UNIVERSITÀ DEGLI STUDI DELLA BASILICATA
SAFE - Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali

Università degli Studi della Basilicata

Ph.D. in
Agricultural, Forest and Food Science

The effects of thermal stresses on aphids and their natural enemies

Scientific-Disciplinary Sector
"AGR/11 - General and Applied Entomology"

Ph.D. Coordinator
Prof. Fabio Napolitano

Ph.D. Student
Pierluigi Forlano

Ph.D. Tutor
Prof. Donatella Battaglia

Cycle XXXIII

TABLE OF CONTENTS

A me e a chi mi è stato accanto!

ABSTRACT

Temperature affects living organisms because it influences all the aspects of the physical environment and governs any process involving energy flows, setting boundaries for all living species. Within not lethal thermal ranges, animals are able to thermoregulate their body.

Since insects are ectotherms, their life histories unfold only if their body temperatures fall within a range that permits the indispensable vital functions. Moreover, they are very susceptible to rapid temperature variations. Insect have evolved an ample array of adaptations, both physiological and behavioral. Insects often experience extreme temperatures that, in the absence of specific mechanisms of thermoregulation, can dangerously injure or kill them. As with all aspects of insect physiology, duration of exposure to thermal variation has effects on the patterns and responsive mechanism to the thermal stress. Moreover, many biological traits of the insects, survived to heat and cold stresses, could be negatively affected, so to affect also their dynamic and structure populations.

Among insects, aphids are particular vulnerable to rapid thermal variations, since they have small body and thin cuticles. Moreover, their limited capability to escape from extreme thermal conditions make them very vulnerable to potential sub-lethal temperatures. Since aphid natural enemies (both predator and parasitoids) share the same habitat of their hosts and preys, they are considered to be potentially exposed to the same stresfull tempertures.

The experiments reported in this thesis are related with the thermal biology of the pea aphid *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), the parasitic wasp *Aphidius ervi* Haliday (Hymenoptera: Braconidae) and the aphidophagous ladybird *Hippodamia variegata (*Goeze) (Coleoptera: Coccinellidae). The aphid *A. pisum*, a model species often used to study the aphid thermal biology, was the species used in all the studies.

In the studies reported in my thesis, the above mentioned insect species were exposed to extreme temperatures (heat and cold, in base to the different aims of the studies) to assess the main effect of these exposure on their vitality, on some life history traits (longevity and prolificacy, as done for the pea aphid in the first chapter) and some biological performances (as the predation activity in the case of the ladybird *H. variegata,* in the last chapter).

Briefly, in the Ch. I, II, III and IV, I exposed the pea aphid to a severe heat temperature, that was 39°C per 30 minutes, under laboratory conditions. At this thermal condition, an aphid mortality of about 80% was obtained in the aphid experimental group (adults, 8-days-old aphids).

I found that when the pea aphid is exposed to this severe high temperature both longevity and prolificacy of survived aphids are significantly reduced, demonstrating the existence of a really strong long-term effect on these two fitness-related life history traits (Ch. I).

In accordance with other studies on the thermal biology of the aphid-endoparsitoid system (Lagos *et al*., 2001; Bensadia *et al*., 2006; Cayetano and Vorburger, 2013), my results show a physiological interaction between the pea aphid *A. pisum* and the immature stages of its endoparasitoid *A. ervi*. I found that, after being parasitized, the pea aphid had a greater thermal resistance to heat (Ch. II) and cold (Ch.V) stress.

Also, I found that a short acclimation phase to a not-lethal temperature increases the thermal resistance of the pea aphid to cope with a subsequent sub-lethal heat stress (Ch. III and V). But, in the case of parasitized aphid this was not found. On the contrary, I discovered that there is a cumulative detrimental effect of the acclimation treatment and sub-lethal stress on the vitality of the parasitoid when it experiences them at an immature stage (Ch. III). Moreover, I found that the same heat exposure not only could determine long-term mortality in parasitoid experiencing a heat stress at the immature stages (egg and larval stages), but that these exposures also affect the body size of the adult parasitoids (Ch. V). Body reduction, assessed as wing and tibia length, and a variation of the normal static allometry were found in the adult parasitoids that were exposed to heat.

