor 2012), *T. evansi* (Gotoh *et al.* 2010), *T. truncatus* (Sakunwarin *et al.* 2003; Gotoh *et al.* 2015) and even other strains of *T. ludeni* (Gotoh *et al.* 2015), the New Zealand strain of *T. ludeni* has relatively lower immature survival rate (Figure 3.2B) and fecundity

(Figure 3.2F) at low and high temperatures. This suggests that the New Zealand strain may have some difficulty to adapt to cool and hot climate. As a result, it may be less likely that this strain could cause serious damage on field crops in spring or become a major pest in the glasshouses during summer in New Zealand.

Increasing temperature elevates the metabolic rate of organisms (e.g., Woods & Hill 2004; Potter *et al.* 2009; Schulte 2015). It is thus not surprising that the developmental time significantly decreases as temperature increases in *T. ludeni* (Figures 2.2 and 3.2C) and other *Tetranychus* species (e.g., Margolies & Wrensch 1996; Gotoh *et al.* 2015; Bayu *et al.* 2017). Adult body size is particularly dependent on the temperature at which their immature stages develop, for example, when the diet remains consistent, lower temperatures generally result in larger adults (Atkinson 1994; Walters *et al.* 2006; Klok & Harrison 2013). This phenomenon may be attributed to slower growth rates and thermal constraints on cellular growth, making cells larger (Angilletta *et al.* 2004). In accordance with the theory, *T. ludeni* adults of both sexes developing at higher temperatures are significantly smaller (Figures 2.3 and 3.2E). In general, female body size and fecundity are supposed to have a positive relationship (Honěk 1993). However, smaller female adults developing from consistent 25 and 30ºC and larger ones at 20ºC have similar fecundity (Figure 2.4) although the former live a shorter life (Figure 2.5B). Moreover, females developing at 15ºC are significantly larger (Figure 2.3A) and survive longer (Figure 2.5B) than those at 25 and 30ºC but they lay significantly fewer eggs (Figure 2.4). These results have two implications: (1) females can adjust their reproductive strategy to maximise reproductive output by elevating reproductive rate in their short life at high temperatures, and (2) *T. ludeni* may quickly build up their populations at moderate temperatures of 20~30ºC independent of the temperature type.

Extreme weather events have been predicted to be more frequent in the near future due to increasing anthropogenic climate change (Battisti & Naylor 2009; Schär 2016; Klockmann *et al.* 2017). The ability of species to tolerate temperature stress is an important factor determining their survival and distributions (Sunday *et al.* 2011; Araújo *et al*. 2013). My results show that compared with other life stages in *T. ludeni*, the egg is the most vulnerable as it only withstands heat shock of ≤ 48°C for a short duration (Table 4.1). The vulnerability of eggs to high temperatures has been documented previously in *T. urticae* and *T. mcdanieli* McGregor (Roy *et al.* 2002; Gotoh *et al*. 2015). Regardless its potential fecundity, the population will go extinct in the areas where extreme temperatures bring the survival of any life stages to zero (Levene 1953; Gilchrist 2000).

Additionally, Zimmermann *et al*. (2009) and Kellermann *et al*. (2012) emphasize that extreme temperature events have stronger effects on species distribution than mean temperatures. Therefore, I suggest that *T. ludeni* may not be able to build up their population in regions with extreme temperature events above 48°C, which is the maximum temperature their eggs can tolerate for a short period. These findings also have implications for the development of heat disinfestation programmes for treating postharvest products and agricultural machineries. Further investigations into the acclimation and hardening effects on *T. ludeni* by exposing them to sublethal temperatures over several generations may provide more information for understanding their performance in future extreme conditions.

7.3 Effects of food stress on life history traits of *Tetranychus ludeni*

Organisms often alter their life history traits in response to ecological environment with intraspecific variations (Stearns 2000; Shefferson 2010; Fabian & Flatt 2012). To understand how *T. ludeni* adjusts its resource allocation under periodical starvation and provide knowledge for evaluation of its invasion success, I have carried out experiments to determine the effects of intermittent fasting (IF) on both sexes of different mating status (Chapter 5). My results reveal that IF extends the lifespan of both mated and virgin females but shortens the lifespan of mated males and has no effect on that of virgin males (Figure 5.2). After fasting, the virgin and mated females trade off their fecundity and egg size but not the number of daughters for survival (Figure 5.4). However, offspring from eggs of different size have similar survival. Moreover, IF has transgenerational maternal effects, which prolong offspring developmental period (Figure 5.5). I suggest that the longer immature developmental period can increase the body size of resultant adults and compensate egg size loss for offspring fitness. Further studies on transgenerational effect of IF on the offspring performance in *T. ludeni* are warranted.

Food supply during adulthood is important for ovarian development and may affect preoviposition period in many species (Danks 2000). I find that IF significantly delays egg laying in both virgin and mated females with more effect on virgin females (Figure 5.3A). Extended preoviposition period in virgin females may also be attributed to a delay in mating as recorded in *Panolis flammea* Denis and Sciffermuller (Leather *et al*. 1985) and *Earias insulana* Boisduval (Kehat & Gordon 1977). Thus, starvation together with the lack of mating may have suspended

the reproductive onset in virgin females longer than in mated females of *T. ludeni.* This implies that under food stress virgin females tend to allocate more resources to somatic maintenance during the early adulthood. These resource allocation strategies in response to IF result in significant declines in the fecundity of mated females (Figure 5.4A), while the virgin females are still able to maintain their fecundity (Figure 5.4A). A significant decline in fecundity caused by IF is also recorded in mated females of *T. urticae* (Li & Zhang 2019a). Normally, a reduction in egg number is corelated with investment in larger egg size (Elgar 1990; Guisande *et al*. 1996; Rasanen 2002; Macke *et al*. 2012; Koch & Meunier 2014). However, my results show that although mated females lay significantly fewer eggs in respond to IF (Figure 5.4A), the egg size is significantly smaller (Figure 5.4B). These results suggest that under food stress mated females *T. ludeni* may trade-off between reproduction and somatic survival to gain longer life span, but do not trade off egg number for size. The mechanisms behind these findings need further investigations.

As in many other invasive animals (Zhang *et al*. 2019; Papach *et al*. 2020), *T. ludeni* may encounter intermittent food shortages during invasion process when senescence of their host plants occurs or when it is carried by external forces such as wind and transportation to new habitats. My results suggest that this species has developed strategies for invasion success in response to periodical starvation, such as delay in egg laying and extension of longevity. This study provides some insight into adaptive resource allocations in response to food shortage. My findings also offer useful knowledge for management of animal survival and reproduction by dietary regulations.

7.4 Effects of predation threat on life history traits of *Tetranychus ludeni*

Predators have strong effects on prey population dynamics by direct consumption and induction of costly defensive strategies (Preisser *et al*. 2005; Pessarrodona *et al*. 2019). Predatory threat may cause stress and change the behaviour, morphology and physiology of prey (Kats & Dill 1998; Eklöv 2000; Lemos *et al*. 2010; Maher *et al*. 2013; Lucon-Xiccato *et al*. 2016). To provide knowledge for evaluation of the non-consumptive contribution to the biological control of *T. ludeni* and for future development of novel spider mite control measures using predator cues, I have conducted experiments on how predator-induced stress affects life history traits of this mite. My results indicate that predation threat significantly reduces the longevity (Figure 6.1), oviposition period (Figure 6.2A), intrinsic rate of increase (r_m), net population growth rate (R_0),

and extends the time to double the population size (D_t) (Table 6.2) in *T. ludeni.* Therefore, predation threat may have significant non-consumptive effects on *T. ludeni* survival and reproduction which will contribute to the effectiveness of biological control. Future studies may include identification and tests of chemical odors from the predator for development of novel materials and methods for the control of spider mites.

However, the response of organisms to risk cues may change over time as prior experience may alter their perception about the risk which determines their behaviour and survival (Sih *et al.* 2011; Lönnstedt *et al.* 2012; Hackl & Schausberger 2014; Ferrari *et al.* 2015; Culshaw-Maurer *et al.* 2020). The ability to recognize predation risk can be innate or learned or a combination of both, but evidence for learned threat sensitivity in terrestrial animals is lacking as most studies in the field do not allow discrimination between predator-experienced and -unexperienced individuals (Walzer & Schausberger 2011). In addition, both innate and learned anti-predator responses can be modified by maternal effects (Roche *et al*. 2012; Stratmann & Taborsky 2014). My study has revealed the non-consumptive effect of predation threat on predator-naïve (unexperienced) *T. ludeni.* Further studies are needed to investigate the magnitude of non-consumptive effects of predation threat on predator-experienced *T. ludeni* and their offspring.

Although *T. ludeni* population may be negatively affected by predation threat, to some extend this species may also build an adaptive response to predation threat for increasing their offspring survival. For example, my findings show that the hatch rate of eggs laid by a mother exposed to predator cues is significantly higher than that in other treatments (Figure 6.4B). This may be due to the ability of embryos to perceive and respond to environmental stimuli (Mathis *et al*. 2008; Ferrari & Chivers 2010; Ituarte *et al*. 2019; Mezrai *et al*. 2020) and adjust their hatching rate in response to the changing risks and opportunities (Ferrari & Chivers 2010; Ferrari *et al*. 2010; Warkentin 2011; Hughey *et al*. 2015; Fukuse & Yano 2019; Noguera & Velando 2019; Mezrai *et al*. 2020; Kostoglou *et al*. 2021). Furthermore, predator cues significantly prolong the developmental period of *T. ludeni* offspring (Figure 6.4C). I argue that the extended developmental period is an adaptive respond to compensate the significant decline in the size of eggs laid by mothers exposed to predator cues (Figure 6.4A). Further investigation on behavioral changes of *T. ludeni* and their offspring in respond to predation threat may be worth studying.

7.5 Limitations of study

This research, however, has some potential limitations. For example, *T. ludeni* used in my study were taken from the colony that had been reared in the laboratory since 2017. They might have adapted to the laboratory conditions during my PhD studies and changed their general performance to some extent. Furthermore, I have not investigated responses of *T. ludeni* to environmental stressors at the physiological and molecular levels. Future studies can explore the physiological and molecular mechanisms behind the effects of environmental stressors on life history of spider mites.

7.6 Conclusion

In this study I have demonstrated that the environmental challenges (i.e., temperature stress, food stress, and predation risk) on *T. ludeni* may act as strong selective pressure defining their population growth, expansion potential, and driving their life history evolution. *T. ludeni* may detect the current and expected conditions of their environment and adjust their life history strategies through differential resource allocations accordingly. However, their responses to environmental conditions vary depending on different life stages, sexes, and mating status. The susceptibilities and negative responses of *T. ludeni* to a certain degree of environmental stressors demonstrated in this study may provide foundation for developing more effective and sustainable management strategies for this pest.

