

**EFFECTS OF ELEVATED TEMPERATURE AND WATER-DEFICIT
STRESS ON THE POTATO APHID *MACROSIPHUM EUPHORBIAE*
(THOMAS) (HEMIPTERA: APHIDIDAE) AND ITS PARASITOID
APHIDIUS ERVI HALIDAY (HYMENOPTERA: BRACONIDAE)**

BY

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DECLARATION

I, Lezel Muller, declare that the thesis which I hereby submit for the degree of Magister Scientiae at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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SUMMARY

Global climate change is expected to increase average surface temperatures by 1.5 to 4.5 °C by the end of the 21st century. Major variation in climatic conditions is predicted to occur, including more frequent droughts and heat waves as well as higher mid-summer temperatures. Water-deficit stress may lead to increased nitrogen levels in plants, which in turn may increase their suitability for insect herbivores. In addition, the higher nutritional quality of the insect herbivore diet may increase the acceptability to parasitoids for parasitism. Parasitoid larvae depend completely on the nutrient quality of the host, which in turn could influence the fitness of the emerging adult. In this study, the influences of water-deficit stress and high day-night temperatures on a tritrophic interaction, potato, *Solanum tuberosum* L. (Solanaceae), the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) and its aphid parasitoid, *Aphidius ervi* Haliday (Hymenoptera: Braconidae, Aphidiinae) were determined. The specific objectives were (i) to determine the water stress levels and nitrogen concentrations in potato plants and to evaluate the effects of water-deficit stress and high temperatures on the potato-aphid interaction by determining nymphal development times, survival rates, adult longevity and life table parameters of *M. euphorbiae*; (ii) to select a suitable parasitoid of *M. euphorbiae* for experiments; (iii) to evaluate the response of *A. ervi* to various densities of *M. euphorbiae* as part of determining a suitable host parasitoid ratio for experiments; and (iv) to determine the effects of water-deficit stress and high day-night temperatures on amino acid concentrations in potato plants and on the number of mummies formed, pupal survival, development time, longevity, total offspring per female and sex ratio of *A. ervi*.

Levels of water-deficit stress in potato plants were measured indirectly by stomatal conductance (g_s). No differences were found in nitrogen concentrations between water-deficit stress and well-watered plants. However, total amino acid concentrations were higher in water-deficit stressed plants compared to well-watered plants. White (2009) proposed the plant stress hypothesis, stating that the performance of senescent feeders and consequently population growth is enhanced on stressed plants, especially under water-deficit stress, due to an increase in phloem nitrogen. In this study, moderate water-deficit stress at ambient temperature had little or no effect on the performance (development time and reproduction) as well as on population growth parameters of *M. euphorbiae*, rejecting the plant stress hypothesis. Fluctuating high day/night temperatures, on the other hand, had a negative effect

on aphid growth parameters, suggesting that *M. euphorbiae* could decline in abundance during average summer mid-day temperatures exceeding 27.3 °C.

In August 2010, *Aphidius ervi* were collected from a sample of the potato aphid, *M. euphorbiae*, in the field on *Malva parviflora* L. (Malvaceae) in Pretoria, South Africa. This is the first record of this parasitoid from South Africa. The heat-stress hypothesis proposed by Roux *et al.* (2010) was tested on *A. ervi*. This hypothesis states that an increase in mortality of aphid parasitoids, especially weaker individuals exposed to above-optimum temperatures, will occur. Those individuals that survive heat stress will pay a cost in their fitness measured as reproductive output. In effect, the survival and percentage parasitism of *A. ervi* were reduced at high day-night temperatures and the hypothesis was accepted. This indicates that field populations of *A. ervi* could decrease during temperatures rising to 30 °C and above. Water-deficit stressed potato plants may provide an enhanced food source for *M. euphorbiae* due to a higher total essential amino acids content. In the current study, pupal survival of *A. ervi*, percentage parasitism and the number of female offspring were the highest on *M. euphorbiae* reared on water-stressed plants at ambient temperature. This, in turn, indicates that parasitoid larvae benefited from the higher amino acid concentrations ingested by their host. However, high day-night temperatures negatively influenced the performance of *A. ervi* and therefore countered the positive effects of high amino acid concentrations in water-deficit stressed potato plants. This study provides some information on the effects of extreme climatic events. However, short-term observations on an individual level are not sufficient to predict long-term effects on insect population dynamics, which depend on complex interactions between biotic and abiotic factors.

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

Potato (*Solanum tuberosum* L., Solanaceae) is one of the most important food crops worldwide, exceeded only by wheat, rice and maize (Rigano et al., 2013). In South Africa it is the single most important vegetable crop (Radcliffe, 1982; Potatoes South Africa 2013). The total world potato production was 321 million tons cultivated on 18.5 million ha in 2010 (Potatoes South Africa 2010). In South Africa, 2.2 million tons of potatoes, with an estimated gross value of R 6 billion, were harvested from about 54 000 ha in 2012 (Potatoes South Africa 2013).

Potatoes are produced in 16 regions spread across South Africa in different climatic areas (Potatoes South Africa 2013), and farmers are able to produce potatoes continuously throughout the year. Potato production includes table potatoes, for processing, or seed potatoes. In South Africa, approximately 10 000 ha are registered annually for seed potato production by the Independent Certification Council for Seed Potatoes (Potatoes South Africa 2013). Africa's population is estimated to double by 2050, with an expected increase in consumption of potatoes. Therefore, it is of vital importance to produce more potatoes from the same or less land.

Potato production worldwide is severely hampered by pests and diseases. Aphids, as phloem feeding insects, are very important economic pests on cultivated plants in agriculture, horticulture and forestry (Millar, 1990). In addition, on potato, aphids are considered to be of even greater economic importance worldwide than defoliators or tuber pests (Radcliffe, 1982; Radcliffe & Ragsdale, 2002; Narayandas & Alyokhin, 2006). Aphids cause direct damage to potato plants by feeding in dense clusters on the stems and leaves, resulting in stunted growth. This in turn results in a reduction in yield due to high consumption of phloem nutrients such as sucrose, nitrogen and water (Radcliffe, 1982; Radcliffe & Ragsdale, 2002; Nguyen et al., 2007). More importantly, aphids cause indirect damage by transmitting plant viruses such as *Potato leafroll virus* (PLRV) and *Potato virus Y* (PVY) (Radcliffe & Ragsdale, 2002; Narayandas & Alyokhin, 2006; Nguyen et al., 2007).

The potato aphid, *Macrosiphum euphorbiae* (Thomas) (Hemiptera, Aphididae), is considered as one of the most important aphid pests on potato due to substantial potato crop losses through feeding damage, honeydew production and transmission of viruses such as PLRV and PVY (Radcliffe, 1982). In general, chemical control has been the most commonly

used strategy to reduce aphid populations in potato crops (Perring et al., 1999). However, releases of biological control agents (natural enemies) in combination with specific chemical applications have proven to be an effective control strategy against aphid pests in open field crops and enclosed structures like greenhouses (van Lenteren, 2012).

Natural enemies, such as *Aphidius ervi* Haliday (Hymenoptera, Braconidae, Aphidiinae), can be successful in controlling aphid populations, and some are commercially available. *Aphidius ervi* is commonly used as a biological control agent for the pea aphid, *Acyrtosiphum pisum* (Harris), as well as other aphid pests in Europe, North America, South America, Australia and New Zealand (Hofsvang & Hågvar, 1975; Marsh, 1977; Schwörer & Völkl, 2001; Takada, 2002; He et al., 2004; Kos et al., 2009).

Aphid population dynamics are regulated by abiotic (e.g. climate) and biotic (e.g. parasitic wasps (parasitoids)) factors. Aphids are thought to respond relatively fast to changes in climatic conditions due to their biology (e.g. short life cycle, high reproductive potential) (Harrington et al., 1995). Predicted climate change in parts of South Africa includes an increase in temperature and in droughts. If any changes occur, whether physiological or physical, in the first trophic level (plant), this will influence the second (aphid) and the third (parasitoid) trophic levels. The aim of this study is to determine the effects of water-deficit stress and high temperature on the tritrophic interactions between potato, *M. euphorbiae*, and the parasitoid *A. ervi*. Determining the influence of water-deficit stressed plants and high temperature on the various trophic levels in the laboratory can help to predict what will happen in the field.

The specific objectives were (i) to determine the water stress levels and nitrogen concentrations in potato plants and to evaluate the effects of water-deficit stress and high temperature on the potato-aphid interaction by determining nymphal development times, survival rates, adult longevity, and life table parameters of *M. euphorbiae*; (ii) to select a suitable parasitoid of *M. euphorbiae* for experiments; (iii) to evaluate the response of the parasitoid *A. ervi* to various densities of *M. euphorbiae* as part of determining a suitable host: parasitoid ratio for experiments; and (iv) to determine the effects of water-deficit stress and high day-night temperatures on amino acid concentrations in potato plants and on the number of mummies formed, pupal survival, development time, longevity, total offspring per female, and sex ratio of *A. ervi*.

1.1 The potato aphid, *Macrosiphum euphorbiae* (Thomas) (Hemiptera, Aphididae)

Macrosiphum euphorbiae is a highly important economic pest known to attack more than 200 host species from 20 different plant families, many of which are vegetable and ornamental crops (Millar, 1990; Srinivasan & Alvarez, 2011). The species originated from North America and has spread throughout the world except for the Indian subcontinent (Millar, 1990). In South Africa, the primary hosts of *M. euphorbiae* are plant species of the genus *Rosa* (Rosaceae). However, potato plants are particularly favoured as secondary hosts (Millar, 1990; Srinivasan & Alvarez, 2011). *Macrosiphum euphorbiae* may cause substantial potato crop losses through feeding damage and honeydew production (Kaloshian et al., 1997; Narayandas & Alyokhin, 2006). Honeydew serves as a substrate for fungal growth such as sooty mould, which may consist of one or several saprophytic ascomycete fungi. These fungi are black in colour and impairs photosynthesis, leading to a reduction in yield by means of lower starch and sucrose synthesis (Brodeur & McNeil, 1994; Radcliffe & Ragsdale, 2002; Nguyen et al., 2007; Zamani et al., 2007). More importantly, *M. euphorbiae* is a vector of potato viruses such as *Potato leafroll virus* (PLRV, genus *Polerovirus*, family *Luteoviridae*) and *Potato virus Y* (PVY, genus *Potyvirus*, family *Potyviridae*) (Radcliffe & Ragsdale, 2002; Nguyen et al., 2007; Zamani et al., 2007). PVY and PLRV can cause a serious reduction in yield, ranging from 10% to 100%. Primary infection is the initial infection of a healthy plant or tuber, whereas secondary infection results from planting infected potato seeds (Radcliffe, 1982). Seed potato producers have to comply with low tolerance levels of PLRV and PVY infection (0-1%) for high quality seed (Radcliffe & Ragsdale 2002; South African Seed Certification Scheme 2010) Therefore, control of *M. euphorbiae* and other aphid vectors is of major concern (Radcliffe & Ragsdale, 2002).

Macrosiphum euphorbiae belongs to the tribe Macrosiphini and is considered to be a relatively large aphid. The wingless morph (apterae) normally measures between 1.7 - 3.6 mm in size, and winged morphs (alatae) 1.7 - 3.4 mm (Figure 1.1) (Millar, 1990). Both alatae and apterae have two siphunculi that are cylindrical and long as well as a cauda with 8-11 setae. Alatae differ from apterae by having a sclerotized thorax modified to accommodate the flight muscles. Alatae also possess ocelli on top of the head, the compound eyes are well developed, and secondary rhinaria on the antennae are more distinct compared to apterae (Millar, 1990). The initiation of wing development is controlled by the mother (pre-natal) or by the nymph (post-natal) (Müller et al., 2001).



Figure 1.1 Nymphal stage (first and second instar), wingless (apterae) and winged (alatae) morphs of *Macrosiphum euphorbiae* (Photo: L. Muller).

There are many factors that influence alatae production. One of these factors is overcrowding when aphid populations on host plants are too high, which results in involuntary tactile stimulation which induces wing development. Poor host quality is another factor encouraging alatae development. The quality of the host plant may decline when aphids deplete the food source due to overpopulation, which can lead to wilting or even death of the plant. Therefore, it is advantageous for aphids in large populations to disperse to new food sources and start new colonies. Interguild competition between different aphid species feeding on the same host could also lead to alatae formation by one species, to find other available hosts (Srinivasan & Alvarez, 2011). In addition, winged morphs are also developed in response to changes in environmental conditions such as day length and temperature. According to MacGillivray and Anderson (1964), alate *M. euphorbiae* are more common in the field when the photoperiod is between 11-13 hours of light. Furthermore, high temperatures (25-28 °C) favour apterae, whereas low temperatures (10-15 °C) favour the production of alatae (Schaefers & Judge, 1971). However, low temperature combined with overpopulation and short light photoperiod may induce development of alatae, whereas temperature alone may have no effect (Schaefers & Judge, 1971).

The life cycle of *M. euphorbiae* in South Africa is mainly anholocyclic during winter and summer and reproduction occurs by parthenogenesis (asexually), females giving birth to live young (Millar, 1990). Holocyclic (sexual) reproduction occurs in the Northern Hemisphere when monthly mean night duration increases (longer dark photoperiod) and temperature decreases (Lamb & MacKay, 1997). Males are produced by parthenogenetic

wingless females, and mating occurs to produce a cold-tolerant egg stage that remains dormant during the winter (Lamb & MacKay, 1997).

Barlow (1962) reported a decrease in development time of *M. euphorbiae* as temperature increases. This reduction in development time occurs until an upper temperature threshold is reached, in which the development time then increases and survival rate decreases. Development of *M. euphorbiae* from first-instar nymph to adult takes about 8.9 days at 25 °C (Barlow, 1962). High temperatures reduce the reproductive period, fecundity, longevity and population growth in general (De Conti et al., 2010). Female *M. euphorbiae* may give birth to up to 50 nymphs or more within two weeks, which may result in a new generation developing on potato every two to three weeks. Apteræ adults develop faster and have more offspring than alatae, which can lead to an increase of 70% in reproductive potential (Müller et al., 2001).

Chemical control is the most commonly used strategy to manage aphid populations in potato and other crops (Perring et al., 1999). However, potato farmers have encountered escalating problems with chemical control strategies, including insecticide resistance, market demand and environmental contamination. Water pollution is a major concern in the semi-arid areas of South Africa, with limited water available for human consumption. The average rainfall across South Africa is 450 mm per year, which is low compared to the world's average rainfall of 860 mm (Benhin, 2008). Therefore, there is an on-going need to find innovative ways to control agricultural pests including aphid populations in potato crops. Natural enemies are successful at controlling aphid populations in natural and semi-natural habitats. However, their efficiency is reduced due to heavy insecticide usage. Agricultural pests, including aphids, are more tolerant to insecticides compared to their natural enemies, and this frequently contributes to increases in pest populations (van Lenteren, 2012).

1.2 The natural enemy *Aphidius ervi* Haliday (Hymenoptera, Braconidae, Aphidiinae)

Aphidius ervi is a solitary, koinobiont generalist endoparasitoid of several economically important aphid pests (Starý, 1970; Boivin et al., 2012b). The species originates from the Palearctic region and has been introduced to other parts of the world such as the U.S.A., Australia and New Zealand (Starý, 1974; Marsh, 1977; Milne, 1986; Starý et al., 2007; Takada, 2002) and has recently also been reported from South Africa (Muller et al., 2014). Although *Aphidius ervi* more commonly parasitizes *Acyrtosiphon pisum* (Harris) than any

other aphid species, several authors reported its polyphagous nature in laboratory and field studies (Bueno et al., 1993; Sigsgaard, 2000; Schwörer & Völkl, 2001; Kos et al., 2009; Sidney et al., 2010; Boivin et al., 2012b).

Aphidius ervi is easily distinguished from other *Aphidius* species by the anterolateral sculpturing of the tergite (petiole) of the first abdominal segment, which is coarsely rugose without any ridges (Figure 1.2A) (Pennacchio, 1989; Starý, 1973). The pterostigma on the forewings is four to five times longer as it is wide (Figure 1.2B). Females of *A. ervi* range from 2.8 to 4.1 mm in length, and the antennae have 17 to 19 segments (Figure 1.3A) (Kos et al., 2009). Males have 19 to 22 segments on their antennae (Figure 1.3B) (Pennacchio, 1989; Starý, 1973). The tergites (petioles) of males are stouter than in females, and males are normally darker in coloration compared to females (Pennacchio, 1989; Starý, 1973).

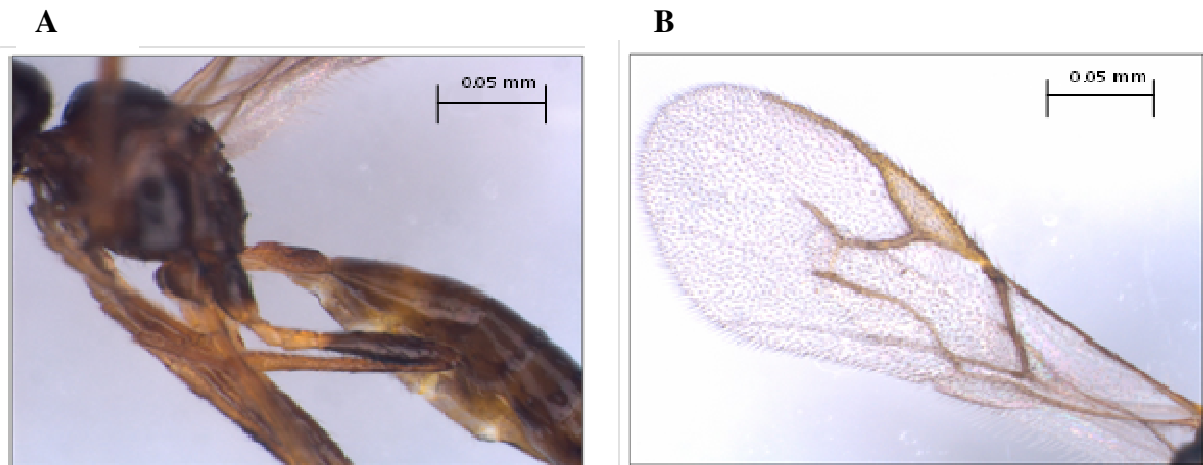


Figure 1.2 A) Rugose petiole of a female *Aphidius ervi* in lateral view. B) Forewing of *A. ervi* (Photo: L. Muller).

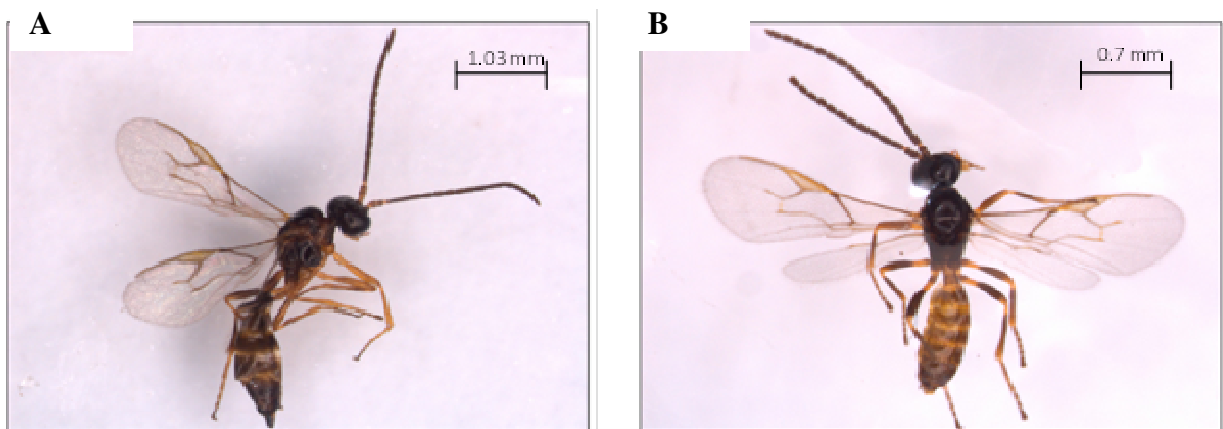


Figure 1.3 *Aphidius ervi* A) female and B) male (Photo: L. Muller).

Aphidius ervi preferentially parasitizes *A. pisum* but the number of offspring produced depends on the previous aphid host (Hofsvang & Hågvar, 1975; Bueno et al., 1993; Kos et al., 2009). Cameron and Walker (1989) observed that within one generation *A. ervi* performed (measured as the number of mummies produced daily, reproductive success) just as well on *A. pisum* compared to *A. kondoi*. However, when different aphid genera were exposed to *A. ervi*, they required four to five generations before they were able to achieve a similar performance on *S. avenae* as on *A. pisum*.

Several studies suggest that interspecific competition, such as interference between searching female parasitoids and larval competition in the case of multiparasitism could result in competitive exclusion by *A. ervi* (Cameron & Walker, 1989; Bueno et al., 1993), which is considered a superior larval competitor (McBrien & Mackauer, 1990). *Aphidius ervi* could displace more specific parasitoids, such as *A. smithi* Sharma & Subba Rao, when competing on *A. pisum* in the field (Cameron & Walker, 1989; McBrien & Mackauer, 1990). However, *A. ervi* is able to discriminate between parasitized and unparasitized aphids and prefers to lay eggs in unparasitized aphids, thus avoiding multiparasitism (McBrien & Mackauer, 1990). On the other hand, Cameron & Walker (1989) reported a decline in reproduction in host-specific species such as *Aphidius eadyi* Starý and *A. smithi* when offered several aphid species as hosts, whereas *A. ervi* was not affected. There is therefore the possibility that *A. ervi* may become the dominant parasitoid by partially displacing other aphid parasitoids in the field. Alternatively, coexistence of different parasitoid species could be beneficial for the natural control of aphids because higher parasitoid diversities in agro-ecosystems may result in higher rates of parasitism (Cameron & Walker, 1989).