I also investigated the role of the thermal acclimation on the predatory activity of the ladybird *H. variegata*, and I found that the aphid consumption is maximum at the temperature at which the predator developed (Ch.VI).

My results confirm that rapid and extreme thermal variations strongly affect some of the main fitness-related life history traits of the pea aphid and two of its natural enemies, the endoparasitoid *A. ervi* and the ladybird *H. variegata*. Moreover, these results represent a contribution to the knowledge of the aphid-endoparasitoid relationship under extreme thermal conditions and on the thermal biology of the ladybird *H. variegata*.

The study reported in the Ch.VI was carried out at the Laboratory of Entomology of the University of Lleida (Catalogna, Spain), from November 2019 to June 2020. All the other studies were carried out at the Laboratory of Insect Etology of the University of Basilicata (Basilicata, Italy).

GENERAL INTRODUTION

Global thermal increment: trend and perspectives

The Intergovernmental Panel on Climate Change (IPCC), an intergovernmental body of the United Nations, reports that, currently, Earth surface temperature is increasing by about 0.2 °C per decade. In particular, from the 2009 to 2018, average Earth temperature was 0.93 ± 0.07 °C higher than the pre-industrial baseline (1850–1900). In the contest of rapid global warming, degree, duration and frequency of high temperatures are predicted to increase substantially (Diffenbaugh *et al*., 2005). IPCC reports that, if global warming will continue to increase at the current rate (Meehl *et al*., 2000; Ballester *et al*., 2009; Marengo *et al*., 2009; Diffenbaugh and Field, 2013), it will likely rise to 1.5 °C above pre-industrial levels in the period 2030-2052 (IPCC, 2018). The average temperature increment of the Earth climate system is defined as "Global warming". This term is often interchangeably and wrongly used with the term "climate change". It is right to specify that the term global warming refers to the human-caused thermal increase of our planet, and also its projected continuation. Instead, the term "Climate change" includes both global warming and its effects occurring over several decades, or longer. Moreover, climate change encompasses other environmental variables such as sea-level rise, changes in precipitation patterns and increases in the frequency of extreme weather events (IPCC WG2, 2018)**.**

Climate change is linked to human activities. In fact, since the Industrial Revolution, the amount of greenhouse gases in the atmosphere substantially increased. These gases (CO_2, methane, nitrous oxide, *etc.*) are above all produced by industrial activities. Greenhouse gases trap heat radiating from the Earth to space. This heat, in the form of infrared radiation, gets absorbed and emitted by these gases in the atmosphere, thus warming the lower atmosphere and the surface.

Scientists inform that land surfaces are heating faster than the ocean surface, leading to heat waves, wildfires and expansion of deserts. Moreover, increasing atmospheric energy and rates of evaporation are causing more intense storm and weather extremes, damaging (Vermeulen *et al*., 2012). Terrestrial surface temperature increases are greatest in the Arctic, which have contributed to the reduction of glaciers, permafrost and ice. Environmental effects of global warming include the relocation of many species since their ecosystems change. Surface temperatures would stabilize and even decline some if emissions were cut off, but other impacts will continue in next decades, including rising sea levels from melting ice sheets, rising ocean temperatures, and ocean acidification, caused by elevated levels of carbon dioxide. Moreover, some impacts, such as loss of snow cover,

increased water vapor, water vapor, cause effects that further increase the rate of global warming. Mitigation efforts to address global warming include the development and deployment of policies to reduce fossil fuel emissions, reforestation, forest preservation.