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Appendix 1: Three Published Papers from PhD Study

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Dynamics of life history traits in *Tetranychus ludeni* Zacher in response to fluctuating temperatures

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Tetranychus ludeni Zacher (Acari: Tetranychidae) is an invasive spider mite that currently occurs in Europe, Asia, Africa, America, and Australasia (CABI 2011; Migeon & Dorkeld 2018). It feeds on more than 300 plant species in 60 families and causes significant damage to a number of economically important crops such as eggplant, pepper, tomato, bean, pumpkin and other cucurbitaceous plants (Zhang 2002, 2003; Gotoh *et al*. 2015). In their study on effects of constant temperatures on several spider mite species, Gotoh *et al.* (2015) suggest that *T. ludeni* may be better adapted to warmer climate and predict that it could replace *T. urticae* to become a major pest of crops in the world. However, temperature in nature is fluctuating, typically higher during the daytime and lower during the nighttime, which could have different effects on organisms (Fischer *et al.* 2011; Paaijmans *et al.* 2013; Bowden *et al.* 2014; Gotoh *et al*. 2014; Zeh *et al.* 2014; Nachman & Gotoh 2015; Bayu *et al*. 2017). So far, how fluctuating thermal conditions affect life history traits of *T. ludeni* is unknown. In the present study, we investigated the plasticity of life history traits in response to fluctuating temperatures in *T. ludeni* to provide information for predicting its potential to invade and to become a major pest in different regions or conditions.

We maintained a breeding colony of *T. ludeni* on 20 potted kidney bean plants (*Phaseolus vulgaris* L.) in the Massey University Entomology and IPM Laboratory, Palmerston North, New Zealand, at 25 ± 1°C temperature and 50 ~ 70% RH with a photoperiod of 16L:8D hours. We performed four fluctuating temperature treatments (15~21, 22~28, 29~35 and 36~42°C) with 20 replicates per treatment in growth chambers (I-36VL, Percival Scientific Inc., Perry, Iowa, USA). The temperature setting protocol is shown in Figure 1. For each replicate, we randomly collected 20 female and 4 male adults from the colony, introduced them onto a bean leaf disc (3 cm × 3 cm) placed upside down on a water saturated cotton pad in a Petri dish (5.5 cm diameter × 1.0 cm height), and allowed them to stay on the leaf disc for 24 hours at 25°C. We kept 50 eggs on the leaf disc, removed the redundant eggs using a soft paintbrush, and then placed the Petri dish with 50 eggs into a growth chamber at a test temperature. We replaced the leaf disc with a fresh one once every five days. We took each Petri dish from the test temperature once a day for a few minutes and recorded egg hatch, immature stage mortality and adult emergence under a stereomicroscope (Leica MZ12, Germany) at 25°C. During the few minute's observation each day, we also sexed and removed emerged adults and recorded development period (from egg to adulthood) of each sex.

To determine the effect of treatments on adult body size, we randomly selected two females and two males of the resultant adults per replicate and measured their size under a digital camera (Olympus SC30, Japan) connected to the stereomicroscope and a computer with adequate imaging software (CellSens® GS-ST-V1.7, Olympus, Japan) installed. To obtain information on the effect of treatments on reproductive outputs and adult longevity, we randomly selected 20 newly emerged females and 20 newly emerged males (< 1 d old) from each of the above treatments and individually

paired them on a leaf disc (2 cm × 2 cm) in a Petri dish as mentioned above. We maintained all these adults under the same treatment conditions. Twenty-four hours later, we transferred the male to a clean leaf disc of the same size in another Petri dish and recorded its longevity. We allowed the female to lay eggs for 72 hours on the same leaf disc and then transferred her to a new leaf disc of the same size in another Petri dish. We repeated this process until her death, and recorded fecundity and egg hatch rate of all eggs laid by each female. Mite transfer, and fecundity and egg hatch recording were performed at 25°C for a few minutes.

All data were analysed using SAS 9.4 with a rejection level set at $\alpha < 0.05$. A Shapiro-Wilk test (UNIVARIATE procedure) was used to test the distribution of data. Data on the immature survival rate, and male and female adult body size were normally distributed and thus analysed using an analysis of variance (ANOVA, GLM procedure) followed by Tukey's Studentized range test. Data on the number of eggs laid were ln(x) transformed to achieve normal distribution before ANOVA. Data on egg hatch rate, and offspring sex ratio and developmental period were not normally distributed even after transformation, and thus analysed using a non-parametric ANOVA (GLM procedure) with a Tukey test for multiple comparisons. Data on adult survival were compared using a Wilcoxon test (LIFETEST procedure).

Our results show that no eggs hatched at the treatment of 36~42°C, indicating that this temperature range is lethal to *T. ludeni* eggs. Similarly, Gotoh *et al.* (2015) report that *T. ludeni* and *T. urticae* eggs do not hatch at the constant temperature of 40°C, and Roy *et al.* (2002) show that *T. mcdanieli* McGregor eggs fail to hatch at ≥ 36°C. These findings suggest that many *Tetranychus* species may not be able to survive in regions with a period of late 30°C to early 40°C in a year, and that air temperature of ca. 40°C can kill spider mite eggs for postharvest disinfestation.

We show that almost 100% of eggs hatched at 15~21 and 22~28°C while only about 70% of eggs hatched at 29~35°C ($F_{2,57} = 69.32$, $P < 0.0001$) (Figure 2A). Among hatched mites, about 75% developed to adults under 22~28°C compared to about 60% and 10% at 15~21°C and 29~35°C, respectively ($F_{2,57} = 224.32$, $P < 0.0001$) (Figure 2B). These findings show that 22~28°C is the optimal thermal condition for survival and development of *T. ludeni*. Based on the results, we propose that this mite may not be able to establish in regions or greenhouses with the daytime temperature ≥ 35°C. The relatively low survival of immature stages at 15~21°C may be attributed to significantly longer development period ($F_{2,1413} = 2328.85$, $P < 0.0001$) (Figure 2C), which may pose higher risk of death (Murphy *et al.* 2018; Esbjerg & Sigsgaard 2019).

Like many haplodiploid species (Roy *et al.* 2003; Macke *et al.* 2011; Tamura & Ito 2017; Zhou *et al.* 2018), *T. ludeni* generally had a female-biased sex ratio (Figure 2D). However, the proportion of female offspring was significantly higher at 29~35°C than at 15~21 and 22~28°C ($F_{2,52} = 22.70$, $P < 0.0001$) (Figure 2D). Roy *et al.* (2003) suggest that an increasing female-biased sex ratio in *T. mcdanieli* at extremely high temperatures could be an evolutionary response of spider mites to deteriorating habitats because females have better capacities than males to disperse and survive under harsh conditions.

Adult *T. ludeni* developed from 29~35°C were significantly smaller ($F_{2,78} = 3.6$, $P = 0.0320$ for male; $F_{2,106} = 149.54$, $P < 0.0001$ for female) (Figure 2E) and laid significantly fewer eggs ($F_{2,37} = 7.88$, $P = 0.0014$) (Figure 2F) than those from 15~21 and 22~28°C. The present study supports both theoretical and empirical findings in many organisms, i.e. adult body size decreases with increasing temperature (Atkinson 1994; Walters & Hassall 2006; Klok & Harrison 2013) and female body size and fecundity are positively correlated (Honěk 1993). Furthermore, we demonstrate that adult longevity and temperature were inversely related in both sexes ($\chi_2^2 = 21.53$, $P < 0.0001$ for males; $\chi_2^2 = 12.00$, $P = 0.0025$ for females) with females being more tolerant than males at high temperatures ($\chi_1^2 = 26.76$, $P < 0.0001$ for 29~35°C) but not at lower temperatures ($\chi_1^2 = 1.91$ and 0.01 for 15~21 and 22~28°C, respectively; $P > 0.05$) (Figure 3).

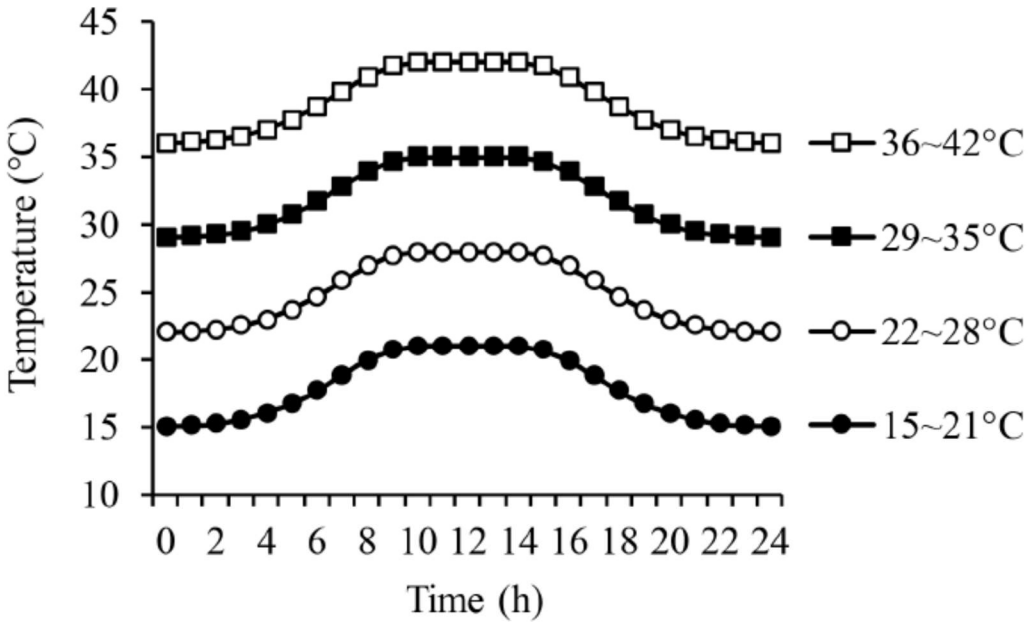

FIGURE 1. Illustration of the hourly temperature change following a modified Gaussian function (Schou *et al.* 2014): Temperature $_{(time)} = a \times e^{-[\frac{(time-b)^2}{2c^2}]} + d$, where a (= 6) is the amplitude, b (= 10 ~ 14) is the time of the peak, c (= 3.16) is the width of the distribution, and d is the nighttime temperature. Time: 0 ~ 4 dark, 4 ~ 20 light, and 20 ~ 24 dark.