Aphidius ervi is commercially available and commonly used as a biological control agent for the pea aphid *A. pisum* as well as other aphid pests in Europe, North America, South America, Australia and New Zealand (Hofsvang & Hågvar, 1975; Marsh, 1977; Schwörer & Völkl, 2001; Takada, 2002; He et al., 2004; Kos et al., 2009). Reasons for the success of *A. ervi* as a biological control agent include its excellent searching ability, rapid response to increase parasitism when host densities increase and its polyphagous feeding behaviour. These factors contribute to its ability to adapt to a wide range of climates and habitats (Gonzalez et al., 1978; He et al., 2006). After emergence, a female parasitoid carries in its ovaries about 60 mature eggs and can lay approximately 300-350 eggs during its life span (He & Wang, 2008; Kos et al., 2009). *Aphidius ervi* has a short life cycle, with a development time from egg to adult of approximately 10 to 15 days (Malina & Praslicka, 2008; Malina et al., 2010). Due to its short life cycle *A. ervi* can react quickly to population changes in its

aphid hosts (Malina & Praslicka, 2008; Malina et al., 2010). Sex ratio in the field is normally 1 male to 1.9 females (He & Wang, 2008).

It has been reported that *A. ervi* parasitizes *M. euphorbiae* in the field and is commercially used as a biological control agent for this aphid pest (Boivin et al., 2012a). However, there are factors influencing the reproductive fitness of *A. ervi* as a biological control agent such as host age, size and quality. *Aphidius ervi* is an obligate parasitoid of aphids and depends completely on the nutrient quality of the host (Sequeira & Mackauer, 1994; Henry et al., 2005). The mode of reproduction of *A. ervi* is arrhenotoky, i.e. fertilized eggs produce diploid females and unfertilized eggs, haploid males. Therefore, mated females can produce both male and female offspring while unmated females produce only males (He & Wang, 2008). Female parasitoids are able to accept or reject a specific host based on its nutritional quality (Sequeira & Mackauer, 1994).

The host size model proposed by Charnov et al. (1981) suggests that female parasitoids lay more female eggs in larger and more male eggs in smaller aphid hosts. Based on this model, later instar nymphs (larger hosts) offer superior food resources for offspring, suggesting that *A. ervi* preferentially oviposits in them. However, recent studies show a non-linear relationship between host age and host quality (Sequeira & MacKauer, 1992a; 1992b; 1993; 1994; Harvey et al., 1994; MacKauer, 1996; MacKauer et al., 1996; Cloutier et al., 2000; Harvey & Strand, 2002; Li & Mills, 2004; Henry et al., 2005; Jervis et al., 2008). Li and Mills (2004) suggested that temperature influences host size. For instance, aphid growth and development were slower at a lower temperature (constant 15 °C), resulting in larger aphids compared to faster growth and development but smaller aphids at a higher temperature (constant 30 °C). Second-instar nymphs reared at a constant 15 °C were similar in size to fourth-instar nymphs reared at a constant 30 °C (Li & Mills, 2004). Consequently, *A. ervi* prefers to oviposit in second-instar rather than fourth-instar nymphs (Li & Mills, 2004). Parasitized aphids continue to feed, grow and develop (Li & Mills, 2004). Host quality is therefore related to the nutrition obtained by the parasitoid larva and not the initial size at parasitism of the host (Sequeira & MacKauer, 1992a; 1992b; 1993; 1994; Harvey et al., 1994; MacKauer, 1996; MacKauer et al., 1996; Harvey & Strand, 2002; Li & Mills, 2004; Henry et al., 2005).

There is a trade-off for female parasitoids when making a choice between a smaller or a larger host regarding the rate of parasitism, development time and sex ratio of offspring, and longevity of adult offspring (Table 1.1). For instance, female parasitoids parasitizing second-

Table 1.1 Reproductive fitness of *Aphidius* species parasitizing second- and fourth-instar aphid nymphs.

Life-history traits of <i>Aphidius</i> species	2nd-instar nymph (small host)	4th-instar nymph (large host)	References
Development time (egg to mummy)	Longer	Shorter	Sequera & MacKauer 1992a; 1992b; 1993; Cloutier et al. 2000
Adult offspring longevity	Shorter	Longer	Sequera & MacKauer 1992a; 1992b; 1993; Cloutier et al. 2000
Pupal survival rate	High	Low	Sequera & MacKauer 1992a; 1992b; 1993; Cloutier et al. 2000
Sex ratio (male : female)	1 : 1	1 : 2	Sequera & MacKauer 1992a; 1992b; 1993; Cloutier et al. 2000
Female offspring size	Small	Large	Sequera & MacKauer 1992a; 1992b; 1993; Cloutier et al. 2000
Female offspring fecundity	Low	High	Sequera & MacKauer 1992a; 1992b; 1993; Cloutier et al. 2000
Percentage mummy formation	High	Low	Sequera & MacKauer 1992a; 1992b; 1993; Henry et al. 2005
Resistance to parasitoid attacks	Less resistant	More resistant	Sequera & MacKauer 1992a; 1992b; 1993; Henry et al. 2005

instar nymphs (small aphids) have a higher rate of parasitism, a longer development time from egg to mummy, a sex ratio of 1:1 and a shorter adult lifespan (Sequeira & MacKauer, 1992a; 1992b; 1993; Cloutier et al., 2000; Henry et al., 2005). As the host size increases, the rate of parasitism decreases and the duration of development and adult longevity increases, and the sex ratio is female-biased (Sequeira & MacKauer, 1992a; 1992b; 1993; Cloutier et al., 2000; Henry et al., 2005). At any time during foraging, female parasitoids are expected to show a preference for smaller hosts (higher reproductive success) rather than larger hosts (female-biased sex ratio and faster development). On the other hand, larger aphid hosts produce larger female offspring which in turn produce more eggs than smaller females (Sequeira & MacKauer, 1992a; 1992b; 1993; Cloutier et al., 2000; Henry et al., 2005). In addition large aphids (third- and fourth-instar nymphs) are able to defend themselves vigorously and effectively compared to younger instars (Stacey & Fellowes, 2002; Henry et al., 2005; He & Wang, 2006). However, not only can aphids physically defend themselves but they also have an immune response to parasitism, a physiological defence response the underlying mechanism of which is still unknown (Guay et al., 2009). In large aphids, parasitoid eggs are killed more effectively by facultative symbiotic bacteria, specifically *Hamiltonella defensa*, compared to smaller, younger and/or less resistant individuals (Guay et al., 2009).

1.3 Influence of water-deficit stress and high temperature on tritrophic plant–aphid–parasitoid interactions

Global climate change models predict that atmospheric CO₂ concentrations will double by the end of the 21st century from 280 ppm in pre-industrial times (Canto et al., 2009; Morison & Lawlor, 1999). As a result, global average surface temperature has been predicted to increase (Hoover & Newman, 2004); the estimate is that it will rise by 1.5 to 4.5 °C by the end of the 21st century (IPCC, 2013). Therefore, major variability in climatic conditions could occur which includes more frequent droughts and heat waves as well as higher mid-summer temperatures (Canto et al., 2009; Hance et al., 2007). The predicted climate change is likely to affect species distributions, life-history traits, tritrophic interactions and ecosystem functions (Hance et al., 2007).

Africa is one of the most vulnerable regions in the world due to its low ability to adjust and respond to extreme weather conditions (Benhin, 2008). South Africa has been getting warmer over the past four decades (1960 to 2003), with an average increase of 0.13 °C per decade resulting in an increase in the number of warmer days per year (Benhin, 2008). Three

General Circulation Models (GCMs), Genesis, HadCM2 and CSM, have been developed to determine the potential impact of climate change in South Africa (Hewitson & Crane, 2006; van Jaarsveld & Chown, 2001). Based on these models an increase in temperature between 2 and 4 °C over the entire country has been predicted, which could result in extended summer seasons and a reduction of 5 to 15% in mean annual precipitation (Benhin, 2008; Magadza, 1994; Turpie et al., 2002). The predicted reduction in rainfall from the current 450 mm per year suggests that South Africa will become even more arid (Magadza, 1994; van Jaarsveld & Chown, 2001; Turpie et al., 2002; Hewitson & Crane, 2006; Benhin, 2008). The western parts of South Africa are expected to become even drier and warmer, which could result in more frequent drought events, whereas increases in temperature and precipitation are predicted for the eastern part of the country, which could result in more frequent floods (Magadza, 1994; Benhin, 2008).

The effect of heat and drought (water-deficit stress) on plants has recently been reviewed (Lipiec et al., 2013). Plants are adversely affected by drought (a period of dry weather that is injurious to plants) and heat stress. Both water-deficit and heat stress lead to the closing of stomata leading to a decrease in the rate of transpiration and photosynthesis, which may result in earlier plant maturity and reduced productivity. Their combined effect influences plants more than each of the stresses individually (Lipiec et al., 2013).

Previous studies have shown that potato plants are adversely affected by water-deficit stress; even low levels of water-deficit stress result in the closure of stomata in leaves (Van Loon, 1981). In addition, nitrogen levels in water-deficit stressed plants are higher due to inhibition of protein metabolism and amino acid synthesis (Hale et al., 2003; Huberty & Denno, 2004). Low turgor pressure in plants during water-deficit stress induces osmotic regulation to increase turgor pressure in plants. This leads to a decrease in osmotic pressure due to the build-up of nitrogen-containing osmoprotectants, resulting in an increase in phloem nitrogen (Mattson & Haack, 1987; McVean & Dixon, 2001; Hale et al., 2003; Huberty & Denno, 2004). In addition, water-deficit stress leads to a reduction in height and leaf area of potato plants which result in overall reduction in growth (Van Loon, 1981; Mattson & Haack, 1987; Hale et al., 2003; Huberty & Denno, 2004; Bale et al., 2007). However, water-deficit stress or drought usually occur in conjunction with high temperature or heat stress, which affects the metabolic balance and interactions between enzymes and hormones, resulting in less photosynthate available for growth (Ewing, 1981).

Phloem sap, which is mainly composed of sugar compounds and low concentrations of amino acids, is generally regarded as a low quality food source (Douglas, 2003). An increase

in nitrogen levels in potato plants may benefit aphids such as *M. euphorbiae* by increasing longevity, development, fecundity and survival rates (Febvay et al., 1999; Sandström & Moran, 1999). Increases in temperature could be beneficial in lengthening the growing season of potatoes, which is likely to result in increased yields at higher latitudes (Morison & Lawlor, 1999; Hijmans, 2003). However, at lower latitudes, a shift in planting dates would be more beneficial for potato crops to limit the negative effects of increased temperatures (Franke et al., 2013). In addition, in a climate change model Van der Waals et al. (2013) suggested that aphid populations may increase due to an increase in temperature, especially during the winter. However, aphid populations will continue to increase until their thermal limits are reached, where after population growth will decrease. An increase in the frequency of PLRV and PVY infections in potato fields is expected due to higher vector numbers during winter seasons (van der Waals et al., 2013).

It is expected that aphid outbreaks will become more common due to more frequent droughts and higher temperatures (Mattson & Haack, 1987; Coley, 1998; Huberty & Denno, 2004). Aphid outbreaks may however in future also be influenced by the indirect effect of temperature and drought. Parasitoid abundance tends to be severely reduced during drought and high temperature (Mattson & Haack, 1987; Weisser et al., 1997; Coley, 1998).

The effects of water-deficit stress and high temperature are likely to influence the third trophic level (parasitoids) more than the second trophic level (aphids), because the third trophic level depends on the ability of the second trophic level to adjust to environmental changes (Hance et al., 2007). Parasitoids are particularly affected because they are dependent on their hosts throughout their development (Li & Mills, 2004).

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CHAPTER 2

MAINTENANCE OF INSECT CULTURES: *MACROSIPHUM EUPHORBIAE* AND *APHIDIUS ERVI*

2.1 Potato plants

Potato (*Solanum tuberosum* L.) (Solanaceae) mini tubers (cv. BP1, G0) were obtained from Rascal Seeds Research Laboratory (Pty) Ltd, Christiana and Potato Seed Production (Pty) Ltd, Lydenburg, South Africa. Single tubers were planted in pots (12.5 cm diameter, 9.5 cm deep) containing 700 ml sandy soil and coconut hair (4:1 ratio), and 1 ml dolomitic agricultural lime, calcium 160 g/kg; magnesium 120 g/kg; KKE/CCE (acid) 88%; (resin) 78% (Wonder™, Agro-Serve (Pty) Ltd t/a Efekto, Rivonia, South Africa). Two and eight weeks after planting, 1.6 g of slow release granular fruit and flower food fertilizer (N:P:K 3:1:5, Grovida KhulaKahle™ Fruit and Flower) was added to the soil of each pot and then every 6 weeks thereafter. From two weeks after planting micronutrients (0.1% Trelmix Trace element solution, 21.3 g Fe/L, 3.0 g Cu/L, 2.7 g Mn/L, 2.3 g Zn/L, 1.0g B/L, 0.3 g Mo/L and 0.3g Mg/L; The Kendal Group, Merrivale, South Africa) were sprayed onto the leaves once a week. Plants were maintained in an environment-controlled room at 25±3 °C, 60–80% RH and 14L:10D photoperiod (Cool white L30W/640, Fluora L30W/77, Osram, Germany). Plants were watered with tap water every second day to maintain high soil moisture.

2.2 Aphid culture

Cultures of *Macrosiphum euphorbiae* (Thomas) (Hemiptera, Aphididae) were collected from *Malva parviflora* L. (Malvaceae) plants at the Experimental Farm (25°45'03.6"S 28°15'28.9"E) of the University of Pretoria (Pretoria, South Africa) in June 2009. Viviparous, parthenogenetic *M. euphorbiae* (Figure 2.1 A-D) were reared on potato plants (cv. BP1, G0 mini tubers) in ventilated glass cages (55 x 42 x 56 cm) with a top glass panel (Figure 2.1 E). The culture was maintained in an environment-controlled room at 25±3°C, 70–80% RH and 14L:10D photoperiod (Cool white L30W/640 and Fluora L30W/77, Osram, Germany) at the University of Pretoria. The aphid culture was maintained for six months before individuals were used in experiments.

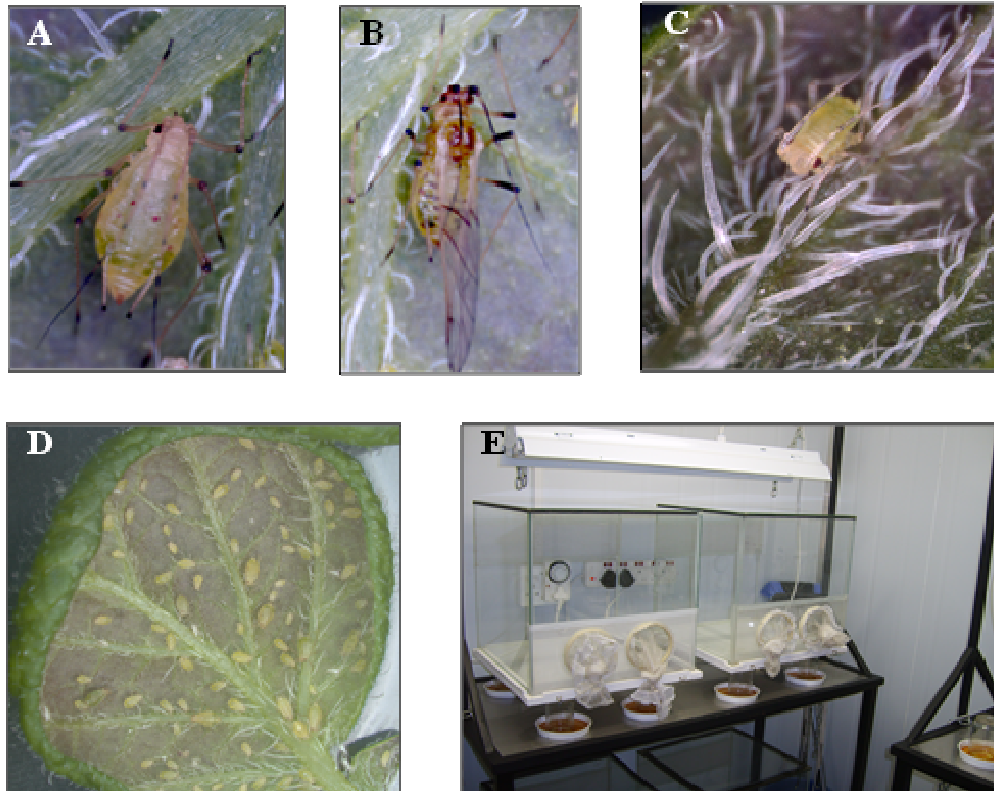


Figure 2.1 Rearing of *Macrosiphum euphorbiae*; A) adult apterae (wingless) *M. euphorbiae*, B) adult alatae (winged) *M. euphorbiae*, C) first instar *M. euphorbiae*, D) colony of *M. euphorbiae*, underneath a potato leaf, E) glass cages used for rearing *M. euphorbiae* (Photo: L. Muller).

2.3 Parasitoid Culture

In August 2010, *Aphidius ervi* Haliday (Hymenoptera, Braconidae, Aphidiinae) parasitoids were reared from *M. euphorbiae* collected from *M. parviflora* plants at the Experimental Farm of the University of Pretoria. *A. ervi* was reared on *M. euphorbiae* in the laboratory on potato plants and maintained at 22 ± 2 °C and 60-80% RH in wooden sleeved cages (55 x 42 x 56 cm) at 14L:10D photoperiod (Cool white L30W/640, Fluora L30W/77, Osram, Germany) at Rietondale experimental farm of the ARC-Plant Protection Research Institute (ARC-PPRI). Parasitoids were kept at a density of 50-80 parasitoids per rearing cage. *Aphidius ervi* parasitize an aphid (Figure 2.2) by laying a single egg through the exoskeleton and the larva subsequently feeds inside the host until it pupates. The larva spins a cocoon inside the mummy, and an adult emerges within 5 to 7 days depending on temperature (Starý,

1974). A few aphid mummies were removed weekly to prevent overcrowding. The parasitoids were maintained for about three generations before being used in experiments.

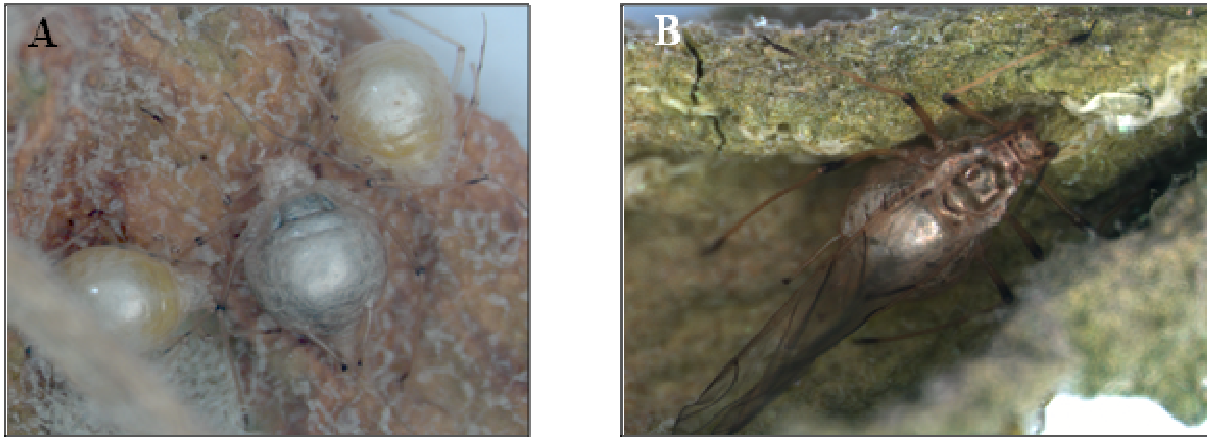


Figure 2.2 A) apterae (wingless aphid) mummies collected for experiments B) alatae (winged aphid) mummy (Photo: L. Muller).

CHAPTER 3

EFFECTS OF PLANT WATER-DEFICIT STRESS AND HIGH TEMPERATURE ON LIFE-HISTORY TRAITS OF THE POTATO APHID, *MACROSIPHUM* *EUPHORBIAE*

Abstract

Climate change may lead to an increase in drought and heat waves in some regions. Their combined effect on insect-plant interactions is not yet fully understood. Water-deficit stress may lead to increased nitrogen levels in plants, which in turn may increase their suitability for insect herbivores. On the other hand, heat stress is thought to have a negative effect on growth parameters of insects. To elucidate the combined effect of drought and heat on aphid-plant interactions, this study tested the effect of moderate water-deficit stress and high day-night temperature on the development, survival and reproduction of the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae), on potato, *Solanum tuberosum* L. (Solanaceae). Water-stressed plants had a lower stomatal conductance, an indirect indicator of water stress, than non-stressed plants. Water stress and high temperature did not result in increased nitrogen levels in leaves. Life history parameters of *M. euphorbiae* were negatively affected by high temperature and moderately water-stressed potato plants.. Nymph-to-adult survival reached 72% on well-watered and 77% on water-stressed plants at ambient (25/15 °C) day-night temperature, and 36% on well-watered and 20% on water-stressed plants at high (30/20 °C) day-night temperature. Adult survival was lowest on water-stressed plants at high temperature. Irrespective of the water regime, the mean number of offspring produced at high day-night temperature was 32 to 55% lower than at ambient temperature. The overall population growth rates were higher on well-watered compared to water-stressed plants, as well as at ambient compared to high temperature. Although lower night temperature may aid in the recovery from high day temperature, the abundance of *M. euphorbiae* may decrease during periods of high day-night temperatures and drought during times of high temperature.

3.1 Introduction

The impact of changing climatic conditions on animals and plants has been central to recent research efforts. Global average surface temperature is predicted to increase by 1.5 to 4.5 °C

by the 21st century (IPCC, 2013). Due to this increase, major variability in climatic conditions could occur which includes more frequent droughts and higher mid-summer temperatures (Hance et al., 2007; Canto et al., 2009; IPCC, 2013). These predicted climate changes are likely to affect species distributions, life-history traits, tritrophic interactions and ecosystem functions (Hance et al., 2007). Of particular relevance is how climate change will affect disease and pest outbreaks as this is of direct concern to human health and food production. Several aphid species cause direct feeding damage and transmit plant diseases to crops. They are ideal study organisms for determining the effects of global warming due to their short development times and high reproductive capacity (Harrington et al., 1995).