Thanks to the effort by the scientists, we now have a good idea of what our future climate might look like. The challenge is now for ecologists to tell us the consequences of these projected changes in climate for our flora and fauna. In particular, plant and animal physiologists and ecologists are focusing on this topics (Parmesan and Yohe, 2003; Pounds and Puschendorf, 2004). Part of these studies are addressed to clarify how climate change will affect phytophagous insects and their communities (Coviella and Trumble, 1999; Lawton, 2000; Menéndez, 2007).

How temperature affects living organisms

Temperature affects living organisms because of it influencing all the aspects of the physical environment and governs any process involving energy flows, setting boundaries for all living species. Probably, due to its pervasive effects on living organisms, temperature is the most studied and measured abiotic factor (Johnston and Bennet, 1996).

The effect of temperature on animal biology drive relationships between body temperature, performance and fitness over a thermal range (Chown and Nicolson, 2004; Terblanche, 2007). For instance, in the case of insects, the increase in growth rate with higher temperatures can allow them to complete more generations per year and achieve higher population growth rates (Fraizer *et al*., 2006). Over an ample range of body temperatures, enzyme-catalyzed chemical reactions become faster with increasing temperature. Above a certain thermal threshold, performances no longer increase with temperature, but reaches a plateaus and then declines. For this reason, temperature-performance relationships are known as thermal performance curves (Huey and Kingsolver, 1989; Angilletta, 2009). Figure 1 illustrates how the relationship between body temperature (*Tb*) and performance in ectothermic organisms is bounded by their critical limits. Maximal performance occurs at an optimal body temperature, and the thermal performance breadth is the range of *Tb* permitting a certain level of performance (Huey and Stevenson, 1979). Performance curves may vary in function of acclimation, changing in position or shape, but the extent of thermal acclimation is often rather modest.

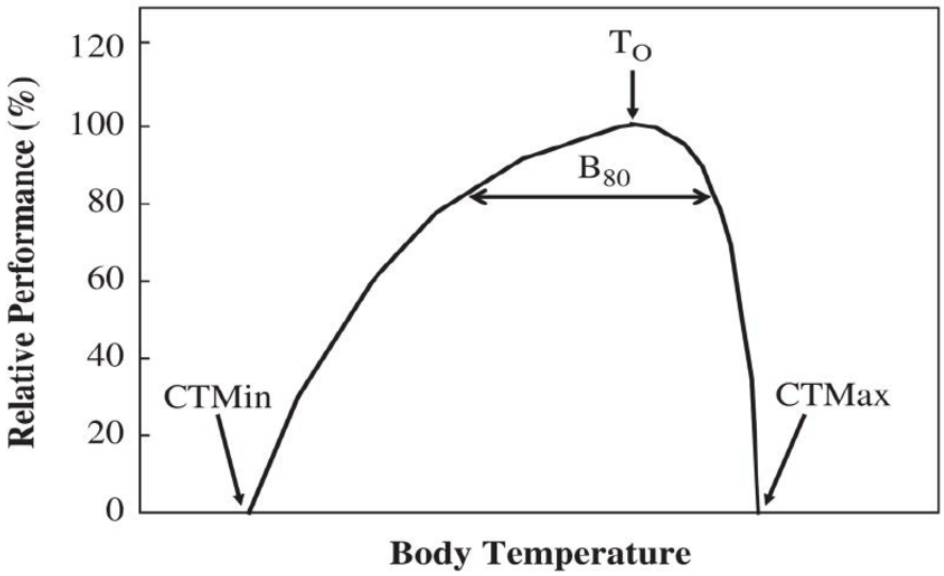

Fig. 1 - Relationship between body temperature and performance in ectotherms, showing the optimum temperature performance (*T0*), the 80% performance breadth (B_{80}), and CT_{min} and CT_{max} (Chown and Nicolson, 2004).