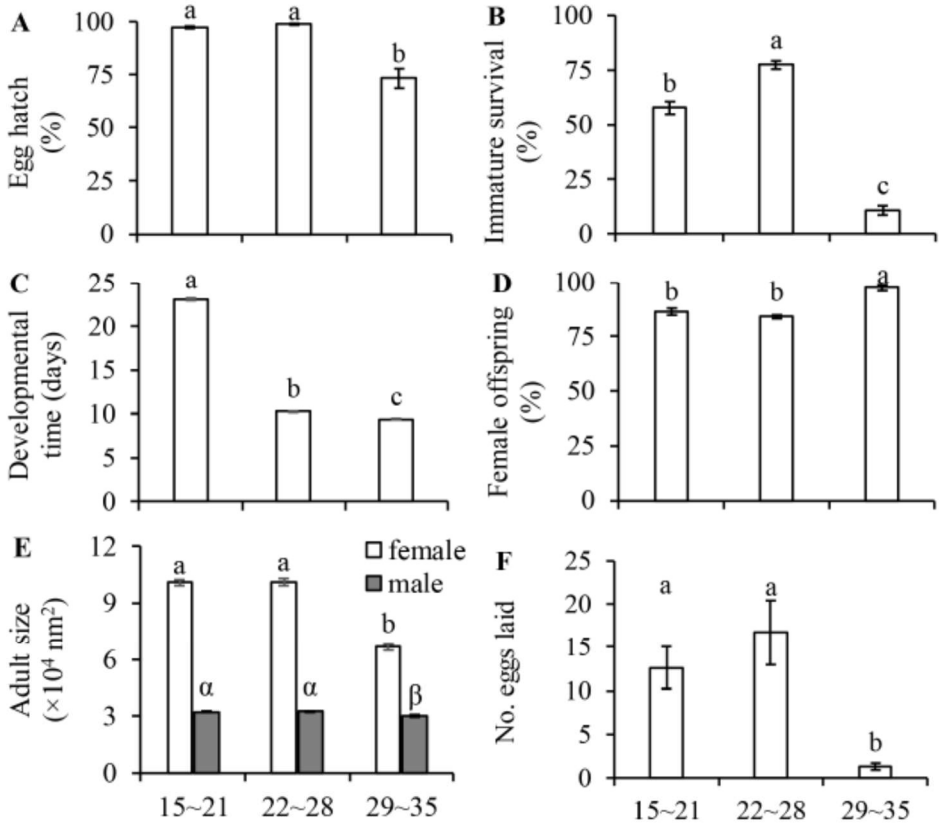

FIGURE 2. Mean (± SE) egg hatch rate (**A**), immature survival rate (**B**), developmental time (**C**), proportion of female offspring (**D**), adult size (**E**), and number of eggs laid (**F**) at different temperature ranges (°C). For each category, columns with the same letters are not significantly different (P > 0.05).

In summary, *T. ludeni* performs the best at 22~28ºC and the worst at 29~35ºC with no eggs surviving at 36~42ºC, suggesting that this mite is less likely to invade hot to very hot regions. The mite can reproduce well at 15~21ºC but at this temperature range, it develops twice slower and suffers 25% higher immature stage mortality than at 22~28ºC, implying that this mite has some difficulty to establish in cool regions and even if it survives in these regions, it is less likely to become a major pest. Our results also suggest that *T. ludeni* can establish in the regions with mild to warm climate after invasion and become an important pest. New Zealand has a mild climate with the South Island being cooler. As a result, the mite population size may be larger in the North Island than in the South Island. Furthermore, it may not be able to survive in the regions with hot climate and greenhouses with a daytime temperature over 30ºC during the summer.

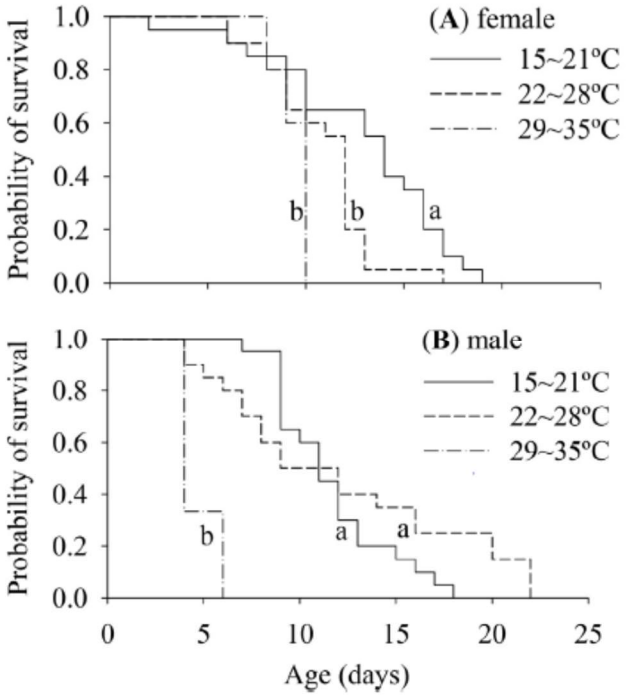

FIGURE 3. Survival of female (**A**) and male adults (**B**) at different fluctuating temperatures. Lines with the same letters are not significantly different ($P > 0.05$).

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Article

Response to thermal environment in *Tetranychus ludeni* (Acari: Tetranychidae)

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Abstract

Tetranychus ludeni Zacher is a spider mite that has invaded all continents except Antarctica and become an economically important pest around the world. Understanding the plasticity of its life history traits as a response to temperatures provides critical information for its risk analysis and management. Here we tested its response to temperatures ranging from 15 to 30ºC over two generations. We found that there was no difference in the egg hatch rate and immature survival rate across temperatures in the first generation. However, the egg hatch rate was lower and immature survival rate was higher at 30ºC in the second generation. The sex ratio (proportion of females) of resultant adults was consistent under all test temperatures in both generations except for 30ºC in the second generation which was lower. Higher temperature accelerated development in both generations but the development at the lower temperatures was faster in the second generation. Adult body size in both generations generally decreased with the increase of temperature, with females being more likely than males to adjust body size in response to temperature changes they first experienced. Temperature-dependent body size was not translated into fecundity, but larger adults lived longer. The thermal threshold was lower and degree days (DD) were greater in the second generation than in the first generation. Our findings indicate that life history traits of *T. ludeni* are highly flexible and adaptive to dynamic thermal environment in successive generations. Furthermore, increasing temperature elevated the intrinsic rate of increase (r_m) but shortened the generation time (T) and the time to double the population size (Dt). The net population growth rate (R_0) was higher at 20 and 25ºC as compared to lower and higher temperatures.

Keywords: *Tetranychus ludeni*, temperature, life history traits, life table, adaptation

Introduction

Tetranychus ludeni Zacher is an invasive mite pest originating from Europe and now present in Asia, Africa, America, and Australasia (Migeon & Dorkeld 2018; CABI/EPPO 2020; Zhou *et al.* 2021). It attacks more than 250 plant species (Gotoh *et al.* 2015) including economically important crops such as carrots, beans, eggplants, pumpkins and other cucurbitaceous plants in warm regions and greenhouses of temperate areas (Zhang 2002; Adango *et al.* 2006; Fathipour & Maleknia 2016). Because temperature is a key factor influencing physiological processes in insects and mites (Ullah *et al.* 2010; Gotoh *et al.* 2015; Zou *et al.* 2018) and determining their distribution and abundance (Bale *et al.* 2002; Roy *et al.* 2002), global warming may further increase the distributional range of *T. ludeni* (Gotoh *et al.* 2015) and favor the spread of other invasive mites (Ghazy *et al.* 2019). Therefore, investigation into the influence of temperature on life history traits provides important information for pest risk analysis and management.

Evidence shows that egg hatch rate, immature survival, developmental time, adult longevity, and life table parameters vary substantially in different mite species and even different strains of the same species in response to test temperatures in the laboratory (e.g., Zhang *et al.* 1999; Da Silva

2002; Roy *et al*. 2003; Gotoh *et al*. 2010, 2015; Ullah *et al*. 2010; Riahi *et al*. 2013; Bazgir *et al*. 2015; Li *et al*. 2015; Liu & Zhang 2016; Bayu *et al*. 2017; Zou *et al*. 2018; Hasanvand *et al*. 2019; Ristyadi *et al*. 2019). This may be attributed to a diverse range of physiological limits in different species (Nguyen *et al*. 2014) and adaptation to temperature changes in different strains (Gotoh *et al*. 2010). However, most studies on the effect of temperature on mite life history traits analyze their data from only one generation, making it difficult to evaluate mites' potential adaptations to variations of temperature over generations. Hence, examination of life history traits in response to different temperatures for more than one generation can provide information on how mites may adapt to dynamic thermal environment for prediction of their potential of invasions and crop damage in the world.

Temperature is also an important factor regulating sex ratio in spider mites (e.g., De Moraes & McMurtry 1987; Margolies & Wrensch 1996; Roy *et al*. 2003; Gotoh *et al*. 2015). Roy *et al*. (2003) suggest that females have better capacities than males to disperse and survive under harsh conditions in some spider mite species, such as *T. mcdanieli* McGregor, and consequently, their sex ratio should be increasingly female-biased at extremely low or high temperatures indicating deteriorating habitats. Yet, it is largely unknown whether this notion applies to other spider mites. Furthermore, body size of adults developing from immatures at different temperatures may vary substantially (Klok & Harrison 2013). For example, body size of geographic strains of a species in warmer regions should be smaller than in cooler regions (Walters & Hassall 2006; Plesnar-Bielak *et al*. 2013; Pequeno *et al*. 2018). However, it is not clear whether such temperature-dependent body size is translated into fecundity in spider mites.

In the present study, we aimed to investigate the life history strategies of *T. ludeni* in response to dynamic thermal conditions in two successive generations. Based on theoretic framework and empirical findings outlined above, we postulate that (1) higher temperature accelerates development and shortens adult longevity; (2) immature mortality is higher and sex ratio is more female-biased at low and high temperatures; (3) adults that develop from lower temperatures are larger and more fecund, and (4) mites can adapt to thermal changes over generations. To test these hypotheses, we carried out a series of experiments and compared various life history traits in response to dynamic temperatures within and between generations. We also created a life table using data collected.