Water-deficit stressed host plants and high temperature are important factors that directly or indirectly influence growth and survival of aphids. Moderate water-deficit stress may influence aphids indirectly as it changes plant physiology, including an increase in nitrogen levels due to the inhibition of protein metabolism and amino acid synthesis (Hsiao, 1973; Hale et al., 2003; Huberty & Denno, 2004; Nguyen et al., 2007). The increase in phloem nitrogen, which consists largely of free amino acids (Sandstrom & Moran, 1999), may benefit the growth and reproduction of phloem-feeding insects such as aphids (Showler, 2013). However, reduced turgor pressure and water content may render access to increased plant nitrogen more difficult and thus negatively affect phloem-feeding insects on water-stressed plants (Huberty & Denno, 2004).

Numerous factors influence the physiology of water-deficit stressed plants and subsequently the performance of insect herbivores. Depending on plant species, cultivar and age, duration and severity of water-deficit stress and aphid species, aphid abundance may be affected positively (e.g. Kennedy & Booth, 1959; Hale et al., 2003; Mewis et al., 2012; Tariq et al., 2012; Rivelli et al., 2013), negatively (e.g. Kennedy & Booth, 1959; McVean & Dixon, 2001; Hale et al., 2003; Nguyen et al., 2007; Rivelli et al., 2013; Simpson et al., 2012) or remain unaffected (e.g. Mewis et al., 2012; Tariq et al., 2012; Rivelli et al., 2013).

White (1969) proposed the plant stress hypothesis, initially stating that herbivore performance and population growth are enhanced on stressed plants, especially under water-deficit stress, due to an increase in phloem nitrogen. White (2009) later restricted the plant stress hypothesis to senescence as opposed to flush feeders. The plant vigour hypothesis at the other end of the spectrum proposes that herbivores feed preferentially on vigorously growing plants (Price, 1991). However, vigorous plants may become stressed and flush feeders could benefit from this nutritionally (White, 2009).

Temperature influences aphid population dynamics directly by affecting their development, reproduction and survival (Bale et al., 2002; Harrington et al., 1995). High temperature (27.5 to 35 °C) reduces aphid performance, such as reproduction and survival (Barlow, 1962a; Asin & Pons, 2001; Davis et al., 2006). Temperatures fluctuate under natural conditions, especially between day and night (Ma et al., 2004). However, few studies have determined the effect of fluctuating temperatures on the biology of aphids. In contrast to constant temperature, fluctuating temperature regimes may improve survivorship and reproduction of aphids at high temperatures (Barlow, 1962a; Davis et al., 2006; Nguyen et al., 2009). Davis et al. (2006) observed that *Myzus persicae* (Sulzer) had a higher optimal development threshold and was able to survive extreme temperatures under fluctuating day temperatures, suggesting that aphids were able to recover from heat stress during cooler periods.

A substantial amount of research has been done on the effects of water-deficit stress (e.g. Kennedy et al., 1958; Kennedy & Booth, 1959; McVean & Dixon, 2001; Nguyen et al., 2007; White, 2009; Mewis et al., 2012; Simpson et al., 2012; Tariq et al., 2012; Rivelli et al., 2013) and temperature (e.g. Barlow, 1962a; b; Elliott et al., 1988; Tang et al., 1999; Asin & Pons, 2001; Ma et al., 2004; Davis et al., 2006; Parajulee, 2007; Nguyen et al., 2009) on the biology of aphids. However, the combined effects of water-deficit stress and fluctuating day-night temperature has received little attention.

The potato aphid, *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) originated from North America and has spread throughout the world (Millar, 1990). In South Africa, the genus *Rosa* L. (Rosaceae) is the primary host of *M. euphorbiae*. This species is known to attack more than 200 plant species from 20 different families, many of which are vegetable crops, including potato (*Solanum tuberosum* L., Solanaceae), and ornamental crops (Millar, 1990; Srinivasan & Alvarez, 2011). *Macrosiphum euphorbiae* feeds on stems and new flush on the upper part of its secondary herbaceous hosts (Quisenberry & Ni, 2007). It is an economic pest of herbaceous crops by causing direct damage and by transmitting plant viruses (Blackman & Eastop, 2007).

Severe water-deficit stressed potato plants can reduce adult survival, abundance and biomass of *M. euphorbiae* (Nguyen et al., 2007). The upper lethal temperature for *M. euphorbiae* is 30 °C, at which no nymphs survived to adult stage in the laboratory (Barlow, 1962b; Nguyen et al., 2009). Furthermore, prolonged high temperatures exceeding 26 °C increased mortality of *M. euphorbiae* in the field (Walker et al., 1984). Although several studies determined the effects of water-deficit stress and high temperature on the biology of

aphids, there is a paucity of research on their combined effects and fluctuating day-night temperature. We tested the prediction that the performance and population growth of *M. euphorbiae* are enhanced on water-deficit stressed plants at fluctuating ambient temperature but reduced at high fluctuating temperature. The nymphal development time and life history parameters were evaluated on *S. tuberosum* under high (30/20 °C) and ambient (25/15 °C) day-night temperatures in the presence and absence of water-deficit stress. In addition, the nitrogen levels and stomatal conductance of the potato plants were determined under the above conditions and related to *M. euphorbiae* life history traits.

3.2 Materials and methods

3.2.1 Potato plants

Potato plants (cv. BP1, G0 minitubers) obtained from Rascal Seeds Research Laboratory (Pty) Ltd and Potato Seed Production (Pty) Ltd), South Africa, were used in the experiments. The potato mini tubers were planted in pots (12.5 cm diameter, 9.5 cm deep) containing 700 ml of sandy soil and coconut husk (4:1 ratio) with 1 ml of dolomitic agricultural lime (calcium 160 g/kg, magnesium 120 g/kg, KKE/CCE (acid) 88%, (resin) 78%; Wonder™ Agro-Serve (Pty) Ltd) was added. Two weeks after planting, c. 1.6 g of slow release granular fertilizer (N:P:K 3:1:5, Grovida Khula Kahle™ Fruit and Flower) was added to the soil of each pot and then every 6 weeks thereafter. From two weeks after planting micro nutrients (Trelmix trace element solution, 21.3 g Fe/l, 3.0 g Cu/l, 2.7 g Mn/l, 2.3 g Zn/l, 1.0 g B/l, 3.0 g Mo/l and 0.3 g Mg/l) were sprayed onto the leaves once a week according the manufacturer's instructions.

Plants were grown in an environment-controlled room at 25 ± 3 °C, 60-80% relative humidity (RH) and 14L: 10D photoperiod (Cool white L30W/640 and Fluora L30W/77, Osram, Germany). When plants were 30 days old they were transferred to 200 l growth chambers (Labcon™ 2 LTGC 20, Laboratory Marketing Services cc, South Africa) to precondition them for 30 days to ambient and elevated day (06:00 to 20:00 h) and night (20:00 to 06:00 h) temperatures of 25/15 °C and 30/20 °C, respectively, 14L: 10D photoperiod (Cool white L18W/640) and ambient humidity. The ambient day-night temperature regime was chosen based on mean ambient summer temperatures in Gauteng, South Africa (Benhin, 2006), which falls into the summer rainfall region with a continental climate.

In addition to the two temperature regimes, two different water regimes were applied to the potato plants during the pre-conditioning phase. Water-deficit stress was induced by maintaining the soil at 25-30% pot capacity. For the well-watered treatment soil was maintained at 80-100% pot capacity. The pot capacity of the soil was calculated by weighing oven-dried soil and water-saturated soil in a pot with an Adam QBW - 15 scale (Adam Equipment Co. Ltd., Milton Keynes, UK). The soil was oven dried at 60 °C for 24 h before weighing. Thereafter it was watered until saturated, and the mass of dry soil subtracted from saturated soil mass to determine the mass of water needed to saturate the soil (100% pot capacity) in the pots. This process was replicated three times for each temperature treatment. To saturate dry soil in the pots (12.5 cm diameter, 9.5 cm), an amount of 180 g of water was required to obtain 100% pot capacity whereas 45 g was needed to maintain plants at 25% pot capacity. Each pot with potato plants was weighed every other day to determine the mass of water needed to obtain either 100% pot capacity (well watered) or 25% pot capacity (water-deficit stress).

Potato plants of approximately the same leaf area at BBCH growth stage 19 with nine or more leaves of main stem unfolded (Meier, 2001) were chosen for the experiments. Prior to pre-conditioning of plants, leaves from a randomly selected subsample of potato plants were harvested and measured with a LI-3100C Leaf Area Meter (LI-COR, Lincoln, Nebraska, U.S.A.). There was no significant difference in the total leaf area between subsamples of plants assigned to different treatments (analysis of variance (ANOVA), treatment: $F_{3,44} = 0.344$, $P = 0.794$, $n = 12/\text{treatment}$). The mean leaf area ranged between 151.5 and 162.8 cm². After completion of the experiments, the leaf area was measured again to determine the effect of treatments on plant growth.

The level of water-deficit stress in potato plants was measured indirectly as stomatal conductance (g_s), the speed at which water evaporates from the pores in a plant (Collatz et al., 1991). A plant under water-deficit stress has a low stomatal conductance compared to a well-watered plant (Schapendonk et al., 1989). Stomatal conductance of one plant (control plant) for each replicate and treatment was measured daily with a Decadon Leaf Porometer System SC-1 (LI-COR, Campbell Scientific Africa, Stellenbosch, South Africa). Measurements were taken from six leaves of each plant throughout the experiment to determine whether plants were stressed compared to the well-watered treatment.

Nitrogen concentrations were analysed for three replicate potato plants for each treatment after completion of experiments (AOAC official method 968.06, Dumas method) by the Nutrilab of the University of Pretoria.

3.2.2 Experimental design

Preconditioned 60-day old potato plants were transferred to ventilated glass cages (25 x 25 x 25 cm). Four glass cages containing a single potato plant were placed in each of four growth chambers. In each incubator three of the four potato plants were used to determine the life-history traits of *M. euphorbiae*, and the fourth plant without aphids served to measure stomatal conductance. The *M. euphorbiae* culture was maintained at specific conditions mentioned in Chapter 2. Each growth chamber simulated different environmental conditions. Two growth chambers were kept at daily cycles of 25/15 °C day-night temperatures and additional two growth chambers were kept at daily cycles of 30/20 °C day-night temperatures and 14L: 10D photoperiod. At each temperature water-deficit stress (25 - 30% pot capacity) and well-watered (80 - 100% pot capacity) regimes were applied to the potato plants. The relative humidity ranged between 35% and 40% for all growth chambers despite efforts to increase relative humidity in the well-watered treatments. The experiment was replicated three times for each temperature and water regime.

A temperature and humidity logger (iButton, Hydrochron Temperature/Humidity Logger, FairBridge Technologies, Sandton, South Africa) was placed in each glass cage within each growth chamber to measure the temperature and humidity at hourly intervals. The mean temperature in the glass cages was 23.4 °C during the day and 15.8 °C at night for the ambient temperature treatment. The mean temperature for the elevated temperature regime was 27.3 °C during the day and 20.5 °C at night.

3.2.2.1 Development and survival of immatures

To obtain first-instar nymphs, one adult aptera (wingless) *M. euphorbiae* was confined to one potato leaf in a mesh-covered clip-on leaf cage with an inside diameter of 1.8 cm and height of 1.5 cm. Six leaf cages containing one aphid each, facing the abaxial surface, were attached to six separate leaves on a potato plant for each treatment. Adult aphids were allowed to larviposit for 24 h, after which the adult and all but one first-instar nymph were removed from the potato leaf. The single first instar nymph was confined to the abaxial surface of a potato leaf in a new clip-on leaf cage. Six clip-on leaf cages with one nymph each were used per plant. Individual nymphs were observed once daily for moulting and survival until the last individual from each treatment moulted into the adult stage. The presence of exuviae was used to determine moulting. When calculating developmental times, nymphs were considered

to be 24 h old when the experiment was initiated. Total nymphal development and instar-specific nymphal development duration in days were determined.

3.2.2.2 Adult longevity and reproductive capacity

For each treatment, immatures that developed into adults were observed daily for reproduction and survivorship. Newly born offspring were counted and removed daily until the last individual adult in each treatment died. The number of nymphs born to each female was recorded as fecundity and the average daily reproduction was calculated for each aphid. After completion of the experiments, all plants from each treatment were used to determine nitrogen concentrations.

3.2.2.3 Life table parameters

Life table parameters were calculated as described by Birch (1948). Adult aphid age (x), age-specific survival rates (l_x) and number of offspring produced per female per day (m_x) were determined for all female aphids used in the study. The life table parameters, gross reproductive rate ($GRR = \sum m_x$), net reproductive rate ($R_0 = \sum l_x m_x$), mean generation time ($GT = \sum (l_x m_x x) / \sum (l_x m_x)$ or $GT = \ln(R_0)/r$), intrinsic rate of increase (r) ($\sum e^{-rx} l_x m_x = 1$), finite rate of increase ($\lambda_F = e^r$) and doubling time ($DT = \ln(2)/r$) were calculated. The intrinsic rate of increase and doubling time are used to measure the influence of different environmental factors on aphid population development (Tang et al., 1999).

3.2.3 Data analysis

Data were tested for normality and homogeneity of variances. Analysis of variance (ANOVA) was used to test for differences between leaf area and nitrogen concentrations after experiments with treatment (temperature and water regime) as fixed effect and trial and treatment by trial as block. Tukey's honestly significant difference (HSD) test was used to separate means. Linear mixed model analysis (REML) (Payne et al., 2009b) was used to analyse the stomatal conductance, to model the correlation over 42 days for repeated measurements. The fixed effects were specified as day, treatment, and day x treatment interaction, while random effects were specified as trial and cage x day interaction. A first-order autoregressive model (AR 1) best modelled the correlation over days. Nymphal development time, adult lifespans, daily reproduction and fecundity were analysed using linear mixed model analysis (REML) with treatment as fixed effect and trial, trial x treatment and trial x treatment x cage as random effects. Survival analyses were carried out to test for

differences in nymphal survival (survival from newly born nymphs to adults) as well as adult survival between different treatments using the Kaplan-Meier method and the log-rank test. The significance level was set at $P < 0.05$ for all analyses. Data were analysed with GenStat® (Payne et al., 2009a) and SPSS (IBM® SPSS® 241 Statistics, version 21).

3.3 Results

3.3.1 Potato plants

Potato plants maintained under water stress and at high temperature grew less vigorously than well-watered plants at ambient temperature based on leaf area (Figure 3.1A). After completion of experiments the leaf area was significantly larger in well-watered plants maintained at ambient temperature compared to high temperature and water-stressed plants ($F_{3,32} = 9.484$, $P < 0.001$, $n = 11$). Nitrogen concentrations did not differ significantly between temperature and water regimes ($F_{3,32} = 0.978$, $P = 0.415$, $n = 11$; Figure 3.1B). Stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$; mean \pm SE) was significantly higher for well-watered plants maintained at ambient (26.93 ± 0.80) and elevated (29.51 ± 1.08) temperatures compared to water-stressed plants at ambient (10.72 ± 0.47) and high (11.97 ± 0.59) temperatures (Wald $X^2 = 111.60$, d.f. = 3, $P < 0.001$) (Figure 3.2). Differences between days and the treatment by day interaction were not significant (day: $X^2 = 20.31$, d.f. = 41, $P = 0.996$, day x treatment: Wald $X^2 = 21.46$, d.f. = 123, $P = 1.000$; $n = 42$).

3.3.2 Development and survival of immatures

Instar-specific development time and development time from birth to fourth-instar nymph did not differ between treatments (Table 3.1). However, nymphal survival varied with treatment ($X^2 = 22.941$, d.f. = 3, $P < 0.001$). At ambient day-night temperatures ($25/15^\circ\text{C}$) 72% of nymphs on well-watered and 77% on water-stressed plants developed into adults (Figure 3.3A, B). Nymphal survival on well-watered plants at high temperatures ($30/20^\circ\text{C}$) was significantly lower than on well-watered plants at ambient temperature. Only 36% of nymphs developed into adults (Figure 3.3C). Nymphal survival was significantly lower on water-stressed plants at high temperatures ($30/20^\circ\text{C}$) than all other treatments; only 20% of nymphs reached adulthood (Figure 3.3D).

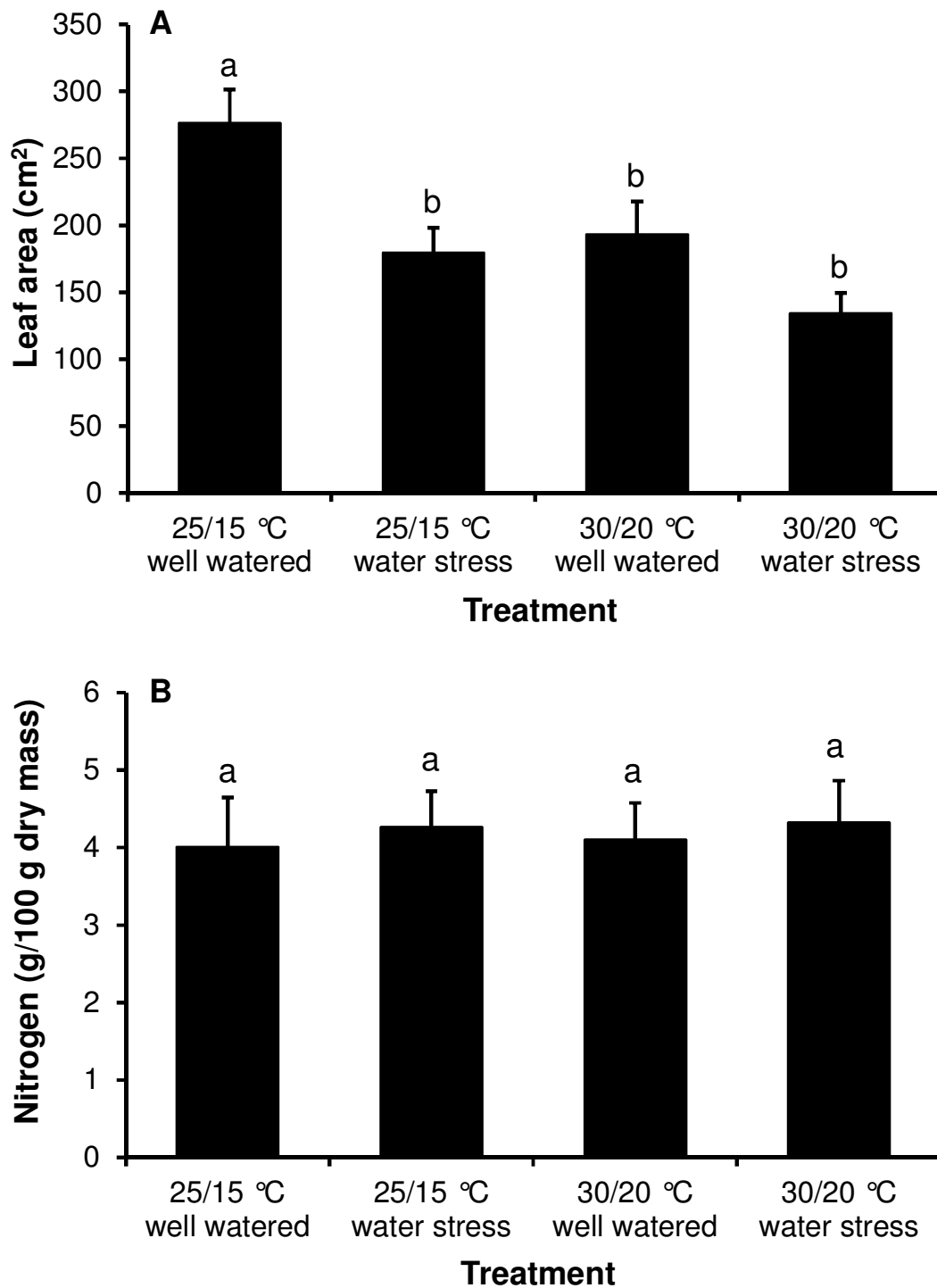


Figure 3.1 (A) Leaf area and (B) leaf nitrogen content (mean \pm SE) of well-watered and water-stressed potato plants maintained at different day-night temperature regimes (n = 9). Different letters above bars indicate significant differences (Tukey's honestly significant difference (HSD) test; $P < 0.05$).

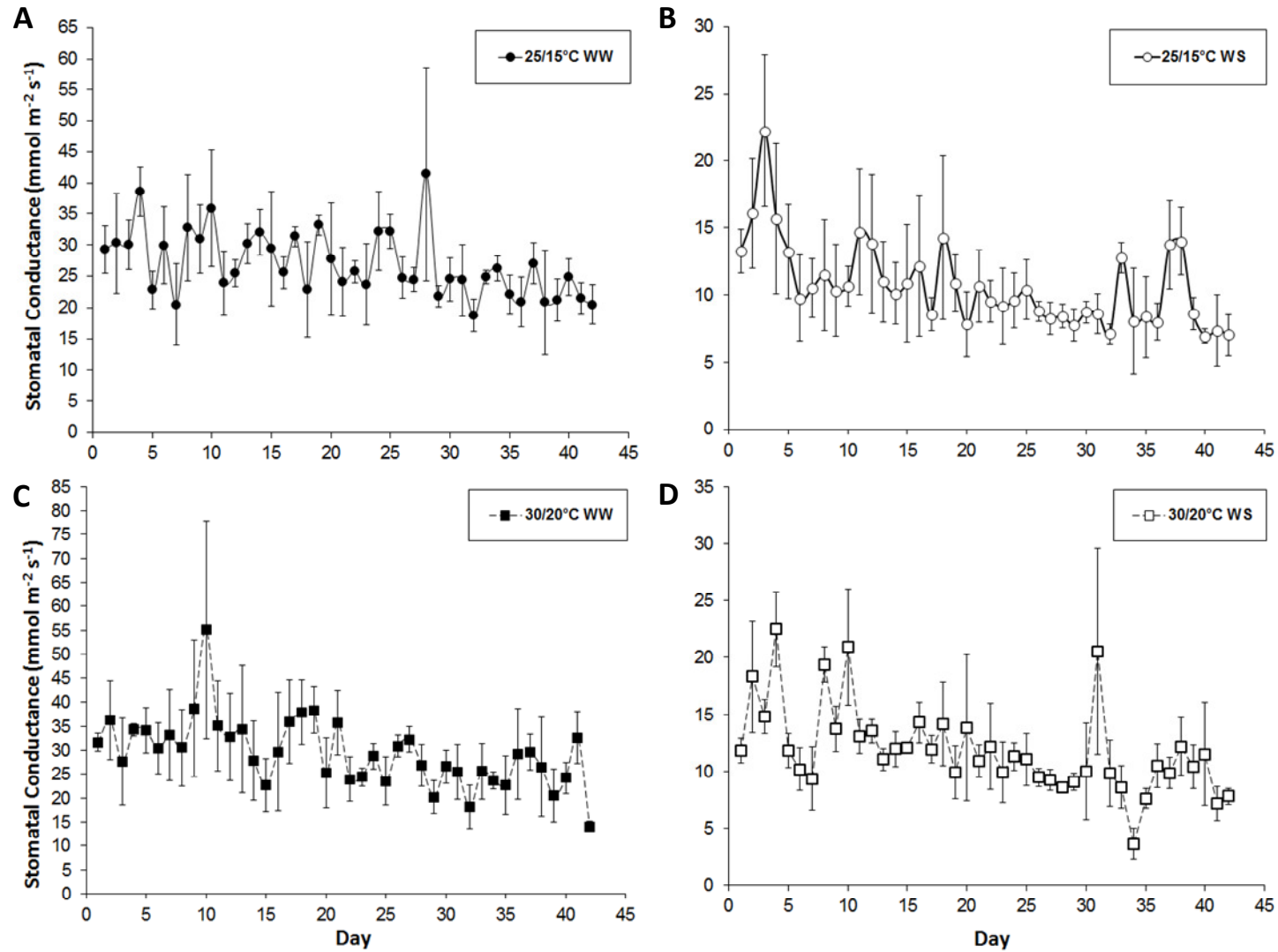


Figure 3.2 Stomatal conductance (mean \pm SE) of potato plants of well-watered (WW) (A) and water-stressed (WS) (B) potato plants at ambient day-night temperature (25/15 °C) and of well-watered (WW) (C) and water-stressed (WS) (D) potato plants at high day-night temperature (30/20 °C) (n = 3).