Thermoregulation: general aspects and how it works in insects

Thermoregulation is the ability of an organism to keep its body temperature within certain boundaries, even when the surrounding temperature is very different. Thermoregulation in organism runs along spectrum from endothermy o ectothermy. The internal thermoregulation process is an aspect of homeostasis, that is a state of dynamic stability in an organism maintained far from thermal equilibrium (Johnston and Bennet, 1996). Thermoregulation depends on the ability to control the amount of heat stored in the body. Ectotherms rely on an external source of heat, usually the sun. Then, in this case, the response is basically based on behavioral strategies. Endothermics generate most of the necessary heat internally as a by-product of metabolic processes. Thermoregulatory mechanisms involve either changes in metabolic heat production or in heat exchange with the environment. Heat exchange takes place by conduction, convection, radiation, and evaporation. In brief, rates of conduction, convection, and radiation depend on the difference between the temperature of the surface of the organism and that of its surroundings: surface area, orientation and geometry, texture, and, in the case of radiation, color of the organism (Bakken and Gates, 1975; Gates, 1969; Porter and Johnston and Bennet, 1996). Heat transfer routes of convention and radiation are the most important, because conduction will be negligible when only tarsi are in contact with the substrate, metabolic heat production is insignificant in small insects, and evaporation is costly and generally assumed to play a minor role. Behavioral mechanisms depend on the possibility to find thermal diversity in the time and the space (Stevenson, 1985). The significance of microclimate, that is the climate of the microhabitat, depends on the moisture

(Willmer, 1982). In particular, the critical parameters are temperature and moisture content of the air. Climate can be modified when vegetation is transpiring and so provides a slow air movement.

Thermoregulation and thermal effects have been one of the main topics in the field of functional biology studies in the last century (Cossin and Bowler, 1987). The importance of thermal biology arises from its relevance for biological systems. In fact, temperature has strong effects on many biological rate processes. Population-level traits that depend on those processes, such as growth and reproduction, may be affected by thermal changes. Biological systems have evolved in and adapted to almost every thermal environment on earth. Individual organisms and their offspring encounter a thermal range during their life cycle varying from daily cycles to seasonal cycles, or long term climate variations (Johnston and Bennet, 1996).

Since many insect species are ectotherms, their life histories unfold only if their body temperatures fall within a range that permits the indispensable vital functions. Insect have evolved an ample array of adaptations, both physiological and behavioral. Insects often experience extreme temperatures that, in the absence of specific mechanisms of thermoregulation, can dangerously injure or kill them. As with all aspects of insect environmental physiology, duration of exposure to thermal variation has effects on the patterns and responsive mechanism to the thermal stress (Harrison *et al*., 2012).

Insects are ectotherms, they principally respond in behavioral way to stressful thermal conditions. In most insects, the exclusive source of metabolic heat of thermoregulatory significance is flight muscle, although walking may elevate body temperature slightly (Stower and Griffiths, 1966). Resting metabolism of insects produces measurable heat, but its magnitude is too small to be important in thermoregulation. Insects have evolved several behaviors to regulate body temperature (May, 1979). Some insects precede flight, and certain other activities, with a period of low-amplitude wing vibrations, called wing-whirring or wing-shivering, or with bouts of high-pitched buzzing or deep abdominal pumping movements (Leston *et al*., 1965; Barber and Pringle, 1966; Kammer, 1970; May, 1979). The behavior is accompanied by a rapid rise in thoracic temperature, but usually by little change in abdominal temperature (Heinrich and Bartholomew, 1971). Most insects actively avoid excessive heat by migrating to a suffer microhabitat (Cloudsley-Thompson, 1970). Sometimes, many insects can be "trapped" by wandering into regions so cold that movement is inhibited, and often peaks of distribution appear in gradients for this reason (Chapman, 1965), or apparent preference may shift downward over time (Prince and Parsons, 1977).

Insect responses to heat stress injuries and related costs

Insects have evolved different physiological strategies to cope with heat stress injuries (Neven, 2000; Wernegreen, 2012). Induction of heat shock proteins (HSPs), a polyfunctional group of proteins conserved in all organisms (Morimoto and Tissières, 1994), is a common and effective physiological response to thermal stress in insects (Hoffmann *et al*., 2003; Zhao and Jones, 2012). Since HSPs are synthesized when environmental temperature exceeds the temperature optimum of the organism, they are considered as a short-term reparative response to thermal stress (Ritossa, 1962; Parsell and Lindquist, 1993; Bahrndorff *et al*., 2009).