Materials and Methods

Breeding colony and test temperatures

We established a breeding colony of *T. ludeni* from field collected adults on *Passiflora mollissima* (Kunth) (Malpighiales: Passifloraceae) in Palmerston North, New Zealand. We maintained the colony on 20 potted kidney bean plants [*Phaseolus vulgaris* L. (Fabales: Fabaceae)] at 25 ± 1°C temperature and 50 ~ 70% RH with a photoperiod of 16L:8D hours. We replaced the 10 oldest plants fortnightly with new ones by cutting leaves of old plants with mites on and placing them on the top of new plants. We tested effects of four temperatures (15, 20, 25, and 30 ± 1°C) under the above RH and photoperiod conditions using four growth chambers (I-36VL, Percival Scientific Inc., Perry, Iowa, USA).

Effect of temperature on life history traits

To determine whether survival, development and reproduction of *T. ludeni* responded to the same temperature treatments differently in different generations, we exposed mites to the above test temperatures for two generations and recorded egg hatch, immature survival and developmental time between oviposition and adult emergence, and sex ratio and body size of resultant adults. The first

generation started from the eggs laid by mites from the breeding colony and the second generation commenced from the eggs produced by the first generation.

We performed 20 replicates per temperature treatment in each generation. For each replicate, we put 50 eggs on a bean leaf disc (3 cm × 3 cm) laid by mated females of ≤ 1-d-old at a test temperature. We placed the leaf disc (3 cm × 3 cm) upside down on a water saturated cotton pad in a Petri dish (5.5 cm diameter × 1.0 cm height) during the entire treatment period. We replaced leaf discs with fresh ones once every five days and examined each leaf disc daily until all individuals reached adult stage. We randomly selected two females and two males of resultant adults per replicate for body size measurement (area from top view) under a digital camera (Olympus SC30, Japan) connected to the stereomicroscope and a computer with Adequate Imaging software (CellSens® GS-ST-V1.7, Olympus, Germany) installed.

To determine fecundity and adult longevity, we randomly selected 20 newly emerged females and 20 newly emerged males from each of the above treatments in the first generation and individually paired them on a leaf disc (2 cm × 2 cm) in a Petri dish as mentioned above. Twenty-four hours after pairing, we individually transferred males onto clean leaf discs of the same size in Petri dishes and replaced leaf discs once every three days until males died. We allowed females to stay on the same leaf discs for three days, after which time, we individually transferred them onto new leaf discs of the same size in Petri dishes. We repeated this process until females died. We counted the number of eggs laid by each female and monitored adult longevity daily. Due to logistical reasons we did not estimate the above parameters for the second generation.

We calculated the life table parameters (Jervis *et al*. 2005) for each temperature by using the above data collected in the first generation. The intrinsic rate of increase (r_m, daughters/female/day) was calculated by solving the Lotka-Euler equation, $\sum e^{-r_m x} l_x m_x = 1$, where x is the pivotal age of females, l_x is the proportion of females surviving to age x, and m_x is the number of daughters produced per female at age x. We also estimated other life table parameters, including the net reproductive rate ($R_0 = \sum l_x m_x$, daughters/female/generation), mean generation time [$T = \log_e(R_0)/r_m$, days], and doubling time [$Dt = \log_e(2)/r_m$, days]. For each treatment, a jackknife method (Caswell 2001) was used to estimate the life table parameters for each female.

Statistical analysis

We tested the distribution of data using a Shapiro-Wilk test (UNIVARIATE procedure) and used SAS 9.3 (SAS Institute 2011) to analyze all data. Data on the male and female adult body size, and ln(x)-transformed number of eggs laid and developmental period were normally distributed and thus analyzed using an mixed-factor analysis of variance (ANOVA, GLM procedure) followed by Tukey's Studentized range test for multiple comparisons. Data on egg hatch, immature survival, sex ratio (proportion of females) of resultant adults and life table parameters were not normally distributed even after transformation, and thus analyzed using a non-parametric mixed-factor ANOVA (GLM procedure). Data on adult survival were compared using a Wilcoxon test (LIFETEST procedure).

To determine the low temperature threshold (T_0) and degree-days (DD) required to start and complete development of immature stages, we fitted the developmental rates (y = 1/developmental time) over temperatures (T) using a linear regression (GLM procedure): $y = a + bT$, where a and b are estimates of the y intercept and slope, respectively. We then calculated $T_0 = -a/b$, and $DD = 1/b$. According to Campbell *et al*. (1974), the standard errors of T_0 and DD were calculated as $(\bar{y}/b)\sqrt{s^2/(N\bar{y}^2)+(SE_b/b)^2}$ and SE_b/b^2, respectively, where s^2 is the residual mean sum of square of y, $\bar{y}$ the sample mean, SE_b the standard error of slope b, and N the total number of samples.

Results

We show that proportions of eggs that hatched and immatures that survived to adults and sex ratio of resultant adults were largely similar across test temperatures in both generations (Figure 1). The only difference occurred at 30ºC in the second generation where egg hatch rate was significantly lower than at 25 and 30ºC in the first generation ($F = 3.21$; $df = 7, 149$; $P = 0.0034$) (Figure 1A), immature survival rate was significantly higher than at 15ºC in the first generation ($F = 2.59$; $df = 7, 148$; $P = 0.0125$) (Figure 1B), and the proportion of resulting females was significantly lower than all other treatments in both generations ($F = 8.68$; $df = 7, 141$; $P < 0.0001$) (Figure 1C).

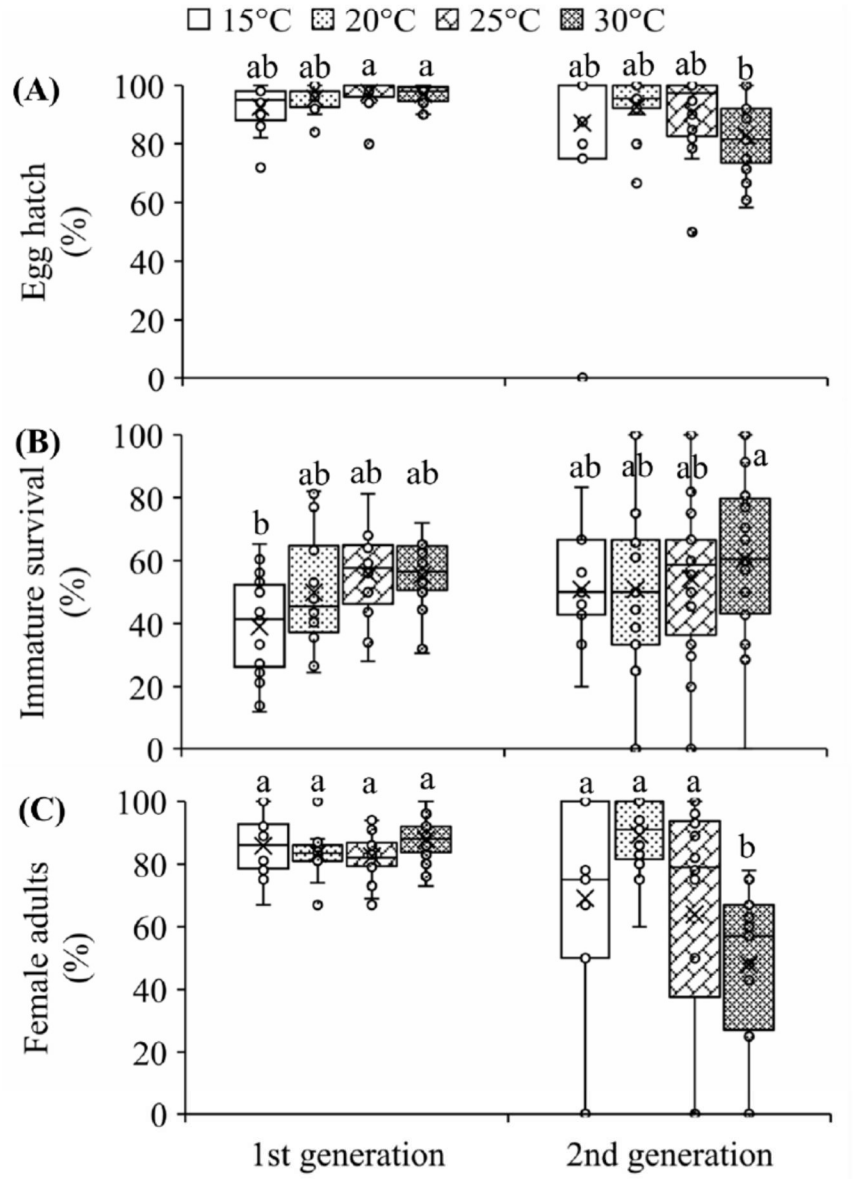

FIGURE 1. Mean (± SE) percentage of egg hatch (**A**), immature survival (**B**) and resulting female adults (**C**) at different temperatures in the first and second generations in *T. ludeni*. Columns with the same letters are not significantly different (P > 0.05). For each box plot, the upper and lower box lines represent 75% and 25% of scores falling beyond the upper and lower quartiles, respectively; the '×' and line in a box indicate the mean and median score, respectively; the '⊤' and '⊥' are the upper and lower whiskers showing scores outside the 50% middle; the circles are the outliers of scores.

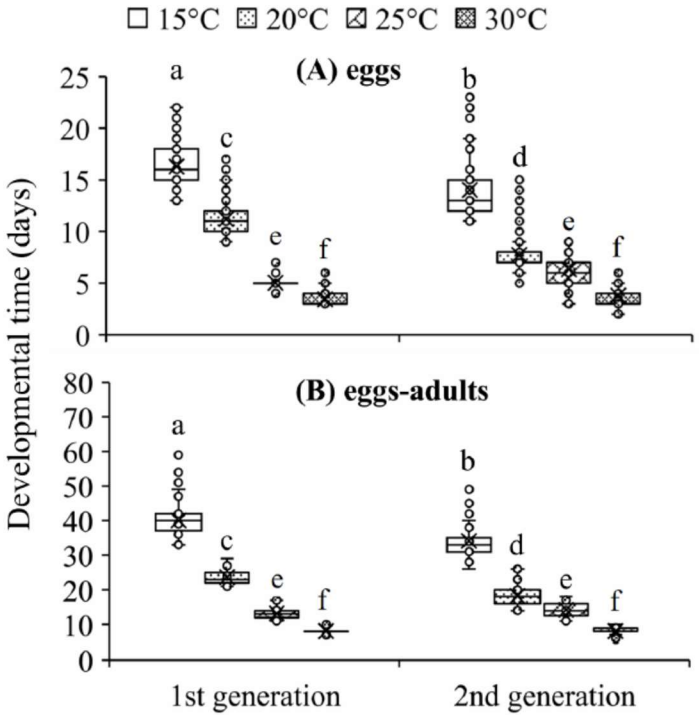

FIGURE 2. Mean (± SE) developmental time of eggs (**A**) and from eggs to adults (**B**) at different temperatures in the first and second generations in *T. ludeni*. Columns with the same letters are not significantly different ($P > 0.05$). For each box plot, the upper and lower box lines represent 75% and 25% of scores falling beyond the upper and lower quartiles, respectively; the '×' and line in a box indicate the mean and median score, respectively; the '⊤' and '⊥' are the upper and lower whiskers showing scores outside the 50% middle; the circles are the outliers of scores.