Table 3.1 Life-history traits of *Macrosiphum euphorbiae* at different day-night temperature and water regimes (n = 34 - 42; F statistic for linear mixed model analysis (REML)). Means (\pm SEM) within a row followed by the same letters are not significantly different (Fisher's LSD test, $P < 0.05$).

	25/15 °C Well watered	25/15 °C Water stress	30/20 °C Well watered	30/20 °C Water stress	N.d.f.	F statistic	P-value
Duration of development (days)							
Nymphal Stage							
Instar 1	2.1 \pm 0.1a	2.0 \pm 0.2a	1.8 \pm 0.1a	1.8 \pm 0.1a	3	0.53	0.680
Instar 2	1.5 \pm 0.1a	1.7 \pm 0.1a	1.6 \pm 0.1a	1.6 \pm 0.1a	3	0.18	0.905
Instar 3	2.0 \pm 0.1a	1.9 \pm 0.1a	1.8 \pm 0.1a	2.4 \pm 0.2a	3	2.06	0.243
Instar 4	2.2 \pm 0.1a	2.2 \pm 0.2a	2.5 \pm 0.2a	2.6 \pm 0.2a	3	1.13	0.412
Total	7.9 \pm 0.3a	7.8 \pm 0.3a	7.7 \pm 0.3a	8.3 \pm 0.3a	3	0.67	0.572
Adult							
Pre-reproductive period	2.5 \pm 0.2a	2.8 \pm 0.2a	3.6 \pm 0.3a	3.6 \pm 0.5a	3	1.47	0.320
Reproductive period	18.0 \pm 1.6a	18.0 \pm 1.6a	16.1 \pm 1.5a	13.9 \pm 1.0a	3	0.37	0.779
Post-reproductive period	1.0 \pm 0.0a	1.0 \pm 0.0a	1.0 \pm 0.0a	1.0 \pm 0.0a	3	0.38	0.767
Longevity	21.3 \pm 1.6a	21.5 \pm 1.5a	20.4 \pm 1.4a	18.3 \pm 1.0a	3	0.26	0.855
Number of offspring produced							
Daily reproduction	2.7 \pm 0.1a	2.6 \pm 0.2ab	2.0 \pm 0.1bc	1.6 \pm 0.1c	3	4.728	0.015
Total fecundity	47.5 \pm 4.5a	43.8 \pm 4.3a	29.6 \pm 3.5b	21.2 \pm 2.6b	3	10.84	<0.001

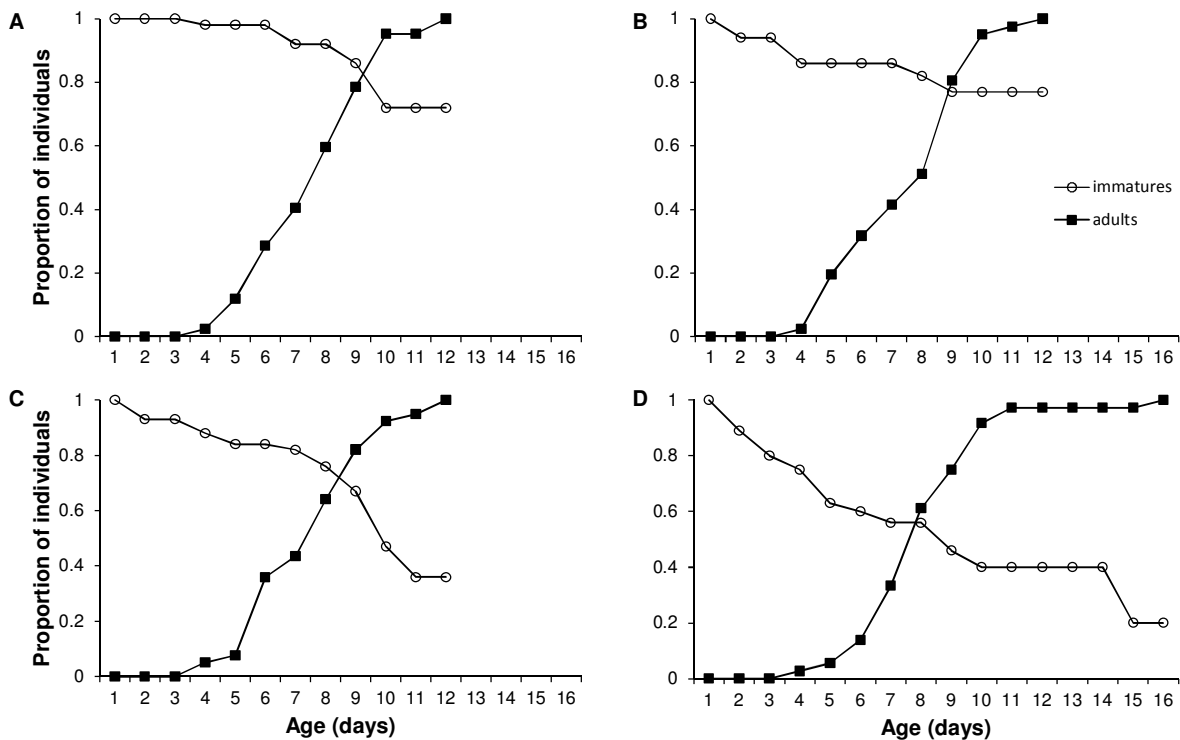


Figure 3.3 Survival curves for immature *Macrosiphum euphorbiae* and recruitment distributions for adults on (A) well-watered and (B) water-stressed potato plants at ambient day-night temperature (25/15 °C) and on (C) well-watered and (D) water-stressed potato plants at high day-night temperature (30/20 °C).

3.3.3 Adult longevity and reproductive capacity

Temperature and water regime did not influence the duration of the pre-reproductive, the reproductive and the post-reproductive periods or adult longevity (Table 3.1). However, daily reproduction was significantly higher on well-watered plants at 25/15 °C day-night temperatures compared to well-watered and water-stressed plants at 30/20 °C and significantly higher on water-stressed plants at 25/15 °C than water-stressed plants at 30/20 °C. Daily reproduction was similar on water-stressed plants at 25/15 °C and well-watered plants at 30/20 °C. The mean number of offspring produced at high temperature was 32 to 55% lower than at ambient temperature and did not differ significantly between water regimes within a temperature treatment. Total fecundity was significantly higher for aphids on well-watered and water-stressed plants at 25/15 °C than on well-watered and water-stressed plants at 30/20 °C (Table 3.1).

3.3.4 Life table parameters

Female aphids on well-watered plants at 25/15 °C showed a high daily fecundity (m_x) from day 2 to day 35 (Figure 3.4A), whereas on water-stressed plants at 25/15 °C daily fecundity increased on day 5 and stayed high until a sharp decline after day 32 (Figure 3.4B). In contrast, on well-watered plants at 30/20 °C daily fecundity only increased after day 9 and declined to day 27 and increased again at day 32 with a sharp decline until day 34 (Figure 3.4C). Daily fecundity of female aphids on water-stressed plants at 30/20 °C increased on day 3 and thereafter declined (Figure 3.4D). Adult survival (Figure 4A-D) was significantly lower for aphids on water-stressed plants at 30/20 °C than the other treatments ($X^2 = 10.717$, d.f. = 3, $P = 0.013$).

Both temperature and water regimes influenced growth parameters of *M. euphorbiae* (Table 3.2). The overall growth rates of *M. euphorbiae* were the highest on well-watered plants at 25/15 °C, followed by water-stressed plants at 25/15 °C. Net reproduction rate (R_0) was more than double for females on well-watered plants at 25/15 °C than for aphids kept at 30/20 °C (both water regimes). The mean generation time of *M. euphorbiae* was shortest on

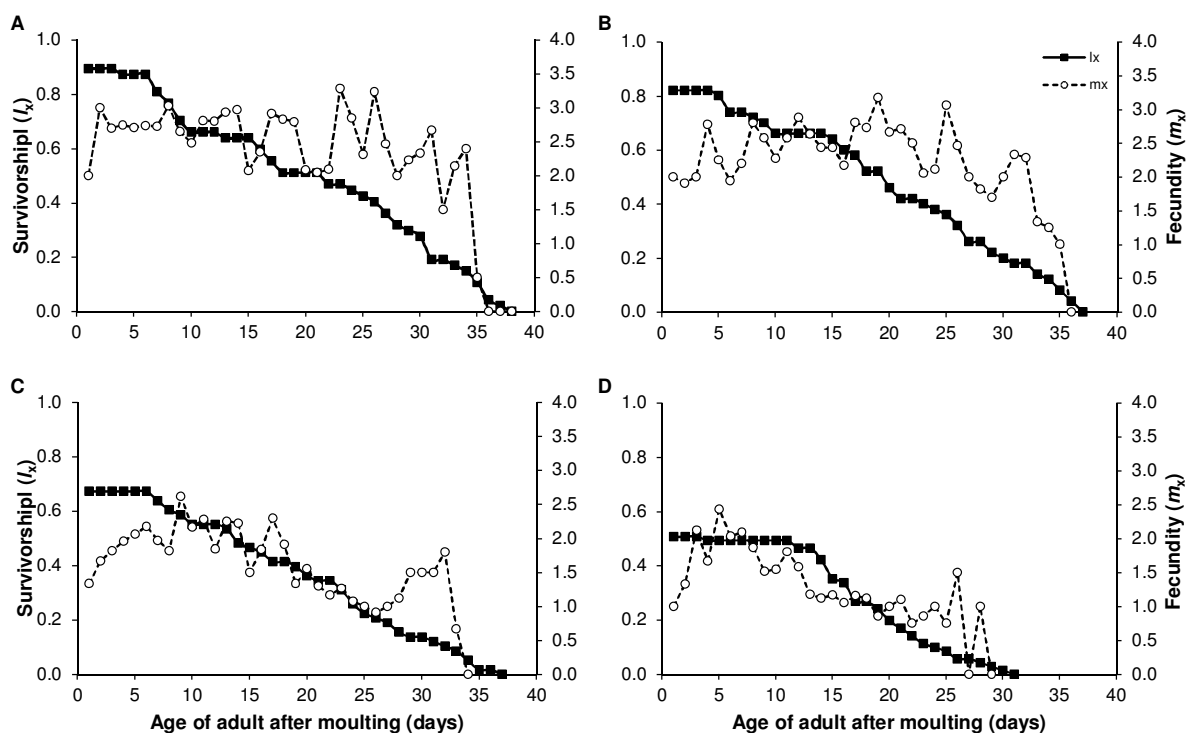


Figure 3.4 Age-specific survival rate (l_x) and fecundity (m_x) of *Macrosiphum euphorbiae* on (A) well-watered and (B) water-stressed potato plants at ambient day-night temperature (25/15 °C) and (C) on well-watered and (D) water-stressed potato plants at high day-night temperature (30/20 °C).

Table 3.2 Life table parameters of *Macrosiphum euphorbiae* at different temperature and water regimes.

Parameters	25/15 °C		30/20 °C	
	Well watered	Water stress	Well watered	Water stress
n (number of females)	42	41	39	36
Gross reproductive rate (GRR)	87.38	79.87	54.40	36.62
Net reproductive rate (R_0)	49.31	41.76	24.47	13.85
Mean generation time (GT)	20.23	20.26	18.40	16.66
Intrinsic rate of increase (r_m)	0.19	0.18	0.17	0.16
Finite rate of increase (λ)	1.21	1.20	1.19	1.17
Doubling time (DT)	3.60	3.76	3.99	4.39

water-stressed plants at 30/20 °C. The intrinsic rate and finite rate of increase was highest on well-watered on plants at 25/15 °C. In addition, the doubling time (DT) with 3.60 was shortest on well-watered plants at 25/15 °C and longest (4.39) on water-stressed plants at 30/20 °C.

3.4 Discussion

High temperature negatively influenced nymphal survival and reproductive success of *M. euphorbiae* but developmental times were similar to those observed at ambient temperature. The performance of phloem-feeding insects tends to be generally reduced on water-stressed plants instead of being enhanced as predicted by the plant stress hypothesis (Huberty & Denno, 2004). In the current study, continuous moderate water-deficit stress at ambient temperature had no significant effect on nymphal or adult survival, development time and reproductive success of *M. euphorbiae*. However, at high temperatures water-deficit stress led to lower nymphal and adult survival and lower population growth rates. Mean generation time and mean duration between birth of the parents and birth of the progeny of *M.*

euphorbiae were shortest on water-stressed plants at high temperature, where the high adult mortality rate resulted in a shorter reproductive period. Mean generation time usually decreases with an increase in temperature (Barlow, 1962a).

Potato plants are considered to be sensitive to water-deficit stress because different physiological processes can be inhibited or completely stopped, such as transpiration and photosynthesis; even at low levels of water-deficit stress, potato plants close their stomata (van Loon, 1981). In addition, water-deficit stress leads to a reduction in growth of potato plants by reducing height and leaf area (van Loon, 1981). In the current study, the stomatal conductance was significantly lower in water-deficit stressed plants under both temperature regimes, indicating that these plants were indeed water stressed. In addition, leaf area was greater on plants grown at ambient day-night temperatures than on water-stressed plants or plants grown at high temperatures. However, nitrogen levels in plants did not differ significantly between treatments.

The lack of differences in nitrogen concentration is in contrast with the findings of Huberty and Denno (2004), who reported that the majority of studies observed a significant increase in leaf nitrogen. However, this may depend on the severity of the drought stress and also the plant species that is being tested. The severity of induced water-deficit stress is important in predicting aphid responses. Continuous severe drought stress on potato plants for 14 days negatively affected performance (development time and reproduction) of *M. euphorbiae* on *S. tuberosum* (Nguyen et al., 2007). This type of water-deficit stress results in low leaf water content, increasing the viscosity of the phloem sap (Nguyen et al., 2007). Nguyen et al. (2007) suggested that the high viscosity of phloem sap led to reduced feeding of *M. euphorbiae* and limited aphid survival, growth and reproduction. Although severe drought stress may affect aphids negatively, moderate water-deficit stressed plants resulted in higher fecundity and an intrinsic rate of increase in *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulzer) on *Brassica oleracea* L. (Brassicaceae) and higher nitrogen concentrations in plants (Tariq et al., 2012). In contrast, moderate water-deficit stress in our study did not enhance nitrogen concentrations in potato plants; however, nymphal survival was highest on water-deficit stress potato plants at ambient temperatures. This could indicate that the specific amino acid concentrations may differ between well-watered and water-deficit stress plants which benefit aphid performance as opposed to the total nitrogen concentration (Hale et al., 2003).

High temperature did influence the performance of *M. euphorbiae*. Both mean daily reproduction and total fecundity were higher at ambient than high day-night temperatures,

suggesting that high temperatures negatively affected reproductive output. Similarly, Barlow (1962a; 1962b) observed a decrease in reproduction with an increase in temperature from 25 to 30 °C. This may in part be explained by the reduced survival of the endosymbiont, *Buchnera aphidicola*, a heat-sensitive bacterium (Nguyen et al., 2009). *Buchnera aphidicola* is located in the abdominal haemocoel of aphids in cells called mycetocytes or bacteriocytes (Bale et al., 2007). Aphids survive on the amino acids in the phloem via the synthesis of essential amino acids by *Buchnera* (Bale et al., 2007; Nguyen et al., 2007; Nguyen et al., 2009). A reduction in the abundance of the endosymbiont usually leads to a concomitant reduction in reproductive output in aphids (Nguyen et al., 2009).

The overall life history parameters (gross and net reproductive rates, intrinsic rate and finite rate of increase) of *M. euphorbiae* were the highest at ambient day-night temperatures and on well-watered plants compared to high day-night temperatures and well-watered and water-deficit stressed plants. This may in part be explained by the effects of high temperatures on aphid mitochondrial processes such as respiration, oxidative phosphorylation and denaturation of proteins and enzymes, which lead to altered functions and cell death (Davis et al., 2006; Nguyen et al., 2009). However, *M. euphorbiae* nymphs were able to survive high temperatures, which could be due to changes in metabolic rates and cellular and physiological processes triggering the production of heat shock proteins, which aid aphids in tolerating temperatures above 27 °C (Nguyen et al., 2007; Nguyen et al., 2009). In addition to this, fluctuating day-night temperatures could also increase survival due to the recovery period from heat stress during cooler conditions at night (Davis et al., 2006). Similar to Barlow (1962a) and Nguyen et al. (2009) no nymphs of *M. euphorbiae* survived to the adult stage in the laboratory at constant 30 °C (Muller et al., unpublished data).

In conclusion, moderate water-deficit stress at ambient temperatures had little or no effect on the performance (development time and reproduction) as well as on population growth parameters of *M. euphorbiae*, contradicting the hypothesis of White (2009) that flush feeders could benefit from feeding on stressed plants. Furthermore, at high temperatures, water-deficit stress resulted in reduced nymphal survival. Although aphids may recover during cooler night temperatures during high temperature periods, high day-night temperatures negatively affected aphid growth parameters. These findings suggest that *M. euphorbiae* is likely to decline in abundance during periods of high day-night temperatures and periods of drought at high temperatures.

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CHAPTER 4

FIRST REPORT OF THE APHID PARASITOID, *APHIDIUS ERVI* HALIDAY (HYMENOPTERA, BRACONIDAE, APHIDIINAE) FROM SOUTH AFRICA

In August 2010, *Aphidius ervi* Haliday (Hymenoptera, Braconidae, Aphidiinae) was reared from a sample of the potato aphid, *Macrosiphum euphorbiae* (Thomas), which was collected in the field on *Malva parviflora* L. (Malvaceae) at the Experimental Farm of the University of Pretoria, Pretoria, South Africa (25°45'03.6"S 28°15'28.9"E). This is the first record of this parasitoid from South Africa. Voucher specimens are deposited in the South African National Collection of Insects, ARC - Plant Protection Research Institute, Pretoria (ARC-PPRI; accession numbers: AcP 9504 - AcP 9509).

Aphidius ervi is a solitary, koinobiont endoparasitoid (Colinet et al., 2005) of several economically important aphid pests (Starý, 1976; Boivin et al., 2012). The species originates from the Palearctic region and has been successfully introduced into North and South America, Australia, New Zealand and Asia (Starý, 1974; Marsh, 1977; Milne, 1986; Cameron & Walker, 1989; Takada, 2002; Starý et al., 2007). *Aphidius ervi* is commercially available and commonly used as a biological control agent for the pea aphid, *Acyrtosiphon pisum* (Harris), *M. euphorbiae*, and several other aphid pests (Wei et al., 2005; Boivin et al., 2012). The parasitoid is used mainly against aphids infesting legumes and, to a lesser extent, against aphids on cereals (Cameron et al., 1984), tomato, sweet pepper, eggplant, gerbera, roses, cucumber and beans (Kos et al., 2009; Boivin et al., 2012).

Several authors reported on the oligophagous nature of *A. ervi* in laboratory and field studies (Sigsgaard, 2000; Lumbierres et al., 2007; Starý et al., 2007). Of the aphid species parasitized by *A. ervi*, at least 15 species are known to occur in South Africa according to Millar (1990). These include the Macrosiphini *Acyrtosiphon kondoi* Shinji, *A. pisum*, *A. malvae* (Mosley), *Aulacorthum solani* (Kaltenbach), *Diuraphis noxia* (Kurdjumov), *M. euphorbiae*, *M. rosae* (L.), *Metopolophium dirhodum* (Walker), *Myzus persicae* (Sulzer), *Sitobion avenae* (F.) and *S. fragariae* (Walker) (Starý, 1976; Marsh, 1977; Takada, 2002; Tomanović et al., 2003; Lumbierres et al., 2007; Starý et al., 2007; Boivin et al., 2012), and four Aphidini, *Aphis gossypii* Glover, *Rhopalosiphum maidis* (Fitch), *R. padi* (L.), and *Schizaphis graminum* (Rondani) (Marsh, 1977; Tomanović et al., 2003; Lumbierres et al., 2007; Starý et al., 2007).

Aphidius matricariae Haliday and *A. rhopalosiphi* De Stefani-Perez are the only recorded *Aphidius* species imported through the Insect Quarantine facility of ARC-PPRI (Pretorius, 2008) and released into South Africa (G. J. Prinsloo, pers. comm.). These parasitoids were introduced during 1988 from Turkey and 1989-90 from the U.S.A. and Australia for the biological control of the Russian wheat aphid, *D. noxia* (Marasas et al., 1997; Pretorius, 2008). In addition, *Aphidius colemani* Viereck has been reported from South Africa (Remaudière et al., 1985). The only previous African records of *A. ervi* are from North Africa (Starý, 1976). *Aphidius ervi* was introduced into Burundi in East Africa but did not establish (Starý, pers. comm.). It is, therefore, not clear when and how *Aphidius ervi* arrived and became established in South Africa but there is no doubt that it can contribute to the natural control of aphid pests.