HSPs were first discovered in 1962 by Ritossa, and they were described as a set of proteins whose expression is induced by heat shock and a variety of other stresses. There is substantial evidence that HSPs play important physiological roles in normal conditions and situations involving both systemic and cellular stress. Most heat shock proteins have strong cytoprotective effects, are implicated in several regulatory pathways, and behave as molecular chaperones (Kregel, 2002). In living organisms, a part from heat stress injuries, HSPs play a significant role in the response to several abiotic stressors, such as: ultraviolet radiation (Rangel *et al*., 2008, Nguyen *et al*., 2009), drought and dehydration (Cornette *et al*., 2010; Cornette and Kikawada, 2011), anhydrobiosis (Gusev *et al*., 2010, Cornette *et al*., 2010; Cornette and Kikawada, 2011), metal (Shu *et al*., 2010), nutrients (Benoit *et al*., 2011), and hypoxia (Michaud *et al*., 2011).

Moreover, HSPs play an important role also in insect responses to biotic stressors, such as the stress induced by parasitic species in their hosts. For instance, the envenomation of the moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) by the ectoparasitoid *Bracon hebetor* (Say) (Hymenoptera: Braconidae), determines the upregulation of the genes codifying for the heat shock proteins in the body of the parasitized moth (Shim *et al*., 2008), which may produce potent factors having indispensable roles in the defensive physiological mechanisms of the host and of the parasitoid.

Temperature effects on insect life history traits

Life history theory is an analytical framework designed to study the diversity of life history strategies of living species. A life history encompasses the life of individuals from its birth to its death, describing the specific patterns of reproduction, survival, and death (Flatt and Heyland, 2011). Life history traits quantitatively represent the properties of organisms that are directly related to the two major components of fitness: survival and reproduction. These traits essentially represent the demographic parameters required to estimate fitness as

defined by the Malthusian parameter. The Malthusian parameter, also known as the "instantaneous rate of natural increase" is the solution to the Euler-Lotka equation, which describes population growth by considering reproductive events and survival probabilities over the individual lifetime (Stearns, 1992). Thus, these two important life history traits are directly related to fitness, with fitness being defined by population growth models.

A key postulate of life history theory informs that life history traits are limited by factors internal to the organism, namely trade-offs (Flatt and Hayland, 2011). A life history trade-off occurs when an increased investment in one fitness component causes an investment reduction in another fitness component. This is due to the fact that energetic reserves of organism are limited, and, consequently, two or more traits cannot be optimized at the same time. A common example of trade-off is that between reproduction and survival (Stearns, 1992), that is the bedrock of the evolutionary theory of ageing (Kirkwood, 1977; Kirkwood and Austad, 2000).

Theory of ageing (Kirkwood, 1977; Kirkwood and Austad, 2000) focuses on the fact that somatic maintenance requires a complex machinery to active and sustain a large number of physiological mechanisms, which maintain the functionality of cells and tissues. Trade-off between reproduction and longevity is central to life-history theory (Williams, 1966; Gadgil and Bossert, 1970; Stearns, 1992; Roff *et al*., 2002), and it basically consists in a heavy investment in current reproduction to exhibit low levels of survival and future reproduction. Physiological explanations for this trade-off are typically based on the assumption that these two fitness components compete for limited energy and nutrients (Reznick, 1992; Zera and Harshman, 2001; Harshman and Zera, 2007). In particular, it happens that reproduction diverts resources away from cellular reparative mechanisms, so that survival and future fertility are subsequently lowered (Partridge and Harvey, 1985).