Tetranychus ludeni significantly accelerated their development with the increase of temperature in both generations, with the second generation developing significantly faster at 15ºC and 20ºC than the first generation ($F = 7341.52$; $df = 7, 4770$; $P < 0.0001$ for eggs, and $F = 9463.52$; $df = 7, 2443$; $P < 0.0001$ for egg-adult) (Figure 2). Adult body size of both sexes generally decreased significantly with the increase of temperature from 20ºC in both generations with it being the largest at 20ºC and smallest at 30ºC ($F = 30.30$; $df = 7, 263$; $P < 0.0001$ for females, and $F = 13.34$; $df = 7, 219$; $P < 0.0001$ for males) (Figure 3). Although the developmental rate and temperature were significantly positively correlated in both generations, the low temperature threshold (T_0) was lower and degree days (DD) were greater in the second generation than in the first generation (Table 1).

Table 1. Relationship between developmental rate (1/d, y) and temperature (T, ºC), and the estimated mean (± SE) low temperature threshold (T_0, ºC) and degree day (DD) required to start and complete development of eggs and from egg to adult stage in *T. ludeni*.

Stage	Equation	R^2	$F_{(df)}$	P	T_0	DD
1st generation						
Egg	y = -0.2064+0.0164T	0.8807	$28221.50_{(1,2695)}$	< 0.0001	12.56±0.03	60.85±0.36
Egg-adult	y = -0.0864+0.0068T	0.9291	$25085.60_{(1,1913)}$	< 0.0001	12.72±0.03	147.15±0.93
2nd generation						
Egg	y = -0.1315+0.0132T	0.4043	$1466.21_{(1,960)}$	< 0.0001	10.23±0.13	75.62±1.98
Egg-adult	y = -0.0749+0.0064T	0.7896	$2003.54_{(1,534)}$	< 0.0001	11.70±0.11	156.28±3.49

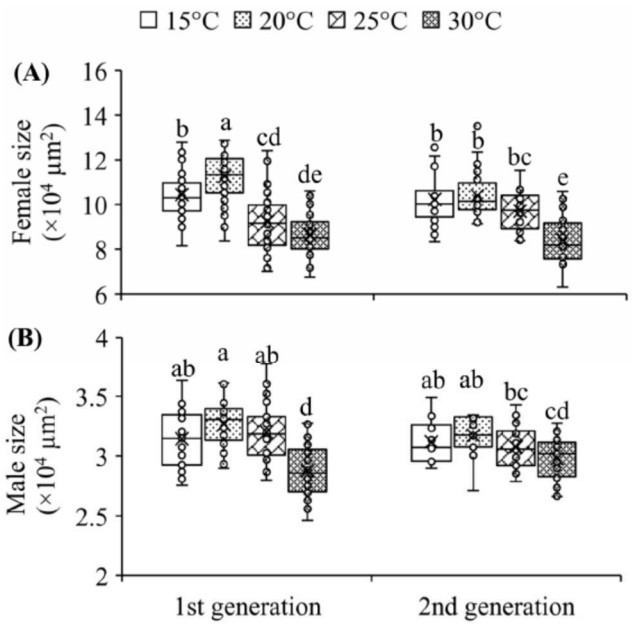

FIGURE 3. Mean (± SE) body size of female (**A**) and male adults (**B**) in *T. ludeni* at different temperatures. Columns with the same letters are not significantly different ($P > 0.05$). For each box plot, the upper and lower box lines represent 75% and 25% of scores falling beyond the upper and lower quartiles, respectively; the '×' and line in a box indicate the mean and median score, respectively; the ' ⊤ ' and ' ⊥ ' are the upper and lower whiskers showing scores outside the 50% middle; the circles are the outliers of scores.

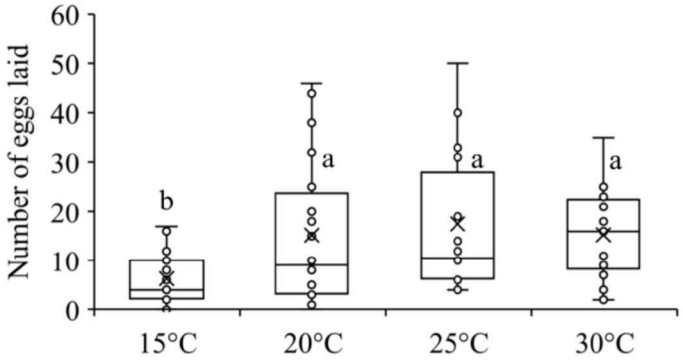

FIGURE 4. Mean (± SE) number of eggs laid by females during their lifetime at different temperatures in *T. ludeni*. Columns with the same letters are not significantly different ($P > 0.05$). For each box plot, the upper and lower box lines represent 75% and 25% of scores falling beyond the upper and lower quartiles, respectively; the '×' and line in a box indicate the mean and median score, respectively; the ' ⊤ ' and ' ⊥ ' are the upper and lower whiskers showing scores outside the 50% middle; the circles are the outliers of scores.

In the first generation, lifetime fecundity of females that developed from 15ºC was significantly lower than that from other treatments ($F = 4.34$; $df = 3, 76$; $P = 0.0071$) where fecundity was similar (Figure 4). Adult males had similar longevity at 15 and 20ºC but their longevity significantly decreased with the increase of temperature from 20 to 30ºC ($x^2 = 52.88$; $df = 3$; $P < 0.0001$) (Figure 5A). Adult females survived significantly longer at 20ºC than at 15 and 25ºC, and their longevity was the shortest at 30ºC ($x^2 = 83.29$; $df = 3$; $P < 0.0001$) (Figure 5B). As shown in Table 2, increasing temperature significantly elevated the intrinsic rate of increase (r_m) but significantly shortened the generation time (T) and the time to double the population size (Dt). The net population growth rate (R_0) was significantly higher at 20 and 25ºC as compared to lower and higher temperatures.

TABLE 2. Mean (± SE) life table parameters of *T. ludeni* at different temperatures.

Temperature (°C)	r_m	R_0	T	Dt
15	0.0219±0.0001 d	1.95±0.02 d	30.35±0.39 a	31.64±0.22 a
20	0.0722±0.0001 c	6.85±0.09 a	26.61±0.16 b	9.60±0.02 b
25	0.1009±0.0005 b	6.55±0.10 b	18.60±0.11 c	6.87±0.04 c
30	0.1281±0.0010 a	4.00±0.05 c	10.81±0.05 d	5.42±0.04 d
$F_{(df)}$	$389.44_{(3,76)}$	$171.55_{(3,76)}$	$302.75_{(3,76)}$	$389.44_{(3,76)}$
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means followed by different letters in columns are significantly different (P < 0.05).

Discussion

We show that the egg hatch rate in the New Zealand strain of *T. ludeni* was > 95% without significant difference between test temperatures from 15–30ºC in the first generation (Figure 1A). Our finding agrees to that for the Japanese strain exposed to similar temperature range for one generation (Gotoh *et al*. 2015). However, the hatch rate was significantly lower at 30ºC in the second generation than at 25ºC and 30ºC in the first generation (Figure 1A), indicating that exposure to 30ºC for more than one generation would reduce egg survival in *T. ludeni*. Immature survival remained similar across test temperatures in the first generation but was higher at 30ºC in the second generation (Figure 1B). This could be an adaptive strategy to compensate lower hatch rate at 30ºC in the second generation.

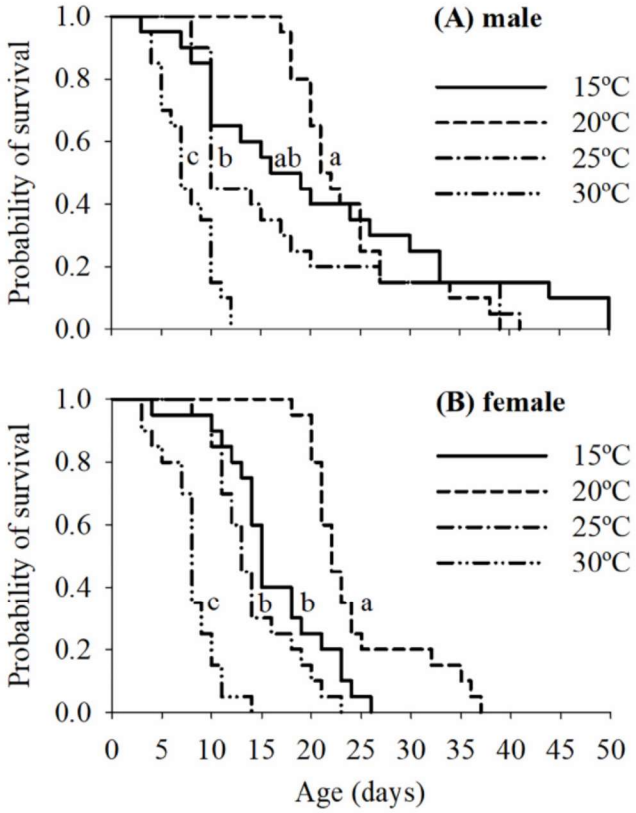

FIGURE 5. Survival of male (**A**) and female adults (**B**) at different temperatures in *T. ludeni*. Lines with the same letters are not significantly different ($P > 0.05$).

Like other haplodiploid species including spider mites (Roy *et al*. 2003; Macke *et al*. 2011; Tamura & Ito 2017), *T. ludeni* generally had a female-biased sex ratio across our test temperatures (Figure 1C). Based on the data from one generation, the sex ratio (proportion of female adults) is higher at 30ºC than at lower temperatures in the Japanese strain of *T. ludeni* (Gotoh *et al*. 2015) and it is more female-biased in *T. mcdanieli* at 15ºC and 34ºC as compared to temperatures between 20ºC and 32ºC (Roy *et al*. 2003). Roy *et al*. (2003) suggest that the increased female-biased sex ratio at extreme temperatures may be an evolutionary response to deteriorating habitats because tetranychid females appear to have better abilities than males to disperse and survive under harsh conditions. However, our findings show that the sex ratio was consistent across all test temperatures in both generations except for 30ºC in the second generation which was significantly lower (Figure 1C). The higher immature survival (Figure 1B) and lower sex ratio (Figure 1C) at 30ºC in the second generation may be an adaptive response to higher temperature over generations in *T. ludeni*.