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CHAPTER 5

RESPONSE OF THE PARASITOID, *APHIDIUS ERVI* HALIDAY (HYMENOPTERA, BRACONIDAE, APHIDIINAE) TO VARIOUS DENSITIES OF THE APHID HOST *MACROSIPHUM EUPHORBIAE* (THOMAS) (HEMIPTERA, APHIDIDAE)

Abstract

The response of the aphid parasitoid *Aphidius ervi* to different host densities of the potato aphid, *Macrosiphum euphorbiae*, was determined by exposing densities of 5, 10, 20, 30, 50, 80 and 100 second- and third-instar nymphs to a single 24 to 48h-old, mated female parasitoid for a 24-h period. The number of parasitized nymphs increased from four to 18, whereas percentage parasitism decreased from 78% to 18% with an increasing host density from five to 100 nymphs. Parasitism was significantly lower at a host density of five nymphs compared to host densities of 30 and 100 nymphs. Therefore, any number of hosts, from 10 to 100 nymphs, can be provided to female *A. ervi* for fecundity trials under laboratory conditions. For the following chapters a host density of 50 nymphs was selected as a standard.

5.1 Introduction

The efficacy of parasitoids as biological control agents is based on their searching ability to find suitable hosts (Waage & Hassel, 1982; Mackauer, 1983). However, the most important quality of a biological control agent is to keep small pest populations below economic threshold levels and not only to decrease already high pest populations (Zamani et al., 2006).

The aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera, Braconidae, Aphidiinae) parasitizes the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Hemiptera, Aphididae), in South Africa (Muller et al., 2014). *Aphidius ervi* has been used for the control of *M. euphorbiae* in Europe since 1996 (van Lenteren, 2003). The success of *A. ervi* as a biological control agent is mainly attributed to its excellent searching ability, increased parasitism when host densities increase and its ability to parasitize many pest aphid species. These factors contribute to its ability to adapt to a wide range of climates and habitats (Gonzalez et al., 1978; He et al., 2006).

The behaviour of individual parasitoids in response to an increase in host density is important in predicting their success as biological control agents (Fernández-Arhex & Corley, 2003; He et al., 2006). In general, parasitoids are able to respond rapidly to an increase in

pest density due to their high fecundity and parasitism rate. Functional response experiments determine the change in the number of hosts parasitized in response to an increasing host density. These experiments are normally carried out at a specific time period, where the parasitoid is confined to a small patch (or arena) with different host densities.

Despite the use of *A. ervi* as a biological control agent for various aphid species, very few studies have determined its functional response. Ives et al. (1999) studied the behavioural and population functional response of *A. ervi* parasitizing the pea aphid, *Acyrtosiphon pisum* (Harris). They reported, a decrease in attack rates by *A. ervi* with higher among-plant variability and a decrease foraging efficiency when average number of aphids per plant was low. He et al. (2006) determined the reproductive response of *A. ervi* parasitizing *A. pisum*, providing information on its oviposition strategy. They reported significant increase in mean number of parasitised aphids with an increase of host density. However a plateau in eggs laid was reached at host density of 75 aphids per cylinder and above. This study is the first to determine the response of *A. ervi* to changes in host densities using *M. euphorbiae* as a host. The aim was to determine the specific number of aphid hosts needed per female parasitoid for subsequent studies on reproduction of this parasitoid.

5.2 Materials and methods

5.2.1 Experimental design

Mummies were collected from parasitoid cultures (Chapter 2) and placed singly in gelatine capsules. The gelatine capsules were kept in growth chambers set at 25/15 °C day-night, 60–80% RH and 14L:10D photoperiod. After adult parasitoids emerged they were sexed using a stereomicroscope; females were distinguished by their protruding ovipositor. Males and females were paired and transferred to glass vials with streaks of honey and a small ball of water-saturated cotton wool to provide food and water. After a 24-h mating period, 24 to 48h-old mated female parasitoids were used in experiments.

Potato leaf cuttings with stems from plants grown as described in Chapter 2 were collected from 60-day-old potato plants, with first individual buds (1-2 mm) of the first inflorescence visible on the main stem (501 BBCH Monograph, Meier). To minimize the effect of host-patch area on host finding, we used potato leaf cuttings of 5-7 cm² (cut under water using a scalpel) and placed them adaxial-side up with aphids on filter paper (90 mm in diameter) in a petri dish (90 mm diameter) saturated with distilled water. Second- and third-instar nymphs (Chapter 2), the preferred stages for parasitism (Henry et al., 2005; Ives et al.,

1999), were transferred to leaf cuttings using a thin camel-hair brush. One 24 to 48h-old, mated female parasitoid was introduced to different aphid densities of 5, 10, 20, 30, 50, 80 and 100 second- and third-instar nymphs per leaf cutting for 24h. The petri dishes were transferred to incubators maintained at 25/15 °C day/night temperature, 60–80% RH and 14L:10D photoperiod (Cool white L30W/640, FluoraL30W/77, Osram, Germany). After removing the parasitoid from each petri dish after 24 h, the stems of the leaf cuttings were placed in water in glass vials (7.4 ml) covered with plastic jars (350 ml, with the bottom cut off and gauze glued on for ventilation) to prevent aphids from escaping. The leaf cuttings were kept for the mummies to develop, and aphids were supplied with fresh potato leaf cuttings when required. Aphids were checked twice daily (once in the morning between 8:00 and 10:00 and once in the afternoon between 14:00 and 16:00) for the presence of newly formed mummies. The experiment was replicated 10 times for each aphid density.

5.2.2 Data analysis

The effects of aphid density on the number and percentage of parasitized aphids were analysed using regression analysis. Data were tested for normality (Shapiro-Wilk's W) and homogeneity of variances (Levene's test). Transformation of data of the number of parasitized nymphs did not result in stabilization of variances. The relationship between host density and the number of nymphs parasitized was therefore analysed using a quadratic regression model (Zar, 2010). The difference between the number of parasitized nymphs at different host densities was analysed with Kruskal Wallis ANOVA followed by a pairwise comparison of mean ranks. Non-linear regression was used to model the relationship between host density and the percentage of nymphs parasitized. Data were analysed with Statistical Package for the Social Sciences SPSS (IBM SPSS Statistics, version 22). Female parasitoids that did not parasitize aphid nymphs were excluded from the analyses because they could have been damaged during handling.

5.3 Results

Parasitism increased with increasing host density (Figure 5.1). The mean number of parasitized nymphs increased from four to 18 nymphs with an increase in host density from five to 100 nymphs. Parasitism was significantly lower at a host density of five nymphs compared to host densities of 30 and 100 nymphs ($X^2 = 17.81$, $df = 6$, $P = 0.007$).

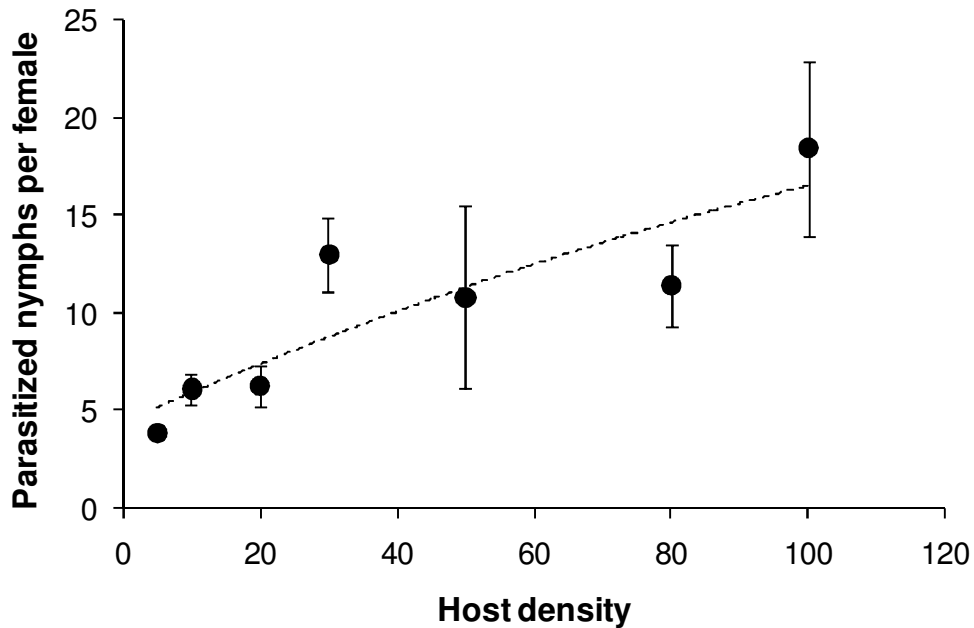


Figure 5.1 Relationship between the nymphal density of *Macrosiphum euphorbiae* and the number of nymphs parasitized (mean \pm SEM) by *Aphidius ervi* ($n = 10$; $y = 4.957 + 0.097x + 0.0002x^2$, $R^2 = 0.226$, $F_{2,56} = 8.159$, $P = 0.001$).

A logarithmic model best fitted the relationship between host density and the rate of parasitism. The percentage parasitized nymphs decreased with increasing host density (Figure 5.2). The highest percentage of mummified aphids (77.5%) was observed at a host density of 5 and the lowest (14.3%) at a density of 80.

5.4 Discussion

Aphidius ervi parasitism increased with an increase in host density of *M. euphorbiae*. Therefore, no sperm limitation seems to have occurred at the host densities tested during the 24-h oviposition period. However, this may change with a larger host density. This finding is in contrast with that of Ives et al. (1999) who reported optimal parasitism of *A. ervi* at host density of 20 aphids (*Acyrtosiphon pisum*) on lucerne (*Medicago sativa* L. Fabaceae). In addition, He et al. (2006) reported that the number of parasitized aphids reached an asymptote at a host density of 75 aphids (*A. pisum*) on broad bean cuttings, *Vicia faba* L. (Fabaceae), in a simple experimental arena. The differences in results between authors and this study could be explained by the complexity of the experimental environment, which could reduce the

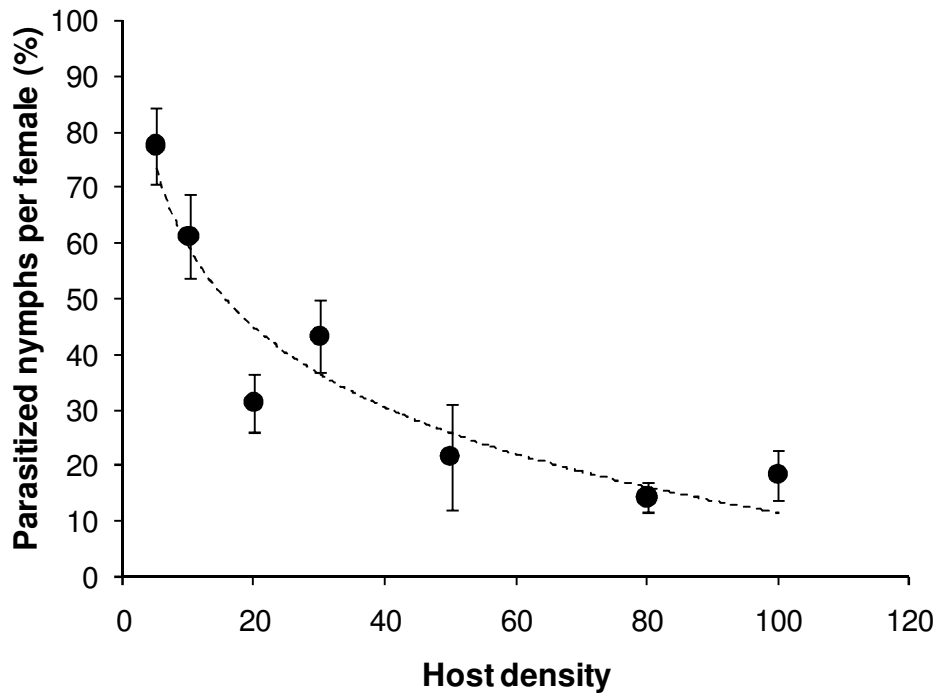


Figure 5.2 Percentage of nymphs parasitized (mean \pm SEM) by *Aphidius ervi* at different host densities of *Macrosiphum euphorbiae* ($y = -20.47 \ln(x) + 106.08$, $R^2 = 0.631$, $F_{1,57} = 97.458$, $P < 0.001$).

probability of a female parasitoid finding a host. For instance, the foraging efficiency of *A. ervi* was reduced on whole plants, where the spatial distribution of the aphid host was more complex compared to leaf cuttings (Ives et al., 1999). This suggests that the simpler the environment the greater is the probability to find aphid host and the higher the rate of parasitism. Furthermore, another possible reason for the higher reproductive capacity of *A. ervi* on *M. euphorbiae* compare to *A. pisum* could be due to the more effective defense methods of the latter (i.e. kicking, dragging and dropping of a surface) when under parasitoid attack (Gross, 1993).

Percentage parasitism decreased with an increase of host density. A reason for this could be due to egg limitation. *Aphidius ervi* emerges with about 60 mature eggs and can only lay a maximum of 60 eggs within a 24-h period after emergence (He & Wang, 2008). However, *A. ervi* females produce more mature eggs as they age and could parasitize a total of 300 to 350 aphids in their lifetime (Sequeira & Mackauer, 1994; He & Wang, 2008; Kos et al., 2009). Furthermore, percentage parasitism of *M. euphorbiae* decreased due to an increase in handling time at densities higher than 30 *M. euphorbiae* per petri dish (Byeon et al., 2011). The parasitoids spent more time cleaning and grooming their bodies due to more volume of

honeydew that was produced by larger aphid groups, particularly at aphid densities of 50, 80 and 100 (Shishehbor & Zandi-Sohani, 2011).

Based on the findings of the current study, host densities of 10 to 100 *M. euphorbiae* nymphs per leaf cutting are adequate for *A. ervi* for experiments evaluating the effects of high temperature and drought stress on a tritrophic interaction (Chapter 6 and 7). A host density of 50 nymphs, which is the median between one to 100 nymphs, was selected as a standard for subsequent experiments.

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CHAPTER 6

EFFECTS OF A HIGH DAY-NIGHT TEMPERATURE REGIME ON THE BIOLOGY OF *APHIDIUS ERVI* HALIDAY (HYMENOPTERA, BRACONIDAE, APHIDIINAE) PARASITIZING *MACROSIPHUM EUPHORBIAE* (THOMAS) (HEMIPTERA: APHIDIDAE)

Abstract

The heat-stress hypothesis proposes an increase in mortality of aphid parasitoids exposed to above-optimum temperatures. However, those individuals that survive heat stress may pay a cost in their fitness measured as reproductive output. The influence of high day-night temperatures on the developmental rate, longevity and parasitism was determined for the aphid parasitoid, *Aphidius ervi* Haliday (Hymenoptera, Braconidae, Aphidiinae) parasitizing the potato aphid *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) on potato (*Solanum tuberosum* L., Solanaceae). The survival of *A. ervi* decreased at high (28/21 °C) compared to ambient (25/17 °C) day-night temperatures. The duration of development from egg to adult was significantly shorter, 1.2 days and 1.5 days for males and females, respectively, at ambient compared with high day-night temperatures. Percentage parasitism and pupal survival were 10% and 20% higher at ambient temperatures. The growth rate and parasitism of *A. ervi* was negatively affected by high day-night temperatures. The results support the heat-stress hypothesis.

6.1 Introduction

Temperature plays a major role in the metabolism and physiology of parasitoids and is one of the most important factors influencing their fitness (Angilletta et al., 2002; Zamani et al. 2007; Malina & Praslicka, 2008; Roux et al., 2010). Generally, parasitoids as well as other organisms have an optimal temperature at which they are able to perform best physiologically and behaviourally (Angilletta et al., 2002; Roux et al., 2010). However, when ambient temperature reaches above-optimal levels parasitoids might experience heat stress that may affect their fitness (Angilletta et al., 2002; Roux et al., 2010). Several studies report a decline in development time, pupal survival and percentage parasitism of *Aphidius* species at temperatures above 25 °C (Hofsvang & Hågvar, 1975; Kambhampati & Mackauer, 1989; Sigsgaard, 2000; Zamani et al., 2007; Malina & Praslicka, 2008; Roux et al., 2010).

Global climate change is expected to result in an increase in average surface temperatures of between 1.5 and 4.5 °C by the end of the 21st century (IPCC, 2013). Therefore it is expected that higher day and night temperatures will occur during mid-summer, December to January.

Aphid parasitoids are generally keystone species representing the third trophic level that depends on the second trophic level, the aphid host, to cope with high temperatures (Hance et al., 2007). *Macrosiphum euphorbiae* (Thomas) (Hemiptera, Aphididae), amongst other aphid species, shows behavioural changes during high temperatures, by attempting to move to cooler areas (Nguyen et al., 2009). Furthermore, Nguyen et al., (2009) found specific cuticle proteins that are more abundant in aphids during heat stress and which could play a role in protecting aphids from desiccation. Nonetheless, parasitoid larvae are dependent on their aphid host for thermal regulation. Therefore, it is expected that high day-night temperature regimes will particularly affect aphid parasitoids. The heat-stress hypothesis proposes that the mortality rate of aphid parasitoids exposed to above-optimum temperatures will increase, specifically that of weaker individuals (Roux et al., 2010). However, those individuals that survive the heat stress will pay a cost in their fitness (reproductive output) (Roux et al., 2010).

The aim of this study was to test the heat-stress hypothesis by determining the effect of high day-night temperatures compared to ambient day-night temperatures on the performance of *Aphidius ervi* on *M. euphorbiae*.

6.2 Materials and methods

6.2.1 Experimental design

The Heat-Stress Hypothesis was developed following determination of the developmental times, offspring sex ratio, pupal survival rate, percentage mummification (percentage of mummies formed) and the longevity of *A. ervi* at two different day/night temperature regimes. The development times of *A. ervi* were determined for firstly, oviposition to mummification, and secondly, mummification to adult emergence.

The ambient temperature was based on the average summer temperature during December and January in Gauteng, South Africa (<https://weatherspark.com/averages/29019/Johannesburg-Gauteng-South-Africa>). Five degrees Celsius was added to the current ambient temperature to represent the high temperature regime, which is the expected temperature increase at the end of the 21st century (IPCC, 2013).

Leafs were cut under water using a scalpel (*ca.* 5-7 cm² in leaf area) from preconditioned potato plants. Potato plants were grown as described in Chapter 2. They were conditioned for 30 days at 25/15 °C and 30/20 °C day (06:00 to 20:00 h) and night (20:00 to 06:00 h) temperature, 60-80% RH and 14L:10D photoperiod (Cool white L30W/640, Fluora L30W/77, Osram, Germany). Fifty randomly selected second- and third-instar *M. euphorbiae* nymphs, reared on potato plants (Chapter 2), were carefully transferred to the leaf cuttings using a thin camel hair-brush. Care was taken not to damage the mouthparts to prevent death of nymphs by starving. The number of nymphs used per leaf cutting was determined in a previous study (Chapter 5). The leaf cuttings were placed ventral-side-up with aphids on filter paper (90 mm in diameter) in a Petri dish (90 mm diameter) saturated with distilled water. The nymphs were given 30 to 60 min to settle on the leaf cuttings and initiate feeding before introducing a single female parasitoid.

Aphid mummies collected from the parasitoid culture (Chapter 2) were placed singly in glass vials (75 ml) and transferred to growth chambers set at ambient 25/15 °C, day (06:00 to 20:00 h) and night (20:00 to 06:00 h) temperature, with 60-80% RH. Within 24 h after emergence, adult parasitoids were sexed using a stereoscopic microscope. One female and one male parasitoid were paired and transferred to a glass vial (75 ml) with a streak of honey and a small piece of water saturated cotton. *Aphidius ervi* females usually mate only once in their life span (He et al., 2004). After a 24-h mating period a single naive 24-to 48-h old female parasitoid was introduced to a potato leaf cutting with 50 nymphs inside petri dishes. Fifty new nymphs were provided daily on fresh leaf cuttings, after the removal of previous leaf cuttings, until the death of the female parasitoid.

After daily removal of the leaf cuttings with nymphs from the Petri dishes, the cuttings were placed in glass vials (75 ml) with water and kept singly in a plastic jar (350 ml) turned upside down with the bottom cut off and gauze glued on for ventilation. The plastic jars containing the leaf cutting and parasitized aphid nymphs were transferred to growth chambers set at the different day-night temperature regimes mentioned above. This was replicated 20 times for each temperature regime. Leaf cuttings were kept under the different temperature regimes until all mummies developed. To keep nymphs alive until mummification, or development into adults, leaf cuttings were replaced with new leaf cuttings when necessary. Aphids were checked twice daily (once in the morning between 8:00 and 10:00 h and once in the afternoon between 14:00 and 16:00 h for newly formed mummies). The mummies were collected with a thin camel hair-brush and placed in glass

vials (75 ml), and returned to the same temperature treatment. Mummies were observed daily until all parasitoids emerged.

The percentage of mummies formed, developmental times, offspring sex ratio, pupal survival rate, and the longevity of *A. ervi* was determined. Individual development time of each parasitoid was recorded from oviposition to mummy formation, and from mummy formation to adult emergence. Offspring parasitoids were sexed using a stereomicroscope. The offspring parasitoids were kept in glass vials streaked with honey until death and their individual longevity recorded. Honey was replenished when necessary. After an observation period of two or three weeks the unemerged mummies were dissected under a stereoscopic microscope to record the total number of dead parasitoids.

6.2.1.1 Temperature and humidity measurements

A temperature and humidity logger (iButtonHydrochron, FairBridge Technologies, South Africa) was used to measure the temperature and humidity inside the Petri dishes containing leaf cuttings with 50 aphid nymphs.

6.2.1.2 Life table parameters

Life table parameters (Birch, 1948; De Conti et al., 2010) were calculated for each parasitoid population at the different day-night temperature regimes. Adult female parasitoid age (x), age-specific survival rates (l_x) and number of offspring produced per female per day (m_x) were determined for all female parasitoids used in the study. The life table parameters, gross reproductive rate ($GRR = \sum m_x$), net reproductive rate ($R_0 = \sum l_x m_x$), mean generation time ($GT = \sum (l_x m_x x) / \sum (l_x m_x)$ or $GT = \ln(R_0)/r$), intrinsic rate of increase (r) ($\sum e^{-rx} l_x m_x = 1$), finite rate of increase ($\lambda_F = e^r$) and doubling time ($DT = \ln(2)/r$) were calculated.