Similar to reports on *T. ludeni*'s Japanese strain (Gotoh *et al*. 2015) and other mite species (Margolies & Wrensch 1996; Da Silva 2002; Bazgir *et al*. 2015; Li *et al*. 2015; Zou *et al*. 2018), we found that the New Zealand strain of *T. ludeni* developed significantly faster with the increase of temperature from 15 to 30ºC in both generations (Figure 2), supporting the notion that increasing temperature elevates metabolic rate and thus shortens developmental time (Woods & Hill 2004; Potter *et al*. 2009; Schulte 2015). However, compared to the first generation, the second generation developed faster at 15ºC and 20ºC (Figure 2). These findings suggest that *T. ludeni* can adjust its developmental period based on its experience in the previous generation. Adaptation to thermal environment over successive generations is also reported in the predatory mite *Amblydromalus limonicus* Garman and McGregor (Acari: Phytoseiidae) (Walzer *et al*. 2020). We propose that *T. ludeni* accelerates its development at lower temperatures to promote an early start of reproduction after experiencing longer development and delayed reproduction in the first generation at these lower temperatures.

We demonstrate that *T. ludeni* adult body size in both generations (Figure 3) followed the so-called temperature-size rule where body size decreases with the increase of environmental temperature, as reported in many ectotherms (Atkinson 1994; Walters & Hassall 2006; Klok & Harrison 2013; Pequeno *et al*. 2018). However, the degrees and patterns of size changes in response to temperature differed between sexes as well as between generations. For example, the degree of size variation was greater in females than in males in the first generation while size changes of both sexes were smaller in the second generation (Figure 3). These findings have two implications: (1) females are more likely than males to adjust body size in response to temperatures they first experience, and (2) both sexes can adapt to temperatures they have experienced in the previous generation.

We found that temperature-dependent female body size did not translate into fecundity in *T. ludeni* because females that developed from 15–20ºC were larger than those from higher temperatures (Figure 3A) but females from 15ºC laid fewer eggs than those from higher temperatures where there was no difference (Figure 4). These findings differ from those for the Japanese strain where highest and lowest fecundity occurs at 20ºC and 30ºC, respectively (Gotoh *et al*. 2015) and for the Brazilian strain where females lay greatest number of eggs at 30ºC (Da Silva 2002). In *T. urticae*, Riahi *et al*. (2013) and Zou *et al*. (2018) report the highest fecundity at 25ºC and 28ºC, respectively. Our results indicate that temperature-dependent adult body size was associated with longevity and such association was different between sexes (Figures 3 and 5). In the first generation, larger male body size at 15–20ºC (Figure 3B) translated into longer longevity at the same temperature range (Figure 5A) while females had largest body size (Figure 3A) and longest longevity (Figure 5B) at 20ºC. In several other mite species (Riahi *et al*. 2013; Sugawara *et al*. 2017) and the Japanese strain of *T. ludeni* (Gotoh *et al*. 2015), longevity decreases with the increase of temperature.

In this study, the lower thermal threshold (T_0) and degree days (DD) for *T. ludeni* development from eggs to adults (Table 1) fell within the range from 7.8 to 13.8ºC and from 110 to 156 DD, respectively, reported for the genus *Tetranychus* for one generation (Sakunwarin *et al*. 2003; Gotoh *et al*. 2010, 2015; Ullah *et al*. 2010; Karami-Jamour and Shishehbor 2012; Riahi *et al*. 2013; Bayu *et al*. 2017; Hasanvand *et al*. 2019). The varying thermal requirements for development between species or strains of the same species probably reflect their adaptations to the local climate (Gotoh *et al*. 2015). Although the developmental rate and temperature were significantly positively correlated in both generations, T_0 was lower and degree day (DD) was greater in the second generation than in the first generation (Table 1). These results also suggest that *T. ludeni* can adapt to temperature changes by adjusting its developmental rate based on its experience in the previous generation. The life table shows that the intrinsic rate of increase (r_m) increased and doubling time (Dt) decreased with the increase of temperatures from 15 to 30ºC (Table 2). These may be attributed to the shorter developmental time (Figure 2) and more daughters produced during the females' early lifespan at higher temperatures (DR unpublished data). Because population size has a significant effect on reproduction and population growth in *T. ludeni* (Weerawansha *et al*. 2020), the population size changes in response to temperatures can further facilitate its invasion success.

In conclusion, life history traits of *T. ludeni* are highly flexible and adaptive to dynamic thermal environment over generations. We show that higher temperature accelerates its development in both generations, but the development at the lower temperatures is faster in the second than in the first generation. This suggests that the mite shortens its developmental time at lower temperatures in the second generation to promote an early start of reproduction after experiencing longer development and delayed reproduction in the first generation at these temperatures. Adult body size in both generations decreases with the increase of temperature, with females being more likely than males to adjust body size in response to temperature changes they first experience. Larger body size results in greater longevity but not higher fecundity. Lower thermal threshold and greater degree days (DD) in the second generation than in the first generation suggest that *T. ludeni* can adapt to temperature changes by adjusting its developmental rate based on its experience in the previous generation.

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Article

Thermotolerance in a spider mite: implications in disinfestation treatment

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Abstract

Tetranychus ludeni Zacher is a European spider mite species and an important invasive pest in horticulture. We investigated the effects of hot air on its survival and reproduction, providing knowledge for development of disinfestation programs using heat. We tested how each life stage responded to heat treatments of five air temperatures (45 to 57°C) and five exposure durations (three to 15 hours). We showed that no eggs hatched after exposed to 45°C for ≥ 15 hours, 48°C for ≥ 12 hours, or 51°C for three hours; no adults survived 51°C or 54°C for ≥12 hours or 57°C for ≥ six hours, and heat tolerance of other life stages fell in between. Higher temperature and longer exposure time also reduced developmental success and fecundity. These findings suggest that we may be able to eradicate the mites of all stages using one hot air treatment at 57°C for six hours or two treatments at 51°C for three hours at a 10-day interval to kill all eggs in the first treatment and those laid by survived adults in the second. The eradication strategy using hot air of 51–57°C may be more suitable for treating plant residues on exported/imported machinery, farm equipment and containers because it may have negative impact on fresh postharvest products. With the knowledge that exposure to 45°C substantially reduced the mites' fecundity, particularly when the younger stages were treated, we suggest that heat treatment of fresh postharvest products with 45°C could still greatly reduce the quarantine risk of this pest.

Key words: Acari, disinfestation, heat treatment, invasive pest, Tetranychidae

Introduction

Heat treatment has been widely used as a non-chemical disinfestation measure for postharvest crops (e.g., Cowley *et al.* 1992; Waddell *et al.* 1993; Jessup *et al.* 1998; Jacobi *et al.* 2001; Macana & Baik 2018). In invertebrates, particularly insects and mites, response to high temperature and exposure duration varies among species (Bertelsmeier *et al.* 2015; Gray 2017; Kingsolver *et al.* 2021) as well as among life stages within species (Heather *et al.* 2002; Kingsolver *et al.* 2011; Gotoh *et al.* 2013; Chiu *et al.* 2014; Hsu *et al.* 2018; Yao *et al.* 2019). Therefore, the effectiveness of heat disinfestation treatment should be determined by temperature, treatment duration (Dentener *et al.* 1997; Lurie *et al.* 1998; Finkelman *et al.* 2006; Hara 2013) and life stages treated (Heard *et al.* 1992; Heather *et al.* 2002; Gotoh *et al.* 2013; Hsu *et al.* 2018). However, heat tolerance benchmarks have not yet been established for most pest species.

Tetranychus ludeni Zacher (Acari: Tetranychidae) is a European spider mite species but has invaded many regions, including Africa, America, Asia, and Oceania (CABI 2020; Zhou *et al.* 2021),

and become an important pest of many crop species globally (Zhang 2003; Kaimal & Ramani 2011). However, some countries still require disinfestation treatment of postharvest products for *T. ludeni*. For example, Korea bans *T. ludeni*-infested fruit and vegetables (MPI 2019). Several workers have tested the effectiveness of heat treatment to disinfest the spider mite *T. urticae* Koch on postharvest products, showing promising outcome (Waddell & Birtles 1992; Waddell *et al.* 1993; Gotoh *et al.* 2013). However, thermotolerance has yet to be established for *T. ludeni*. It is also unknown how high temperature could affect its reproduction.

In the present study, we investigated how each life stage responded to high temperature exposure in *T. ludeni*. We exposed all life stages to five temperatures for five durations and recorded their mortality rates, and developmental success and reproductive fitness of survived individuals. Information presented here is essential for development of heat disinfestation programs for this important pest. It may also provide knowledge for future evaluation of its invasion potential in relation to heat waves caused by climate change.

Materials and Methods

Experimental mite preparation

We collected *T. ludeni* adults from *Passiflora mollissima* (Kunth) (Malpighiales: Passifloraceae) in Palmerston North, New Zealand, in 2017. A breeding colony from these adults was established and maintained on 20 potted kidney bean plants *Phaseolus vulgaris* L. (Fabales: Fabaceae) in the Entomology and IPM Laboratory of Massey University, New Zealand. We replaced ten oldest plants with new ones every two weeks by cutting the infested leaves of old plants and placing them on the top of new ones. The mite colony was maintained, and experimental mites prepared at 25 ± 1°C and 50 ~ 70% RH with a photoperiod of 16L:8D hours.

We randomly collected 20 adult females and four adult males from the colony and transferred them onto a bean leaf disc (3 cm × 3 cm) positioned upside down on a water-saturated cotton pad in a Petri dish (5.5 cm diameter × 1.0 cm height). The adults were allowed to stay on the leaf disc for 24 hours and then removed. The larvae were transferred to a new leaf disc of the same size immediately after the eggs laid by these adults hatched. We reared them at 25°C for 1, 4, 6 and 8 days to obtain larvae, protonymphs, deutonymphs and female adults, respectively, for experiment. In total, we set up 800 such leaf discs. To obtain adult males, we randomly collected 20 female deutonymphs from the colony, placed them on a leaf disc as above and allowed the newly emerged virgin females to lay eggs for 24 hours. The newly hatched larvae were then transferred to a new leaf disc and allowed to develop to adult males for the experiments.

Heat-dependent mortality rate in each life stage

To determine heat-dependent mortality rate of mites of each life stage, we treated eggs, larvae, protonymphs, deutonymphs, virgin adult males and females with five temperatures (45, 48, 51, 54 and 57°C) for five heat durations (3, 6, 9, 12 and 15 hours), resulting in a total of 150 treatments (6 life stages × 5 temperatures × 5 durations). There were 20 replicates for each treatment. For each replicate, 10 individuals were introduced onto a bean leaf disc (3 cm × 3 cm) on a water-saturated cotton pad in a Petri dish (5.5 cm diameter × 1.0 cm height) and then the dish was transferred into an incubator (Series Five, Contherm Scientific Company, New Zealand) with a treatment temperature. Immediately after treatment, we moved the Petri dishes to 25°C, and transferred all life stages except eggs onto new leaf discs in Petri dishes as above. Eggs that did not hatch in 10 days and individuals of other life stages that had no sign of movement 48 hours after treatment were considered as dead.

Effect of heat treatment on immature development to adult stage

To determine the probability of immature mites surviving heat-treatment to develop to adulthood, we randomly took up to 20 individuals that survived from each treatment of the above experiment and reared them individually at 25ºC on leaf discs as above. The leaf discs were replaced with new ones every 4 days and the total number of adults that emerged from these juveniles was recorded. However, if no juveniles survived in some treatments, we did not follow their development.

Effect of Heat Treatment on Reproduction

To determine how heat treatment affected reproduction, we individually transferred up to 20 females that were heat-treated during the adult stage and 20 newly emerged females that developed from each heat-treated immature stage in the above experiment onto leaf discs and reared them at 25 on leaf discs as above. The mortality of females was monitored daily and leaf discs were replaced with new ones once every 4 days if they were still alive. The total number of eggs laid by each female was recorded. However, if no individuals survived in some treatments, we did not follow their reproduction in those treatments.

Statistical Analysis

All data analyses were performed using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC). We analyzed the mortality rate (%) of different life stages using a generalized linear mixed model (GLIMMIX procedure) with temperature, exposure duration and their interactions as the fixed factors and replicate as a random effect followed by a Binomial distribution and a Logit link function for the model. A Tukey-Kramer test was used to compare the difference in the mortality rate between temperatures of a given duration and between durations of a given temperature within each life stage. The same method was used to compare the difference in overall mortality rate between life stages. The proportions of individuals that developed to adult stage after being treated at different temperatures for various durations were analyzed using a likelihood ratio test in a logistic regression model (GENMOD procedure) with a Binomial distribution and a Logit function used to the model and the CONTRAST statement for multiple comparisons. The generalized linear mixed model (GLIMMIX procedure) with a Poisson distribution and a Log link function was applied to determine the combined effect of temperature and duration on the number of eggs laid, followed by a Tukey-Kramer test for multiple comparisons.

Results

Heat-dependent mortality rate in each life stage

In each life stage, temperature and exposure duration significantly interacted ($F_{16,456}$ = 4.26 ~ 14.10, $P < 0.0001$), with higher temperature ($F_{4,76}$ = 8.26 ~ 136.64, $P < 0.0001$) and longer exposure ($F_{4,76}$ = 26.96 ~ 162.94, $P < 0.0001$) causing significantly higher mortality (Table 1). At any treatment duration no eggs hatched when temperature was 51°C or higher, and all life stages died at 57°C of any treatment duration except about 10% of adult females and 1% of deutonymphs which survived 57°C for 3 hours (Table 1). Our results also show that younger life stages, particularly eggs, were significantly more susceptible to heat treatment [overall mean (± SE) mortality rate (%) = 93.1 ± 0.9 for egg, 83.1 ± 0.9 for larva, 75.9 ± 1.1 for protonymph, 75.4 ± 1.1 for deutonymph, 71.8 ± 1.5 for adult male and 62.7 ± 1.7 for adult female; $F_{5,2975}$ = 529.33, $P < 0.0001$] (Table 1).

TABLE 1. Mean (± SE) mortality rates (%) of different life stages of *Tetranychus ludeni* after treatment at different temperatures for various durations*

Life stage	Duration	Temperature (ºC)					$F_{4,76}$	P
	(hours)	45	48	51	54	57		
Adult female	3	11.5 (±3.2) D d	14.0 (±2.7) D d	25.0 (±5.6) D c	42.5 (±5.2) C b	89.0 (±3.5) B a	123.90	< 0.0001
	6	13.0 (±3.2) D d	14.0 (±3.1) D d	47.0 (±4.8) C c	85.5 (±4.3) B b	100 A a	162.94	< 0.0001
	9	21.5 (±3.4) C c	23.5 (±4.0) C c	54.5 (±4.4) B b	100 A a	100 A a	143.12	< 0.0001
	12	34.0 (±3.7) B c	61.5 (±6.3) B b	100 A a	100 A a	100 A a	129.14	< 0.0001
	15	47.0 (±3.8) A c	85.5 (±4.2) A b	100 A a	100 A a	100 A a	98.55	< 0.0001
	$F_{4,76}$	42.08	136.64	114.95	109.68	12.99		
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
Adult male	3	15.5 (±2.8) D e	29.5 (±3.7) D d	68.0 (±6.0) C c	86.5 (±5.3) C b	100 A a	147.17	< 0.0001
	6	15.0 (±3.9) D e	36.5 (±4.9) C d	81.0 (±3.2) B c	90.0 (±4.2) C b	100 A a	156.14	< 0.0001
	9	27.5 (±3.4) C e	37.5 (±3.2) C d	85.0 (±2.9) B c	94.5 (±3.1) B b	100 A a	145.79	< 0.0001
	12	44.5 (±4.4) B b	49.0 (±6.2) B b	100 A a	100 A a	100 A a	118.12	< 0.0001
	15	54.5 (±6.1) A c	79.5 (±4.3) A b	100 A a	100 A a	100 A a	80.03	< 0.0001
	$F_{4,76}$	52.45	54.25	40.01	13.91	0		
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	1		
Deutonymph	3	40.5 (±4.0) D c	43.5 (±5.8) D c	56.5 (±2.0) C b	59.0 (±3.6) B b	99.0 (±0.9) A a	54.92	< 0.0001
	6	52.0 (±4.8) C c	53.0 (±3.9) C bc	59.5 (±2.3) C b	64.5 (±4.9) B b	100 A a	40.46	< 0.0001
	9	54.0 (±4.1) BCd	67.0 (±3.3) B c	85.0 (±4.4) B b	96.5 (±1.8) A a	100 A a	66.22	< 0.0001
	12	59.0 (±3.0) B d	72.5 (±4.1) ABc	88.5 (±2.7) B b	95.5 (±1.5) A a	100 A a	55.57	< 0.0001
	15	68.5 (±2.7) A d	76.5 (±4.1) A c	94.0 (±1.8) A b	100 A a	100 A a	47.20	< 0.0001
	$F_{4,76}$	16.6	29.66	53.4	72.59	0.16		
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.9572		
Protonymph	3	38.0 (±2.8) C c	40.5 (±4.3) Dbc	44.0 (±3.9) D bc	45.5 (±3.5) C b	100 A a	54.52	< 0.0001
	6	47.5 (±4.9) C e	60.5 (±3.9) C d	76.5 (±3.6) C c	82.0 (±3.4) B b	100 A a	57.21	< 0.0001
	9	54.5 (±4.7) B e	62.0 (±4.2) C d	78.0 (±3.8) BCc	91.0 (±2.8) A b	100 A a	56.79	< 0.0001
	12	61.5 (±4.0) B e	76.5 (±3.6) B d	82.5 (±2.9) B c	89.5 (±3.1) A b	100 A a	38.33	< 0.0001
	15	81.5 (±2.8) A b	86.5 (±3.1) A b	100 A a	100 A a	100 A a	26.96	< 0.0001
	$F_{4,76}$	38.6	46.2	61.98	78.47	0		
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	1		
Larva	3	51.5 (±6.1) C b	56.0 (±3.8) D b	58.0 (±4.2) D b	90.0 (±3.1) B a	100 A a	64.92	< 0.0001
	6	62.5 (±4.0) B c	64.0 (±3.7) C c	78.0 (±3.0) C b	93.0 (±2.2) B a	100 A a	47.34	< 0.0001
	9	64.5 (±3.9) B c	71.0 (±2.7) B c	89.5 (±2.6) B b	96.5 (±1.3) A a	100 A a	49.63	< 0.0001
	12	76.5 (±3.5) A b	76.5 (±3.3) B b	95.0 (±1.4) A a	100 A a	100 A a	37.46	< 0.0001
	15	75.5 (±2.9) A c	82.5 (±3.6) A b	98.5 (±0.8) A a	99.5 (±0.5) A a	100 A a	36.74	< 0.0001
	$F_{4,76}$	17.27	17.99	54.22	8.26	0		
	P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	1		
Egg	3	32.0 (±6.8) D c	67.0 (±4.8) B b	100 A a	100 A a	100 A a	128.52	< 0.0001
	6	60.0 (±7.4) C c	95.0 (±2.2) A b	100 A a	100 A a	100 A a	75.79	< 0.0001
	9	76.5 (±5.0) B b	98.0 (±0.9) A a	100 A a	100 A a	100 A a	38.31	< 0.0001
	12	99.0 (±1.0) A a	100 A a	100 A a	100 A a	100 A a	0.16	0.9572
	15	100 A a	100 A a	100 A a	100 A a	100 A a	0	1
	$F_{4,76}$	107.95	55.57	0	0	0		
	P	< 0.0001	< 0.0001	1	1	1		

* Mortality rate with the same small letters in rows or the same capital letters in columns within each life stage are not significantly different ($P > 0.05$)