6.2.2 Data analysis

Development times, longevity, percentage mummification and percentage pupal survival were analysed by Mann-Whitney U test (STATISTICA 10) (Rosner & Grove, 1999).

6.3 Results

6.3.1 Temperature and humidity measurements

The actual mean temperature in the Petri dishes differed from the temperature originally set for the growth chambers. The mean temperatures were 25 °C and 17 °C for the ambient day-

night temperature and 28 °C and 21 °C for the high day-night temperature (Table 6.1). The mean relative humidity in the Petri dishes was lower at ambient than high temperature. It increased from 34% during the day to 41% during the night at ambient and from 48% during the day to 52% during the night at high temperature.

Table 6.1 Temperature and relative humidity (mean \pm SEM) inside Petri dishes kept in growth chambers set at 25/15 °C and 30/20 °C day night temperature regimes.

Day-night temperature regime		Temperature (°C)	Temperature range (°C)	Relative humidity (%)	Relative humidity range (%)
25/15 °C	Day	23.4 - 26.5	24.67 \pm 0.09	76.2 \pm 0.7	43.4 - 100
	Night	15.7 - 17.7	16.5 \pm 0.6	81.9 \pm 3.4	56.4 - 100
30/20 °C	Day	21.9 - 31.2	27.5 \pm 0.11	57.2 \pm 0.4	15.6 - 99.6
	Night	17.7 - 24.3	21.3 \pm 0.15	60.0 \pm 2.5	21.5 - 100.0

The total development time (egg to adult) and the time from mummy formation to adult eclosion were significantly longer, 1.2 days and 1.5 days for both male and female, respectively at high day-night temperature compared to ambient temperatures (Table 6.2). There were no significant differences in the longevity of male and female parasitoids between temperature regimes (Table 6.2).

Table 6.2 Development times and longevity (mean \pm SE) in days of male and female *Aphidius ervi* reared on *Macrosiphum euphorbiae*.

	Day-night temperature regime							
	25/15 °C				30/20 °C			
	n	Male	n	Female	n	Male	n	Female
Egg to mummy	249	9.9 \pm 0.1 a	163	9.9 \pm 0.1 a	81	10.8 \pm 0.2 b	62	10.9 \pm 0.4 a
Mummy to adult	249	4.3 \pm 0.1 a	163	4.6 \pm 0.1 a	81	5.5 \pm 0.3 b	62	6.1 \pm 0.6 b
Egg to adult	249	14.3 \pm 0.1 a	163	14.6 \pm 0.2 a	81	16.3 \pm 0.4 b	62	16.9 \pm 0.7 b
Adult longevity	217	5.5 \pm 0.2 a	129	6.7 \pm 0.4 a	73	5.1 \pm 0.4 a	51	5.8 \pm 0.6 a

Values followed by the same letter in rows within a day-night temperature regime do not differ significantly (Mann-Whitney U test; $P < 0.001$).

The mean percentage of mummified aphids and pupal survival were 10% and 20% higher at ambient than high day-night temperatures (Table 6.3). The sex ratio at both temperature regimes was male biased with a mean proportion of males ranging from 0.60 to 0.66 (Table 6.3).

Table 6.3 Mummification and pupal survival (mean \pm SE) and the sex ratio of *Aphidius ervi* reared on *Macrosiphum euphorbiae*.

Day-night temperature regime	n	Mummification (%)	Pupal survival (%)	Sex ratio ($\sigma^{\text{♂}}$: $\sigma^{\text{♀}}$)
25/15 °C	53	19.1 \pm 2.5 a	72.9 \pm 4.5 a	1.53 : 1
30/20 °C	52	10.0 \pm 1.8 b	50.5 \pm 5.6 b	1.33 : 1

Values of means followed by the same letter in columns do not differ significantly (Mann-Whitney U test; $P < 0.001$).

6.3.2 Life table parameters

The number of aphids parasitized per day at ambient day-night temperature regimes fluctuated (Figure 6.1). The number of mummified nymphs recorded was highest on days 1, 5 and 8. Only one female survived until day 10.

The percentage mummified aphids was higher for female parasitoids exposed to high day-night temperature regime on day 1 with a gradual decrease to day 3 followed by an increase on day 4 (Figure 6.2). No offspring was produced on day 5. The mean number of female parasitoid offspring was nearly double for females kept at ambient (6.11 ± 2.11) than at high (3.2 ± 1.13) day-night temperatures.

The high temperature regime had a negative effect on growth parameters of *Aphidius ervi* (Table 6.4). Gross reproductive rate (GRR) was three times higher at ambient than at high day-night temperatures. Likewise, the net reproductive rate (R_0) was twice as high (Table 6.4). In addition, generation time was shorter, intrinsic and finite rates of increase were higher and doubling time was shorter at 25/15 °C compared to 30/20 °C day-night temperature.

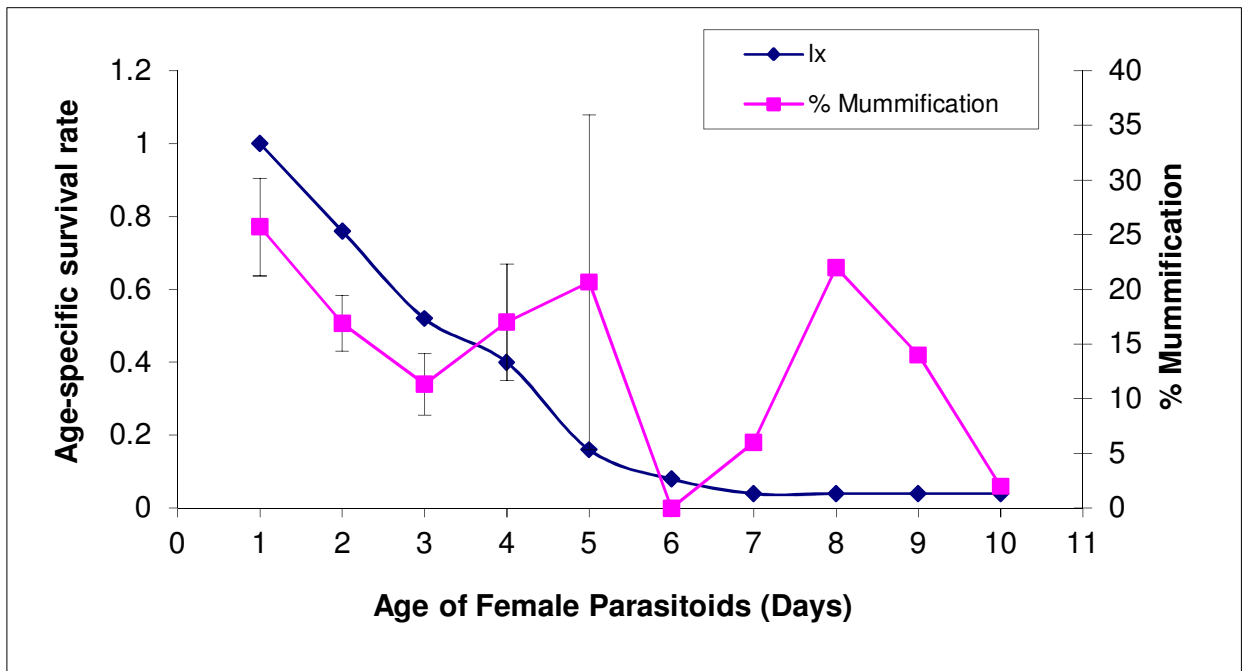


Figure 6.1 Survival rate (l_x) (mean \pm SE) of female *Aphidius ervi* and percentage aphid mummification (mean \pm SE) on *Macrosiphum euphorbiae* at 25/15 °C day-night temperature (n = 22).

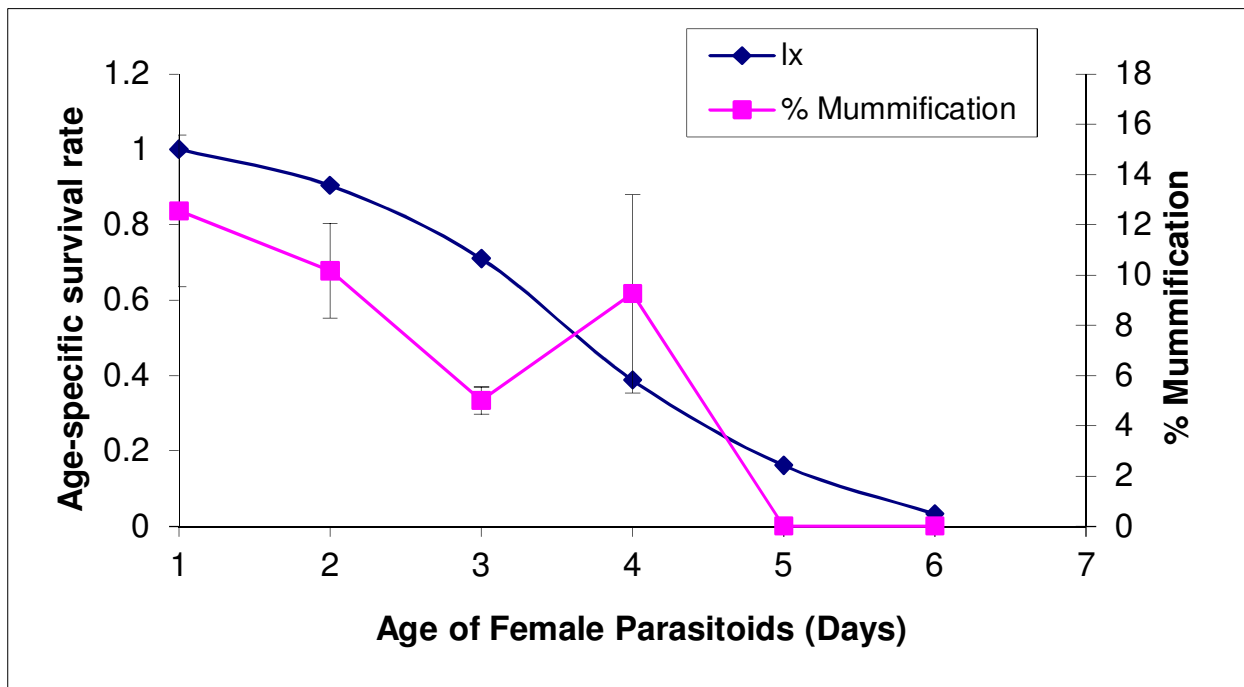


Figure 6.2 Survival rate (l_x) of female *Aphidius ervi* and aphid mummification (mean \pm SE) on *Macrosiphum euphorbiae* at 30/20 °C day-night temperature (n = 22).

Table 6.4 Life table parameters of *Aphidius ervi* reared on *Macrosiphum euphorbiae* at different day-night temperatures regimes.

Parameters	25/15 °C	30/20 °C
n (number of females)	21	22
Gross reproductive rate (GRR)	136.00	36.87
Net reproductive rate (R_0)	49.62	23.64
Mean generation time (GT)	15.77	17.83
Intrinsic rate of increase (r)	0.25	0.18
Finite rate of increase (λ)	1.28	1.19
Doubling time (DT)	2.80	3.91

6.4 Discussion

The developmental time, percentage mummification and life history traits of *A. ervi* on *M. euphorbiae* have not been reported before. High temperature had a negative effect on the biology of *A. ervi*. The aphid parasitoid experienced a reduction in fitness, measured as fecundity and development time, during high day-night temperatures. This is in agreement with Malina & Praslicka (2008), who reported that developmental time from mummy formation to adult eclosion, as well as total development time from egg to adult were shorter at higher temperature of 30 °C compared to 25 °C. The authors also observed that adult longevity of *A. ervi* was greater at a constant temperature of 25 °C compared to 30 °C. However, adult longevity in the current study for both temperature regimes was less than half that reported by Malina and Praslicka (2008). One possible reason for this could be the different sugar concentrations in the food source. Malina and Praslicka (2008) supplied a 15% honey solution to the *A. ervi* whereas we did not dilute the honey.

Aphid parasitoids may experience heat stress when exposed to temperatures above their optimum (Roux et al., 2010). Mummies exposed to high day-night temperatures in the current study had a higher pupal mortality compared to those maintained at ambient day-night temperature, in addition to the longer development time. Parasitoids exposed to the high day-night temperature regime experienced a reduction in fertility (measured as gross and net reproductive rate). Roux et al. (2010) reported a 50% mortality and reduction in fitness of

adults exposed to an elevated temperature of 36 °C; the authors suggest that an increase in activity at the high temperatures may lead to physiological changes that reduce offspring production.

The findings of the current study indicate that female *A. ervi* have a three-day fertility cycle, i.e. females need three days to mature an additional 60 eggs. This is in accordance with previous observations that directly after emergence, female *A. ervi* carry about 60 mature eggs and when they deplete this initial egg load another 60 eggs mature after 3 days (He & Wang, 2008). This rhythmic cycle appears to carry on until about 300 eggs have been laid in a lifespan of 15 days (He & Wang, 2008). However, additional research is necessary to confirm this observation based on laboratory conditions only.

In conclusion, *A. ervi* had high pupal mortality, most likely death of weaker individuals caused by high (day-night) temperature. The surviving adults showed a reduction in reproductive output. Therefore the heat-stress hypothesis is accepted. These results indicate that field populations of *A. ervi* may decrease during summer when temperatures rise to 30 °C and above.

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CHAPTER 7

EFFECTS OF WATER-DEFICIT STRESSED POTATO PLANTS AND HIGH TEMPERATURE ON THE BIOLOGY OF *APHIDIUS ERVI* HALIDAY (HYMENOPTERA, BRACONIDAE) PARASITIZING *MACROSIPHUM EUPHORBIAE* (THOMAS) (HEMIPTERA, APHIDIDAE)

Abstract

It has been proposed that water-deficit stressed potato plants provide an enhanced food source for phloem-feeding insects such as aphids due to an increase in phloem nitrogen. This in turn may lead to a higher rate of parasitism. High temperature on the other hand may reduce insect fitness, although insects may recover during cooler periods. To gain insight into the effects of drought and heat stress on a plant-aphid-parasitoid interaction, the influence of water-deficit stress and high day-night temperatures on life history parameters of *Aphidius ervi* Haliday (Hymenoptera, Braconidae) parasitizing the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Hemiptera, Aphididae) on potato (*Solanum tuberosum* L., Solanaceae) was determined. Amino acid concentrations were higher in water-deficit stressed plants than in well-watered plants at ambient and high day-night temperatures. Pupal survival of *Aphidius ervi* was higher on water-stressed plants at ambient than well-watered plants at high temperatures. The number of female offspring produced was highest on *M. euphorbiae* reared on water-deficit stressed plants at ambient day-night temperatures. The development time from egg to adult and adult longevity were not affected by high day-night temperatures. High day-night temperatures negatively influence the performance of *A. ervi* and therefore countered the positive effects of high amino acid concentrations in water-deficit stressed potato plants. Nonetheless, the combined positive effects of higher parasitism, pupal survival and increased female ratio could result in an increase in the rate of parasitism of *M. euphorbiae* during drought periods at ambient temperatures.

7.1 Introduction

The effects of water-deficit stress and high temperature are likely to influence the third trophic level (aphid parasitoids) more than the second trophic level (aphids), because the third trophic level depends on the ability of the second trophic level to adjust to environmental changes (Hance et al., 2007). Parasitoids are particularly affected because they are dependent on their hosts throughout their development (Li & Mills, 2004).

The physiological and metabolic responses to water-deficit stress in plants were reviewed in detail by Hsiao (1973). More specifically, the effects of water-deficit stress on aphids have been documented by a number of authors (Girousse et al., 1996; McVean & Dixon, 2001; Hale et al., 2003; Huberty & Denno, 2004; Nguyen et al., 2007), who reported that water-deficit stress caused an increase in amino acid concentrations in the phloem sap of plants. Although enhanced fitness (increased fecundity, development, and longevity) of aphids has been associated with increased concentrations of amino acids in phloem sap (Douglas & Prosser, 1992; Karley et al., 2002), in some cases aphids were unable to ingest the phloem sap due to low turgor pressure (Huberty & Denno, 2004). However, moderate water-deficit stress may not only lead to elevated amino acid concentrations in stressed plants but the still sufficiently high turgor pressure allows for adequate ingestion of phloem sap (Hale et al., 2003).

Nitrogen is mainly available to aphids as free amino acids in phloem sap; generally, essential amino acids make up about 20% of the total composition in the phloem sap (Febvay et al., 1999; Sandström & Moran, 1999). Ten amino acids are considered essential to insects, *viz.* arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Sandström & Moran, 1999). However, some non-essential amino acids, such as tyrosine and cysteine, are also important as they are synthesized from the essential amino acids phenylalanine and methionine, respectively (Sandström & Moran, 1999).

Aphids are able to synthesize amino acids when present in low amounts in their diet via their intracellular symbionts (e.g. *Buchnera aphidicola*, *Serratia symbiotica* and *Hamiltonella defensa*) in mid-gut cells called mycetocytes (Douglas & Prosser, 1992; Sandström & Moran, 1999; Guay et al., 2009). However, since these symbionts cannot fix nitrogen, they only improve aphid nutrition by adjusting the composition of ingested amino acids (Douglas & Prosser, 1992; Sandström & Moran, 1999). For instance, an increase in glutamic acid or glutamine and asparagine in the phloem sap due to water-deficit stress increases the synthesis of several other essential amino acids provided by the symbionts in the aphid tissues (Douglas & Prosser, 1992; Febvay et al., 1999; Hale et al., 2003). This increase in essential amino acids in the mid-gut could increase aphid fitness (Connor, 1988; Hale et al., 2003; Ponder et al., 2001). Elevated amino acid concentrations in aphid bodies may ultimately increase the nutrient quality for parasitoid larvae developing in these aphids. Therefore, searching female parasitoids are more likely to accept these based on the higher nutrient quality (Charnov & Skinner, 1985).

As discussed in Chapter 2, female aphid parasitoids prefer host quality over host size and are expected to have a higher rate of parasitism on good quality hosts. In addition, a female-biased offspring ratio is expected on higher-quality hosts (Charnov & Skinner, 1985). Therefore, higher amino acid concentrations in phloem sap of moderately water-deficit stressed plants may increase the nutritional quality for aphids, which in turn could lead to a higher percentage of parasitism, an increase in pupal survival and longevity, as well as a shorter development time and a higher female progeny ratio for the aphid parasitoid.

In addition, plant nutritional quality, including levels of foliar nitrogen, may increase at elevated temperatures and enhance aphid abundance and parasitism. Bezemer et al. (1998) observed an increase in the abundance of the peach-potato aphid, *Myzus persicae* (Sulzer), and an increase in parasitism by *Aphidius matricariae* (Haliday) (Hymenoptera: Aphidiidae) at elevated temperatures (+2 °C). Conversely, high day-night temperature (+5 °C) decreased survival and reproduction of *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) and its parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) (Chapters 3 and 6). However, the latter parasitoid study (Chapter 6) was carried out with leaf cuttings and not intact plants.

This study aimed to determine the combined effect of water-deficit stress at ambient and high day-night temperatures on the performance (developmental time, rate of mummification, pupal survival, female offspring ratio and longevity) of *A. ervi* parasitizing *M. euphorbiae* on potato (*Solanum tuberosum* L., Solanaceae). The aim was to test, firstly, whether moderate water-deficit stress at ambient day-night temperatures will enhance parasitoid performance and secondly, whether high day-night temperatures and the combined effect of high temperatures and water-deficit stress will benefit *A. ervi*.

7.2 Materials and methods

7.2.1 Potato plants

Refer to Chapter 2 for general maintenance conditions. Thirty-day-old potato plants (cv. BP1), BBCH growth stage 19 with 9 or more leaves of main stem unfolded (Meier, 2001) were placed in growth chambers (200 l, Labcon™ 2 LTGC 20, Laboratory Marketing Services cc, Roodepoort, South Africa) to precondition them for 30 days to ambient and high day-night temperatures of 25/15 °C and 30/20 °C, respectively. The ambient temperature regime was chosen based on the mean day-night ambient summer temperatures in Gauteng, South Africa (Benhin, 2006); <https://weatherspark.com/averages/29019/> Johannesburg-

Gauteng-South-Africa). High temperatures were based on those prevalent during the potato growing season in Gauteng, South Africa.

In addition, potato plants were preconditioned at two different water regimes, well-watered (80-100% pot capacity) and water-deficit stress (25-30% pot capacity) treatments for 30 days. Pot capacity was calculated by weighing the pot and oven-dry soil on an Adam QBW – 15 scale (15 kg x 5 g). The soil was oven-dried at 60 °C for 24 h before it was weighed. The soil was then watered until saturated and the mass of dry soil was subtracted from the saturated soil mass to determine the mass of water needed to saturate the soil (100% pot capacity) in the pots. We determined that 180 g of water was needed to saturate soil in 12.5 cm diameter and 9.5 cm deep pots. Therefore, for a 25% pot capacity 45 g of water is needed. Each pot and potato plant was weighed every other day to determine the mass of water needed to obtain either 25% or 100% pot capacity.

7.2.2 Experimental design

All experiments were carried out in growth chambers (200 l, Labcon™ 2 LTGC 20, Laboratory Marketing Services cc, South Africa) at two different temperature regimes of 25/15 °C (ambient) and 30/20 °C (high) day-night temperatures with a photoperiod of 14L:10D. Temperatures were set to correspond with the photoperiod (day: 06:00 to 20:00 h, night: 20:00 to 06:00 h).

Potato plants were grown as described in Chapter 2. After preconditioning the potato plants, 60-day old plants, BBCH growth stage 51 with first individual buds (1-2 mm) of first inflorescence visible on main stem (Meier, 2001) were placed in glass cages (25 x 25 x 25 cm). Temperature and water-deficit stress were randomly assigned to each cage. Four glass cages were placed in each growth chamber and set at either ambient or elevated temperature regimes and 60-70% RH for the well-watered treatment and 40-45% RH for the water-deficit stress treatment (Figure 7.1).