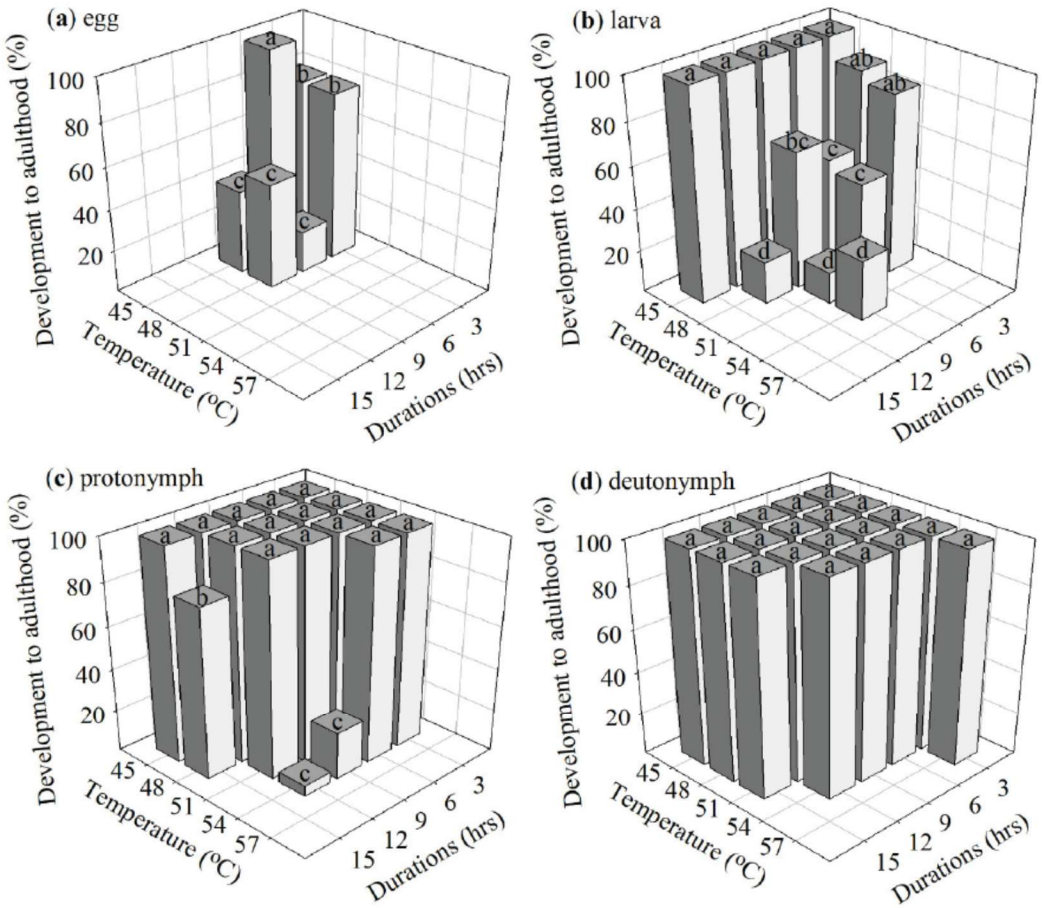

FIGURE 1. Proportion of immatures surviving heat treatment that developed to adult stage in *T. ludeni*. For each life stage, columns with the same letters are not significantly different ($P > 0.05$).

Effect of heat treatment on immature development to adult stage

We show that increasing treatment temperature and exposure duration significantly reduced the probability of immatures to develop to adulthood ($x^2_5 = 34.71$, $P < 0.0001$ for egg; $x^2_{12} = 136.25$, $P < 0.0001$ for larva; $x^2_{17} = 180.34$, $P < 0.0001$ for protonymph; $x^2_{19} = 0$, $P = 1$ for deutonymph) (Figure 1). These findings also indicate that older immatures were significantly more likely to complete development across treatments ($x^2_3 = 158.64$, $P < 0.0001$) (Figure 1).

Effect of heat treatment on reproduction

Females that resulted from eggs treated at > 48°C (Figure 2a) or larvae treated at > 51°C for any duration (Figure 2b) did not lay any eggs. Those that developed from other treated life stages laid significantly fewer eggs with the increase of temperature and exposure duration ($F_{14,271} = 45.48$, $P < 0.0001$ for protonymph; $F_{16,310} = 26.68$, $P < 0.0001$ for deutonymph; $F_{13,266} = 54.32$, $P < 0.0001$ for adult female) (Figure 2c–e).

Discussion

Here we reported the responses of *T. ludeni* to air temperatures ranged from 45 to 57°C for three to 15 hours. We showed that higher temperature and longer exposure caused higher mortality to all life stages, but younger life stages were more sensitive to heat than older ones, with adults being most

tolerant and eggs most susceptible (Table 1). All adults died after exposure to 51°C or 54°C for 12 hours or 57°C for ≥ six hours, while no eggs survived 45°C for ≥ 15 hours, 48°C for ≥ 12 hours, or ≥ 51°C for any duration. We also demonstrate that higher temperature and longer exposure time reduced the probability of heat-treated juveniles to successfully develop to adulthood, but older immatures were more likely to complete development across treatments (Figure 1). Females that developed from heat-treated eggs or larvae laid only a few eggs and those from other treated life stages laid fewer eggs with increasing temperature and exposure time (Figure 2).

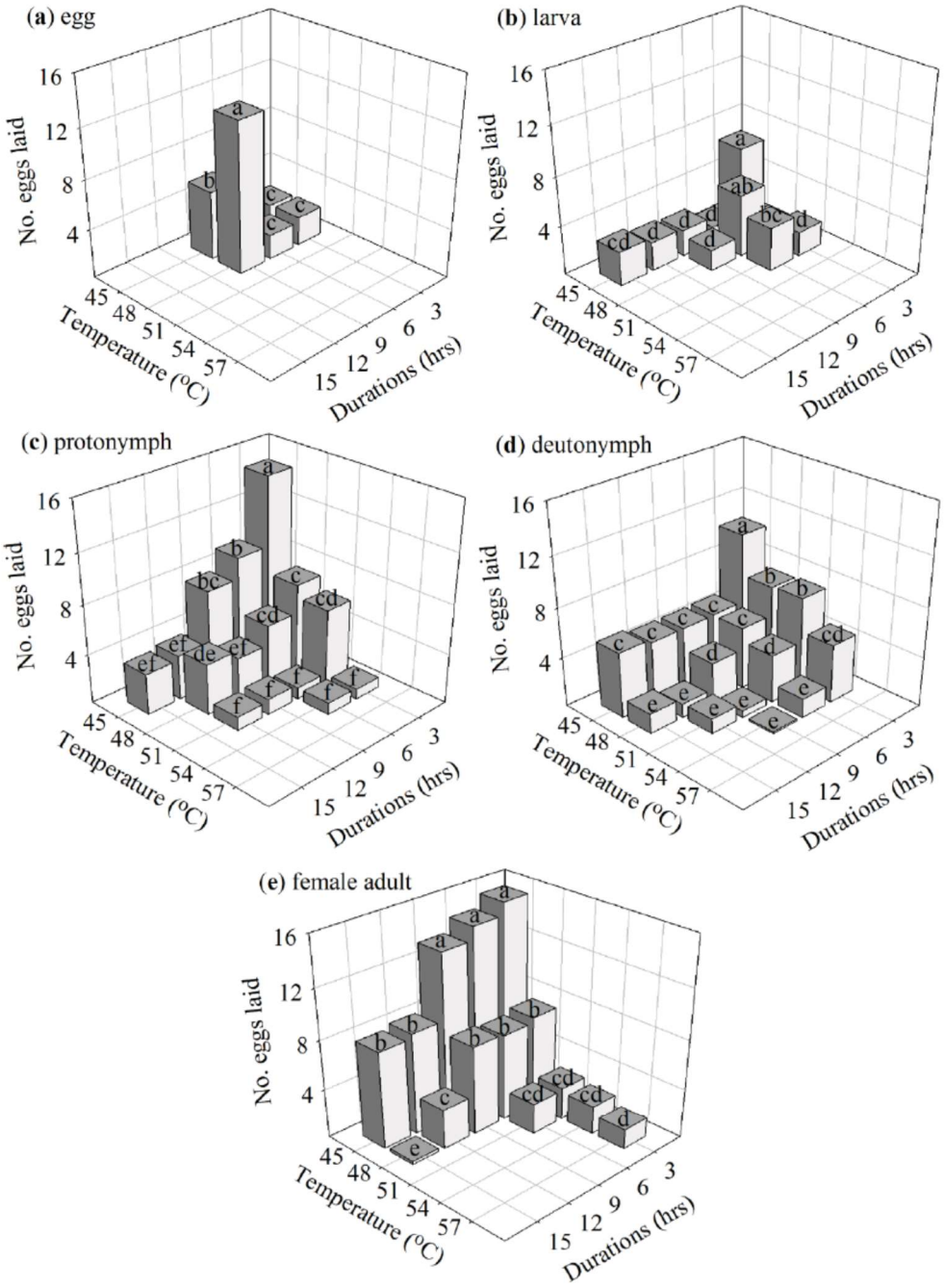

FIGURE 2. Mean number of eggs laid by *T. ludeni* females after their or their juvenile stages' exposure to different temperatures for various durations. For each life stage, columns with the same letters are not significantly different ($P > 0.05$).

Heat treatment has been used or trialled for killing insects (e.g., Cowley *et al.* 1992; Dentener *et al.* 1997; Jessup *et al.* 1998; Jacobi *et al.* 2001; Hara 2013; Macana & Baik 2018) and mites (Waddell & Birtles 1992; Waddell *et al.* 1993; Gotoh *et al.* 2013) on postharvest products. In *T. ludeni*, the egg stage was the least tolerant to air heat with all eggs killed at 51C within three hours (Table 1). Because all life stages may be present at the same time due to short life cycle and overlapping generations (Adango *et al.* 2006; Ristyadi *et al.* 2019) and some individuals of life stages other than eggs may survive this temperature (Table 1), two treatments of 51C for three hours at a 10-day interval can fully disinfest products with all eggs being killed in the first treatment and those laid by survived individuals destroyed in the second. Alternatively, we can eradicate the mites of all stages using one treatment of 57°C for about six hours.

However, the full disinfestation strategy using hot air of 51–57°C may be more suitable for treating plant residues on exported/imported machinery, farm equipment and containers because it may have negative impact on fresh postharvest products. With the knowledge that exposure to 45C for a few hours substantially reduced the mites' fecundity, particularly when the younger stages were treated, we suggest that heat treatment of fresh postharvest products with 45°C could still considerably reduce the quarantine risk of this pest. Moreover, Auger *et al.* (2003) report that wettable sulphur can kill all stages of *T. urticae* at the air temperature of 35C. We thus predict that air temperature much lower than 45°C for shorter than three hours can achieve complete disinfestation for *T. ludeni* if a chemical like wettable sulphur is also used. Further investigation into possible combinations of chemical and heat treatments for this pest would provide valuable information for exporters.

In conclusion, we demonstrate that heat shock tolerance is stage-dependent in *T. ludeni* with eggs being the most vulnerable and adults the most tolerant. Heat shock also reduces developmental success and fecundity. We may fully disinfest plant residues on machinery, equipment and containers using two treatments of 51°C for three hours at a 10-day interval or one treatment of 57°C for about six hours, and substantially reduce quarantine risk by treating fresh products with 45°C for a few hours. This study provides important knowledge for development of hot air disinfestation programs of this invasive pest. Information presented here may offer a reference for future evaluation of its invasion potential in relation to heat waves caused by climate change.

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