To compare life history parameters of *A. ervi* at different water and day-night temperature regimes, 100 apterae (wingless) aphids (2nd instar to adult stage) were transferred to each potato plant, which was then placed singly inside a glass cage. One 24- to 48-h old mated female parasitoid was introduced into each cage, and each female was given a 5-h oviposition period, after which they were removed from the cage. The control potato plants were used to determine water stress levels and stomatal conductance in each treatment. Aphids were checked twice daily for mummies formed as an indirect measure of parasitism.

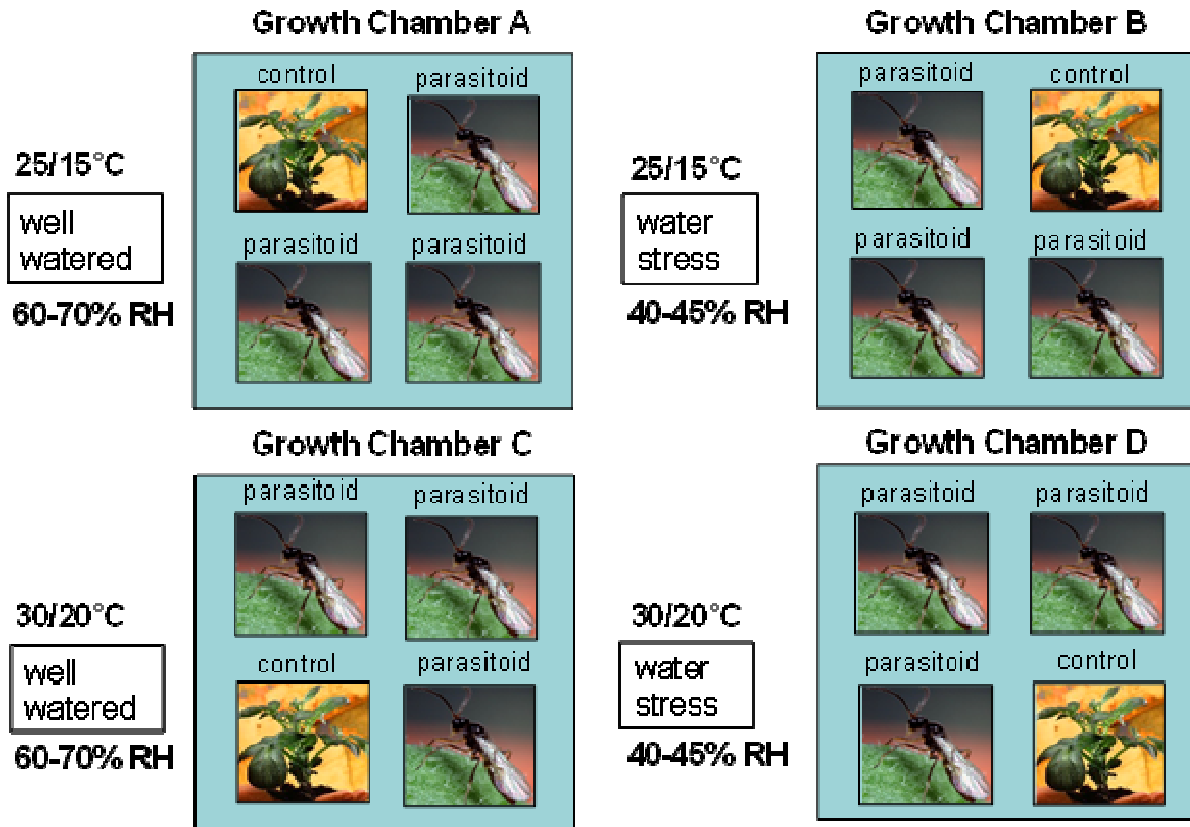


Figure 7.1 Two different temperature regimes, ambient (25/15 °C) and elevated (30/20 °C) day-night temperature, were assigned to growth chambers. Water-deficit stress (WS) (pot capacity of 25-30%) and well-watered (WW) (pot capacity of 80-100%) treatments were induced on potato plants in growth chambers at each temperature regime. One plant was assigned as a control and the remaining three plants were used in the parasitoid experiment.

The mummies were collected using a fine camel hair paintbrush and placed individually in glass vials (75 ml). The glass vials with the mummies were maintained at the two different temperature regimes described above. The date and time of mummy formation, pupal survival, the number of male and female parasitoids emerged and adult longevity were recorded. Developmental time was recorded from time of oviposition to time of mummy formation, and from time of mummy formation to time of adult emergence. Each temperature and water treatment was replicated twice.

7.2.2.1 Temperature and humidity measurements

Temperature and humidity were recorded hourly by means of a logger (iButton, Hydrochron Temperature/Humidity Logger, FairBridge Technologies, Sandton, South Africa) that was placed in each glass cage within each growth chamber.

7.2.2.2 Leaf area measurements, stomatal conductance and amino acid concentration

Potato plants of approximately the same leaf area were chosen for the experiments. Prior to pre-conditioning, the leaf area of the potato plants was measured with a LI-3100C leaf area meter (cm²) (LI-COR Environmental, Nebraska, U.S.A.). Six plants per temperature and water regime were chosen each time before pre-conditioning commenced. After completion of the experiments, the leaf area was measured again to determine variation between leaf areas caused by different treatments.

Levels of water-deficit stress in potato plants can be measured indirectly by stomatal conductance (g_s). This is defined as the speed with which water evaporates from the pores of a plant. Plants under water-deficit stress have low stomatal conductance values compared to well-watered plants (Collatz et al., 1991; Salon et al., 2011). The stomatal conductance of the control plants was measured daily for each replicate with a Decadon Leaf Porometer System SC-1 (LI-COR Biosciences, Campbell Scientific, Inc., Stellenbosch, South Africa). Stomatal conductance measurements were taken from six leaves of each control plant.

Amino acid composition and concentrations were measured as an indicator of plant nutritional status at the Southern African Grain Laboratory (Pretoria, South Africa). After completion of each trial, potato leaves were harvested from eight potato plants and analysed by the Pico-Tag method using Waters Breeze HPLC with Empower software (Waters, Milipore Corp., Milford, MA). Amino acids were extracted with 70% ethanol from potato leaves (500 mg), and then derivatized with phenylisothiocyanate (PITC) to produce phenylthiocarbamyl (PRC) amino acids. These derivatized amino acids were subsequently analysed by reverse phase HPLC (SAGL IN HOUSE METHOD 008, 2000-2004; Cohen et al., 1988). All samples were extracted in duplicate over two days.

7.2.5 Data analysis

Data were tested for normality and homogeneity of variances. The effect of different treatments (combined water and temperature regimes) on stomatal conductance was analysed with repeated measures analysis of variance (two-way ANOVA) with day as within factor.

Greenhouse-Geiser correction was used for degrees of freedom to account for violation of the assumption of sphericity. Means were separated using pairwise comparisons with Bonferroni adjustment for multiple comparisons. Leaf area measurements were analysed with a generalized linear model (GLM). Means were separated with Fisher's least significant difference (LSD) test. Linear mixed model analysis (REML) with treatment as fixed effect and cage as random effect was used to test for differences in developmental times and adult longevity of *A. ervi*. Means were separated using Fisher's LSD test. The percentage of mummies formed and pupal survival, and the number of male and female offspring produced between different temperature and water regimes were analysed with Kruskal-Wallis ANOVA followed by multiple comparisons of mean ranks for all groups. Percentage parasitism was calculated as follows: percentage parasitism = number of mummies x 100/number of aphids. Offspring sex ratio was also determined. The significance level was set at $P < 0.05$ for all analyses. Data were analysed with SPSS (IBM® SPSS® 24.1 Statistics, version 21).

7.3 Results

7.3.1 Temperature and humidity measurements

Temperature and humidity gradually increased or decreased during day and night inside the glass cages within the growth chambers, resulting in a wide range of temperature and humidity parameters experienced by the potato plants, aphids, and parasitoids (Table 7.1). The observed tendencies of fluctuation in temperature and humidity are closer to what is experienced under natural conditions, than the constant temperatures the growth chambers were set at.

7.3.2 Leaf area measurements, stomatal conductance and amino acid concentration

The leaf area of potato plants prior to pre-conditioning did not differ significantly between plants (Wald $X^2 = 3.434$, d.f. = 3, $P = 0.329$). Mean leaf area ranged between 115 and 125 cm² (n = 6) for potato plants before assigning to experiments. Potato plants grown under water stress and high temperature grew less vigorously than well-watered plants at ambient temperatures. After completion of the experiment, the leaf area was significantly smaller for water-stressed plants at high day-night temperatures compared to high (well-watered plants)

Table 7.1 Temperature and relative humidity (mean \pm SE) inside the glass cages at different temperature and water regimes.

Temp and water treatment	Day/Night	Temperature ($^{\circ}$ C)	Temperature range ($^{\circ}$ C)	Humidity (%)	Humidity range (%)
25/15 $^{\circ}$ C WW	Day	23.1 \pm 0.1	12.6 - 27.7	49.0 \pm 0.3	10.3 - 100
	Night	18.1 \pm 0.1	12.6 - 27.7	53.5 \pm 0.4	11.8 - 100
25/15 $^{\circ}$ C WS	Day	24.6 \pm 0.1	11.1 - 26.6	30.8 \pm 0.2	13.4 - 72.9
	Night	17.4 \pm 0.1	12.6 - 26.6	36.7 \pm 0.2	14.2 - 74.1
30/20 $^{\circ}$ C WW	Day	28.6 \pm 0.1	15.1 - 32.7	60.4 \pm 0.2	13.7 - 100
	Night	20.3 \pm 0.1	15.1 - 32.7	64.8 \pm 0.3	20.4 - 100
30/20 $^{\circ}$ C WW	Day	28.3 \pm 0.1	13.1 - 31.6	37.1 \pm 0.3	11.4 - 79.0
	Night	18.2 \pm 0.1	12.7 - 31.6	43.7 \pm 0.3	14.7 - 80.1

WW = well-watered, WS = water-deficit stress.

and ambient day-night temperatures, the latter irrespective of water treatment (Wald $X^2 = 11.65$, d.f. = 3, $P = 0.023$). The leaf areas of water-stressed plants did not differ between water regimes at ambient day-night temperatures (Figure 7.2). Total leaf areas after the experiment were reduced by 50% due to senescence caused by aphid feeding and handling.

Stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$; mean \pm SE) differed significantly between treatments ($F_{2,097, 10,487} = 148.06$; $P < 0.0001$). It was significantly higher for well-watered potato plants compared to water-stressed plants at both temperature regimes (Figure 7.3). In addition, water-stressed plants had a lower stomatal conductance at ambient than at high temperatures. Differences between days and the treatment-by-day interaction were not significant (day: $F_{4,03, 20,149} = 1.691$, $P = 0.191$; day x treatment: $F_{4,247, 21,233} = 1.528$, $P = 0.228$; $n = 21$).

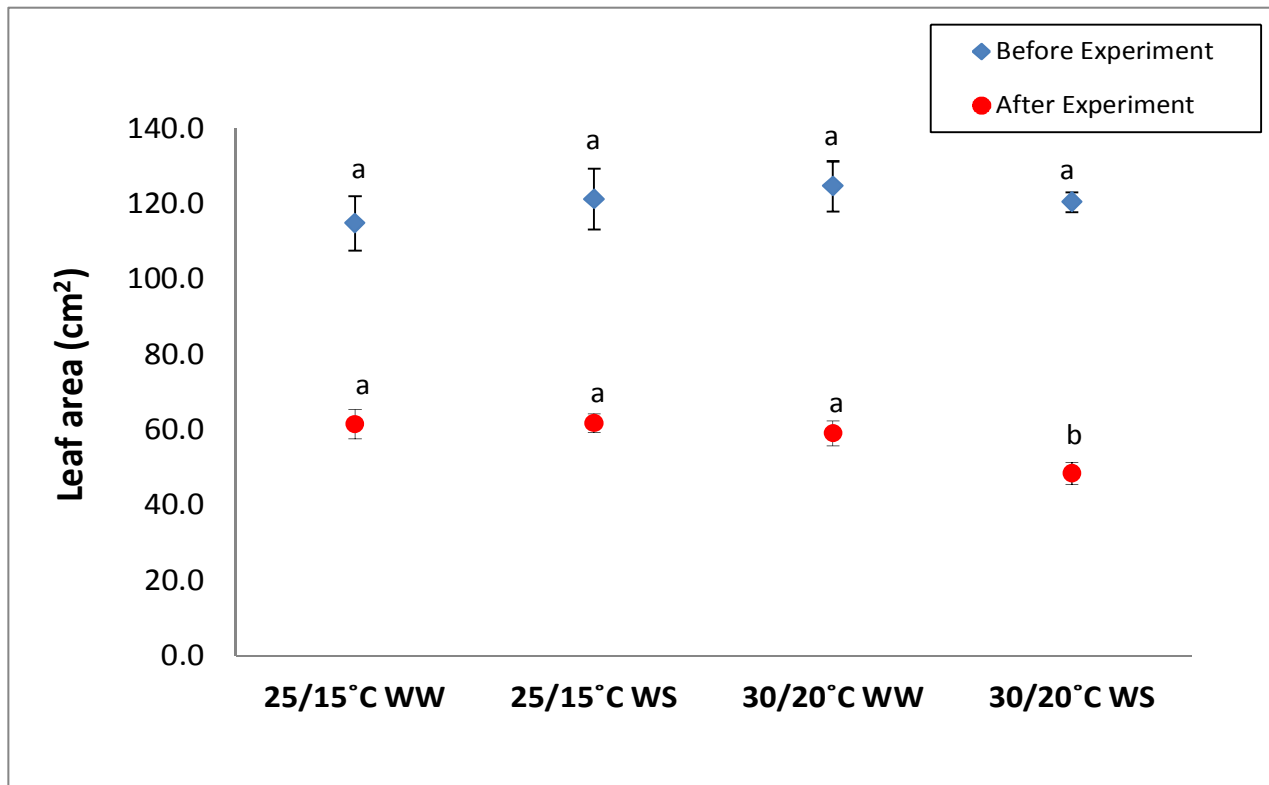


Figure 7.2 Leaf area of potato plants before pre-conditioning and after completion of experiments (mean \pm SE); WW = well-watered, WS = water-deficit stress. Letters above markers indicate significant differences (Generalized Linear Models (GLM), Wald $X^2 = 11.65$, $df = 3$, $P < 0.05$).

Twenty-seven amino acids were identified in the potato plants at different temperature and water regimes (Table 7.2). Total essential amino acid concentration was highest in water-deficit stressed plants compared to well-watered plants. The highest percentage of essential amino acids was recorded in water-deficit stressed plants at high (21.8%) and ambient (19.4%) day-night temperatures. The lowest percentage (13.9%) was recorded from leaves from well-watered plants at high day-night temperatures. Amino acid composition was dominated by the non-essential amino acids asparagine and glutamine. Potato plants exposed to high day-night temperatures at both water regimes had the highest concentration of arginine, asparagine, and glutamine compared to the ambient temperature treatment at both water regimes. High day-night temperatures and water stress had the greatest effect on the essential amino acids in the potato plants. Leaves had the highest concentrations of histidine, isoleucine, lysine, phenylalanine, serine and tryptophan; higher concentrations of two essential (leucine and valine) and one non-essential (tyrosine) amino acid were observed in water-deficit stressed plants irrespective of temperature.

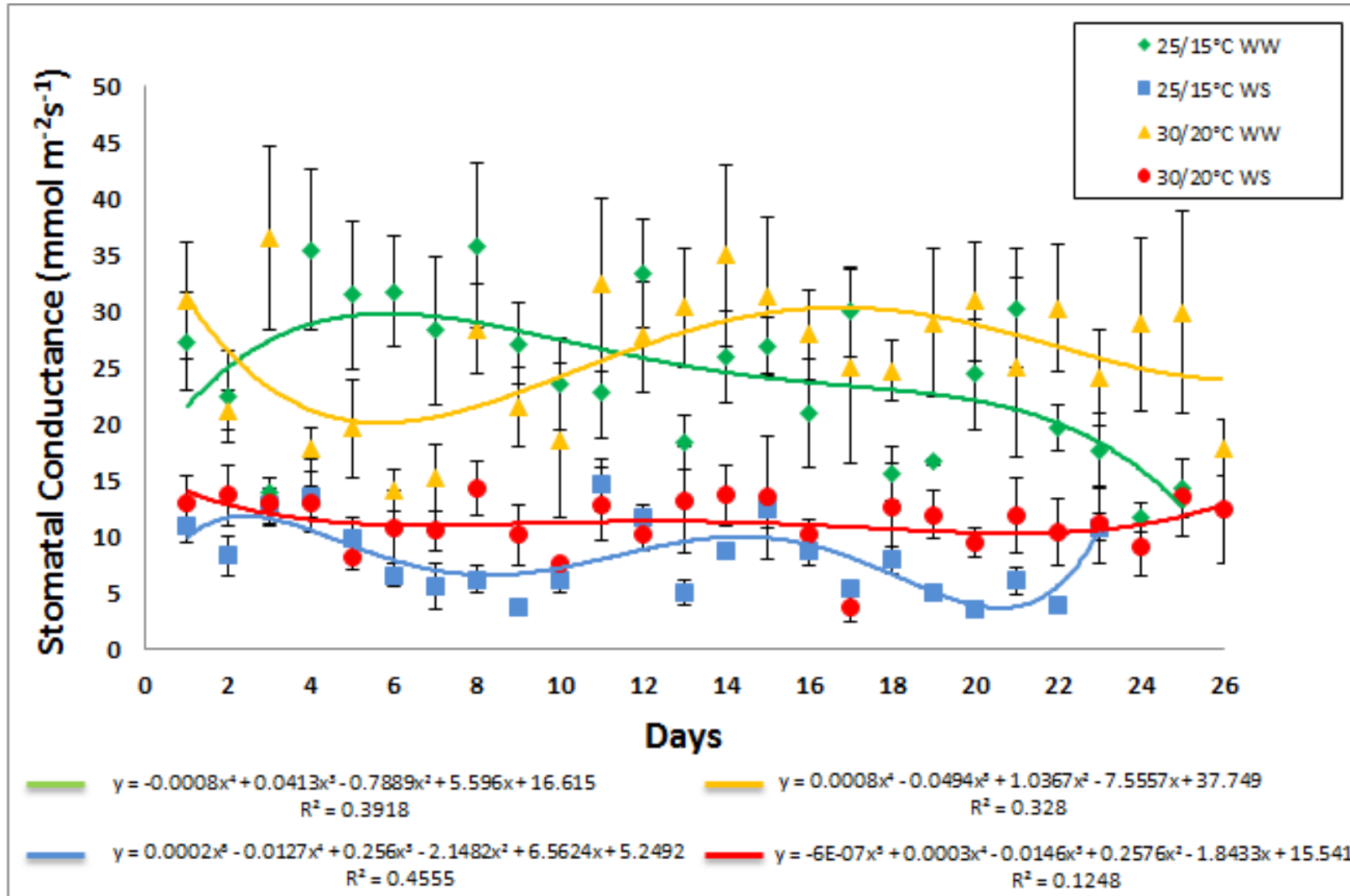


Figure 7.3 Stomatal conductance measurements (mean \pm SE) of well-watered and water-deficit stressed potato plants for each temperature and water regime; WW = well-watered, WS = water-deficit stress.

Table 7.2 Amino acid composition (mg/100 g) of potato plants exposed to ambient (25/15°C) and high (30/20 °C) day-night temperatures and different water treatments.

Temperature and Water stressed Treatments				
Amino Acid	25/15 °C WW	25/15 °C WS	30/20 °C WW	30/20 °C WS
	mg/100g	mg/100g	mg/100g	mg/100g
Alanine (Ala)	20.0	24.0	22.7	20.6
Arginine (Arg)*	143.3	100.2	280.7	982.2
Asparagine (Asn)	1622.2	1516.9	3238.8	5180.2
Aspartic Acid	141.8	181.0	202.9	173.1
Cystine/Cysteine (Cys)	ND	6.4	4.4	ND
Glutamine (Gln)	248.7	177.5	474.3	1160.4
Glutamic Acid	207.1	248.6	260.9	219.8
Glycine (Gly)	ND	ND	ND	ND
Histidine (His)*	55.2	68.3	92.3	302.3
Isoleucine (Ile)*	31.4	82.4	51.2	136.6
Leucine (Leu)*	39.6	67.0	46.4	90.9
Lysine (Lys)*	31.5	54.3	60.9	145.2
Methionine (Met)*	13.3	11.7	7.6	2.5
Phenylalanine (Phe)*	11.2	29.8	13.1	57.1
Proline (Pro)	10.9	27.9	7.6	4.7
Serine (Ser)	66.2	77.7	88.4	115.1
Threonine (Thr)*	19.5	20.0	27.4	22.2
Tryptophan (Trp)*	119.8	114.3	111.0	237.6
Tyrosine (Tyr)	77.8	125.7	95.6	290.8
Valine (Val)*	27.5	77.3	47.3	107.9
Beta-Amino-isobutyric Acid	2.9	4.4	4.4	3.0
Gamma-Aminobutyric Acid	23.7	32.4	24.7	27.6
Ominthine	3.5	2.2	6.7	10.4
Phosphoethanolamine	3.2	4.3	5.8	5.7
Phosphoserine	19.0	17.6	23.2	21.5
Alpha-Aminobutyric Acid	ND	2.3	1.4	ND
Taurine	ND	1.9	2.9	ND
Essential Amino Acids (% of total)	16.4	19.4	13.9	21.8

ND indicates amino acids that were not detected. Asterisks denote essential amino acids. WW = well-watered, WS = water-deficit stress.

7.3.3 Effects of high temperature and water-deficit stress on *A. ervi*

The highest mean percentage of aphid mummies occurred on water-stressed plants at ambient temperature (61%), followed by well-watered plants at high (35%) and ambient (33%) day-night temperatures. The lowest mean percentage mummification was recorded from aphids on water-deficit stressed plants at high temperatures (27%); however, differences were not significant ($H = 3.490$, d.f. = 3, $P = 0.3221$). Pupal survival was significantly higher on water-stressed plants at ambient than on well-watered plants at high temperatures (Figure 7.4; $H = 9.419$, d.f. = 3, $P = 0.0242$). The number of males emerged was significantly higher at ambient temperature, irrespective of water regime ($H = 9.031$, d.f. = 3, $P = 0.0289$). Significantly more females emerged from parasitized aphids reared on water-stressed plants at ambient temperature than any other treatment (Figure 7.5). The lowest number of female parasitoids was recorded from aphids reared on well-watered plants at ambient and water-stressed plants at high temperature. The number of male and female parasitoids emerged was significantly lower on water-stressed than well-watered plants at high temperatures.

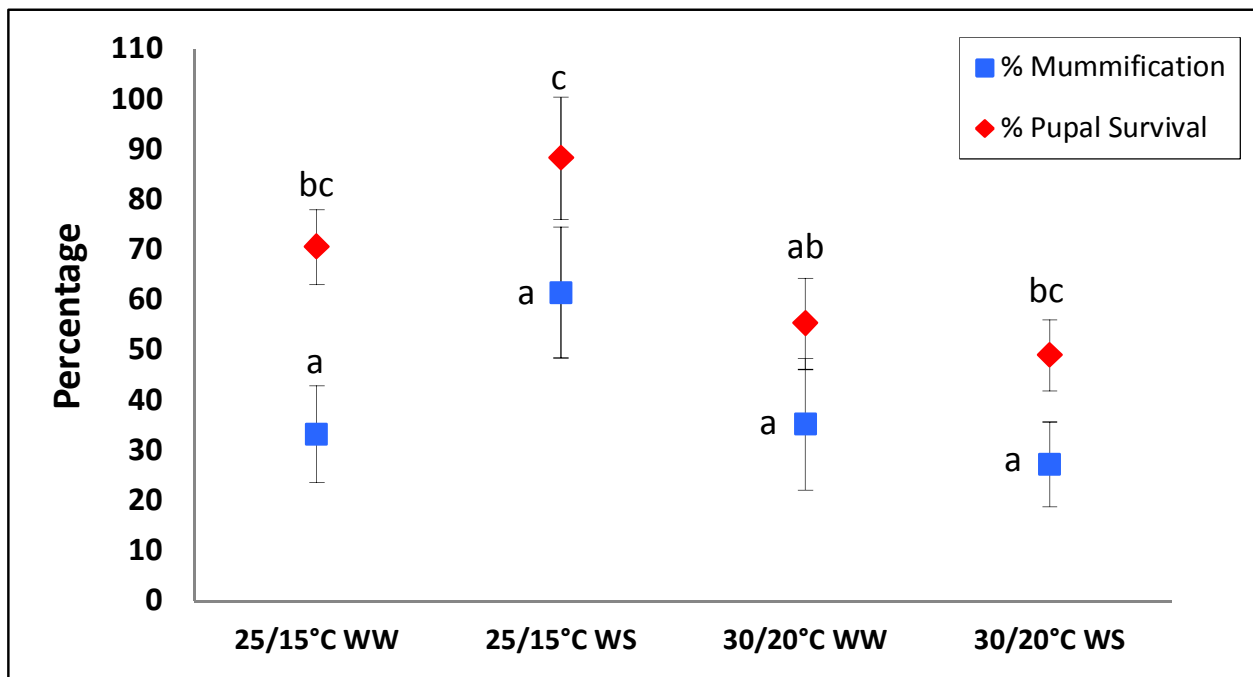


Figure 7.4 Percentage mummification and percentage pupal survival (mean \pm SE) of *Aphidius ervi* at different temperature and water regimes. WW = Well-watered, WS = Water-deficit stress. Letters above markers indicate significant differences (Kruskal-Wallis ANOVA, $P < 0.05$).

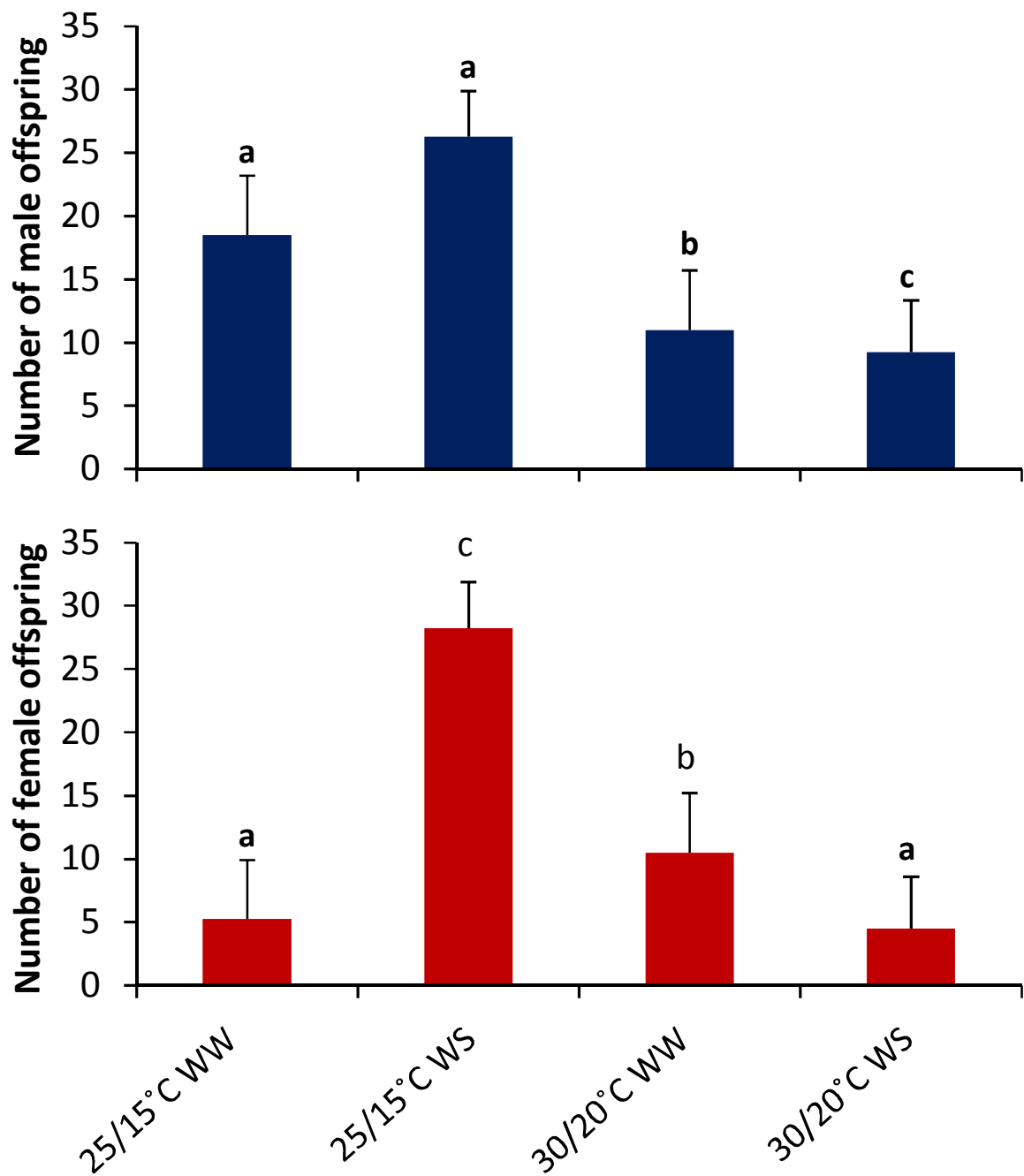


Figure 7.5 Male and female offspring produced (mean \pm SE) by *Aphidius ervi* parasitizing *Macrosiphum euphorbiae* at ambient (25/15 °C) and high (30/20 °C) day-night temperatures on well-watered (WW) and water-deficit stressed (WS) potato plants. Letters above bars indicate significant differences (Fisher's LSD test; $P < 0.05$).

High temperature and water-deficit stress had little influence on male and female developmental times and adult longevity (Tables 7.3, 7.4). Male development times from egg to mummy and from mummy to adult were significantly faster at ambient temperature for both water regimes compared to the 30/20 °C temperature and water regimes. However, there was no significant difference when comparing egg-to-adult development time, and no other differences were observed for female development times. Male and female adult longevity was not affected by high temperature and water-deficit stressed plants. The number of male offspring produced at 25/15 °C at both water regimes was significantly higher compared to 30/20°C at both water regimes (GLM, Pairwise comparison, Wald $X^2 = 41.990$, d.f. = 3, $P < 0.05$). Female offspring produced at 25/15°C water deficit stress treatment was significantly higher (Wald $X^2 = 99.014$, d.f. = 3, $P < 0.05$) than at 25/15 °C well-watered and 30/20 °C temperature treatment at both water regimes.

7.4 Discussion

Aphidius ervi development times from egg to adult and adult longevity were not affected by the increase in concentrations of amino acids in water-deficit stressed plants. In addition, water-deficit stress at high temperatures resulted in lower numbers of males and females emerging on water-stressed compared to well-watered plants at high day-night temperatures, suggesting that parasitoids did not benefit from increased amino acid concentrations. Elevated temperatures may reduce the reproductive capacity of *A. ervi* due to higher physiological activity, e.g. feeding, resting, and grooming caused by heat stress (Roux et al., 2010).

Pupal survival of *A. ervi* was higher in aphids reared on water-deficit stressed plants at ambient day-night temperatures, indicating that higher amino acid concentrations of leucine, proline, tyrosine and valine may have increased the aphids' nutritional quality, which may have benefited the parasitoid. This is supported by the higher female offspring ratio found at ambient temperatures and water-deficit stress treatment as female parasitoids tend to lay more fertilized eggs in high-quality aphid hosts (Sequeira & MacKauer, 1992a; 1992b; 1993; Cloutier et al., 2000; Henry et al., 2005).

Table 7.3 Development time and longevity of male and female parasitoids in days (mean \pm SE) of *Aphidius ervi* parasitizing *Macrosiphum euphorbiae* at ambient (25/15 °C) and high (30/20 °C) day-night temperatures and on well-watered (WW) and water-deficit stressed potato plants.

Temperature & Water Regime		Development (days)							
		Egg - Mummy		Mummy - Adult		Egg - Adult		Adult Longevity	
		Male	Female	Male	Female	Male	Female	Male	Female
25/15°C WW	Range	8.1 - 15.0	9.9 - 16.2	1.2 - 8.9	1.0 - 8.0	11.1 - 22.8	13.0 - 18.8	1.1 - 18.1	3.0 - 17.0
	Mean	10.20 a \pm 0.2 (74)	10.64 a \pm 0.4 (21)	5.55 a \pm 0.3 (74)	5.83 a \pm 0.4 (21)	15.75 a \pm 0.3 (74)	16.5 a \pm 0.3 (21)	5.24 a \pm 0.5 (66)	9.69 a \pm 1.3 (15)
25/15°C WS	Range	4.5 - 15.8	7.9 - 17.8	1.4 - 10.2	1.0 - 12.2	9.5 - 23.7	13.8 - 21.7	1.1 - 18.7	1.1 - 21.0
	Mean	9.96 a \pm 0.2 (105)	10.65 a \pm 0.2 (113)	5.56 a \pm 0.2 (105)	6.21 a \pm 0.2 (113)	15.52 a \pm 0.2 (105)	16.9 a \pm 0.2 (113)	7.32 a \pm 0.4 (92)	7.93 a \pm 0.4 (95)
30/20°C WW	Range	8.8 - 16.1	8.7 - 14.8	1.7 - 8.1	1.7 - 8.1	13.1 - 22.7	13.1 - 21.1	1.1 - 13.3	1.1 - 16.3
	Mean	12.04 b \pm 0.3 (44)	11.79 a \pm 0.3 (42)	5.17 b \pm 0.3 (44)	4.68 a \pm 0.2 (42)	17.21 a \pm 0.3 (44)	16.5 a \pm 0.3 (42)	5.02 a \pm 0.5 (43)	5.61 a \pm 0.6 (40)
30/20°C WS	Range	9.8 - 15.1	9.9 - 13.8	1.8 - 8.0	3.0 - 8.2	13.8 - 20.1	13.8 - 20.8	1.1 - 13.3	1.2 - 11.2
	Mean	12.25 b \pm 0.2 (37)	11.86 a \pm 0.3 (18)	4.30 b \pm 0.2 (37)	5.00 a \pm 0.4 (18)	16.54 a \pm 0.3 (37)	16.9 a \pm 0.4 (18)	4.88 a \pm 0.6 (35)	4.65 a \pm 1.0 (13)

Values followed by the same letter in columns or in rows within a development stage do not differ significantly according to Kruskal-Wallis test ($P < 0.05$).

WW = Well-watered, WS = Water-deficit stress.

Table 7.4 Results of linear mixed model analyses (REML) for development times and adult longevity of *Aphidius ervi* parasitizing *Macrosiphum euphorbiae* on potato at different temperature and water regimes.

Treatment	Sex	N	Ndf	Ddf	F	P
Egg to mummy	Male	74	3	6.182	20.647	0.001
	Female	21	3	12.003	3.026	0.071
Mummy to adult	Male	105	3	6.182	20.647	0.001
	Female	113	3	3.840	5.261	0.075
Egg to adult	Male	44	3	7.296	4.033	0.056
	Female	42	3	10.494	0.787	0.527
Adult longevity	Male	37	3	11.222	1.753	0.213
	Female	18	3	6.190	1.563	0.291

Concentrations of the non-essential amino acids asparagine and glutamine were found to be highest at elevated temperatures and in water-deficit stressed plants; these are the most abundant amino acids to be excreted by aphids, implying that certain non-essential amino acids reduce the nutritional quality of a plant when abundant (Douglas, 1993; Karley et al., 2002).

In addition, pupal survival was higher at ambient temperatures in aphids reared on water-deficit stressed plants compared to well-watered plants at high temperatures. Tyrosine was recorded in higher concentrations in water-stressed compared to well-watered plants. This amino acid is known to be incorporated into cuticular material including protein and sclerotization metabolites (Rahbé et al., 2002). Eggs and first-instar parasitoid larvae can be encapsulated and killed through the immune response of the aphid host, which is driven by facultative symbiotic bacteria such as *Hamiltonella defensa* (Guay et al., 2009). However, it is possible that the increase in tyrosine in the larval diet of the parasitoid could improve resistance to this defence mechanism, resulting in a higher larval survival rate. Rahbé et al. (2002) suggested that endophagous parasitoids such as *A. ervi* may alter the nitrogen metabolism of their hosts in order to satisfy the needs of their developing larvae. The initial stages of the larval development of aphid parasitoids may be controlled by free amino acids in the haemolymph of their hosts. Rahbé et al. (2002) reported that tyrosine accumulation in the haemolymph of *Acyrtosiphon pisum* was the result of changes induced by *A. ervi* larvae, a phenomenon known as “parasitic syndrome”.

Therefore, an increase of tyrosine in the aphid diet consumed by the growing parasitoid larvae may result in the formation of stronger cuticles, thus improving pupal survival.

The increase in amino acids did not improve parasitoid survival, longevity and development time at high temperatures in the current study. Although levels of the essential amino acids histidine, isoleucine, leucine, lysine, phenylalanine, tryptophan, and valine were highest in plants at elevated temperatures as well as in water-deficit stress treatments, the negative effects of temperature cancelled out any positive effects of high amino acid concentration in water-deficit stress plants.

High temperatures may reduce the positive effects of increased nutrient quality. This could lead to a reduction in natural populations of *A. ervi* in the field when confronted with high temperatures and drought. Although increased nitrogen levels under moderate water-deficit stress at ambient temperature were not beneficial to aphids (Chapter 5), parasitoids may indirectly benefit through enhanced fitness such as female-biased progeny, high pupal survival and higher percentage parasitism.

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CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

To determine the effect of changes in climatic conditions such as drought and heat stress on a bi-trophic plant herbivore and tritrophic plant-herbivore interaction, the present study evaluated the effect of water-deficit stress and high day-night temperatures on the interaction between potato (*Solanum tuberosum* L., Solanaceae), the potato aphid, *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae), and the parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). Plants and insect species have specific climatic requirements for growth, survival and reproduction that limit their geographic distribution, abundance and interactions with other species (Voigt et al., 2003). Global climate change is expected to influence phytophagous insects directly through changes in physiology, behaviour and life history parameters, as well as indirectly through physiological and morphological changes in their host plants (Bale et al., 2002; Cornelissen, 2011; Thomson et al., 2010). Climate change models predict different regional situations that include more frequent heat waves, drought and extreme precipitation events (Canto et al., 2009). Aphid parasitoids have to deal with the response of their hosts as well as their own to changes in climatic conditions. They may be less effective in controlling aphid pests during less stable and highly unpredictable climatic conditions (Hance et al., 2007; Thomson et al., 2010). More frequent drought events could lead to herbivorous insect pest outbreaks caused by increased nitrogen concentrations in water-deficit stressed plants (White, 1969; Huberty & Denno, 2004). Due to an increase in phloem nitrogen and a reduction in aphid parasitoid parasitism, the frequency and intensity of aphid outbreaks could increase as climate becomes more variable (Huberty & Denno, 2004; Hullé et al., 2010). In contrast to high constant temperatures, high fluctuating temperatures, such as lower night-time temperatures, may improve survivorship and reproduction of insects. The rationale for the current study was to provide insight into the combined effect of moderate water-deficit stress and high fluctuating temperatures, and test predictions made about drought and heat stress on phloem-feeding insects and their parasitoids.

In the current study, the potato aphid showed a greater population growth response to temperature than to moderate water-deficit stressed potato plants (Chapter 3). Unlike suggested by White (1969), the performance of *M. euphorbiae* did not improve on water-deficit stressed plants at either ambient (25/15 °C) or high (30/20 °C) day-night temperatures, and development

time was not influenced by water regimes and high temperature. In addition, the reproductive period and longevity were similar between water-deficit stressed and well-watered potato plants at both temperatures regimes. However, the reproductive output and survival rate were reduced at high day-night temperatures due to heat stress. Therefore, it is expected that the abundance of *M. euphorbiae* will be reduced at fluctuating high temperatures but that moderate water-deficit stress will have little or no effect on aphid performance.

At ambient day-night temperatures *A. ervi* could successfully control *M. euphorbiae* when comparing life-history parameters. Generally, the intrinsic rate of increase of *A. ervi* is higher and doubling time shorter than that of *M. euphorbiae* (Table 8.1). However, at high day-night temperatures the gross reproductive rate of *A. ervi* was more negatively affected than that of *M. euphorbiae*. According to Roux et al. (2010), the reduced fitness of aphid parasitoids is due to resisting heat stress as they need to overcome their own thermal stress as well as that of their host. The reduction in reproductive output suggests that *A. ervi* will be less effective in controlling *M. euphorbiae* at high temperatures. The results are in agreement with those of Roux et al. (2010) and Thomson et al. (2010), who reported a decline in parasitoid population density at high temperatures.

However, during drought at ambient day-night temperatures, *A. ervi* could increase parasitism due to higher concentrations of amino acids such as leucine, proline, tyrosine and valine in their aphid host feeding on water-deficit stressed potato plants (Chapter 7). Although the performance of *M. euphorbiae* did not benefit from increased amino acid levels, the higher nutritional quality in the diet of *M. euphorbiae* may have resulted in a higher acceptability for parasitism. Higher pupal survival was also observed, and this could be due to an increase in tyrosine, which is known to be incorporated in cuticular material (Rahbé et al., 2002). In addition to this, an increase in female offspring ratio was recorded on aphids reared on water-deficit stressed potato plants and at ambient temperature. Therefore, the combined positive effects of higher parasitism, pupal survival and increased female : male ratio could result in an increase in the rate of parasitism of *M. euphorbiae* during drought at ambient temperature. However, high day-night temperatures had a negative influence on the performance of *A. ervi* and therefore countered the positive effects of high amino acid concentrations in water-deficit stress potato plants.

Table 8.1 Life-history parameters of *Macrosiphum euphorbiae* (Chapter 3) and its parasitoid, *Aphidius ervi* (Chapter 6) at different day-night temperature regimes on well-watered potato plants. *M. euphorbiae* was reared on potted potato plants, whereas *A. ervi* was reared on *M. euphorbiae* on potato leaf cuttings.

Parameters	25/15 °C		30/20 °C	
	<i>M. euphorbiae</i>	<i>A. ervi</i>	<i>M. euphorbiae</i>	<i>A. ervi</i>
Number of females (n)	42	21	39	22
Gross reproductive rate (GRR)	87.38	136	54.4	36.87
Net reproductive rate (R_0)	49.31	49.62	24.47	23.64
Mean generation time (GT)	20.23	15.77	18.4	17.83
Intrinsic rate of increase (r)	0.19	0.25	0.17	0.18
Finite rate of increase (λ)	1.21	1.28	1.19	1.19
Doubling time (DT)	3.6	2.8	3.99	3.91

Macrosiphum euphorbiae could become more abundant in the absence of effective parasitism during high temperatures. Although reproductive and survival rates will be reduced during heat stress conditions, the aphids are still expected to show an increase in population density over time. Not only will there be *M. euphorbiae* population density increases during periods of heat stress, but aphid population peaks may shift to occur earlier in the season (Canto et al., 2009). According to Van der Waals et al. (2013), the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) will become more abundant, resulting in higher incidences of *Potato virus Y* (PVY) and *Potato leaf roll virus* (PLRV) infection during warmer winter seasons. Therefore, *M. euphorbiae* together with other aphid vectors could pose a greater economic threat due to a higher risk of PVY and PLRV transmission in some regions due to changing climatic conditions.

The current study was laboratory-based and findings need to be confirmed with field data, amongst others, because the predictions made on the effect of climate change on herbivores and their parasitoids assume that life history parameters such as development time, reproductive capacity and thermal tolerance are static and cannot evolve (Thomson et al., 2010).

During the course of the study, new ideas and questions have arisen. Some of these are outlined here to provide suggestions for future research.

1. Studies on the effects of water-deficit stress on plants should measure amino acid concentration rather than nitrogen concentration as aphids obtain their nitrogen through different amino acid concentrations.
2. The effect of moderately water-deficit stressed potato plants on aphids transmitting potato viruses should be evaluated to test whether transmission efficiency increases in water-deficit stressed potato plants and to determine the effect of high temperatures.
3. Preference tests of aphids should be carried out to determine if aphids prefer to feed on water-deficit stressed rather than on well-watered plants. Furthermore, it would be of interest to know whether colour and plant volatile profiles differ between water-deficit stressed and well-watered potato plants, and whether this influences their attractiveness to aphids.

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