In literature there are several studies reporting the trade-off between aphid longevity and reproduction (Dixon and Kundu, 1997; Snell and King, 1977; Ward *et al*., 1983). Ward *et al*., 1983 demonstrated that in the vetch aphid *Megoura viciae* Buckton an increase in reproduction investment reduces aphid survival, both on the host plants and without feed/nutriment. Moreover, they demonstrated the marginal cost of reproductive investment. Dixon and Kundu (1997) reported that reproductive investment reduces the longevity in the carrot aphid *Cavariella aegopodii* Scop, both in female and male.

To allow an adequate function of the organism (i.e., to ensure survival and reproduction), both during and after a thermal stress, energetic resources can be differently allocated between the life history traits, then, trade-offs are triggered (Hercus *et al*., 2003). Once an organism has ensured its survival after a thermal stress, its metabolism and other

physiological features are altered (Brown *et al*., 2004) and energetic resources will be allocated to the different life history traits to optimize its fitness (reviewed in Roff *et al.,* 2002; West-Eberhard, 2003). Under thermal stressful conditions, insect longevity and reproduction could be affected, and trade-off between these life history traits could be displayed.

Reproductive output is an important component of insect fitness, and it is a valid trait to evaluate the costs associated to stressful thermal conditions in aphids (Zhou and Carter, 1992; Ma *et al*., 2004; Mehrparvar and Hatami, 2007;). Many laboratory studies on insect thermal biology observed the effects of high temperature on aphid fecundity by estimating the intrinsic rate of increment (r_m) (Zhou and Carter, 1992; Ma *et al*., 2004; Mehrparvar and Hatami, 2007). Intrinsic rate of increment is a common measure of aphid fitness considering nymph production during a period of time equal to the pre-reproductive period (Wyatt and White, 1977). For many aphid species reared in laboratory at controlled thermal conditions, the pre-reproductive period ranges from few days to some weeks. Intrinsic rate of increment is commonly considered a valid tool to estimate aphid fecundity (Pons and Tatchell, 1995; Asin and Pons, 2001).

Effects of high and extreme temperatures on the ecology of phytophagous insect species

Phytophagous insects are those able to feed on plants. They are highly diverse and the total species number is at least 500,000 (Bernays, 2009). All green plants are eaten by one or more species of phytophagous insects. The great diversity of insects feeding on plants is matched by a remarkable diversity of lifestyles, mouthparts and gut morphological adaptations to the food eaten, cuticular morphology and coloration adapted for crypsis or aposematism, and behavioral adaptations for use of particular plants and escape from natural enemies. Phytophagous insects are commonly distinguished in three wide categories: monophages, oligophages and poliphages. Commonly, species that use only one plant genus or species are called monophagous, and species that use plants within a tribe or family are called oligophages, species that use plants of several botanical families are called poliphages (Bernays, 2009).

For phytophagous insect species, the ability to complete their life cycle represents a successful adaptation to both their host plant and to the climatic environment in which they live (Bale *et al*., 2002). To understand the effects of rising temperature on phytophagous insects, it is necessary to consider that phytophagous insect species generally employ one of six life-history strategies (Bale *et al*., 2000), that are:

1) In some phytophagous species, life cycle may run over several years with growth and development dependent on climatic conditions, above all on temperature (Kukal and Kevan, 1987). Temperature range favorable for growth differs among species and is often related to climatic range and habitat. Within this temperature range, temperature elevation increases the speed of development during the growth phase but the rate of increase differs between species (Butterfield and Coulson, 1997).

2) Phytophagous insect growth and development are controlled by environmental cues, starting and stopping in synchrony with seasonal changes. The life cycle can go through more than one stop-go cycle but each time the cycle length is synchronized and of a fixed duration (Miles *et al*., 1998).

3) The host plant is only available as a food resource for a limited interval within a growing season. Then, close synchrony of the insect life cycle with plant phenology is vital and usually there is only a single generation per year. Moreover, the seasonal cycle may be linked to moisture availability or temperature (Dennis, 1993).

4) The host plant remains suitable for continuous development throughout a limited growing period, allowing two or more insect generations per season. Rates of development vary within season, depending on temperature and host plant condition (Bryant *et al*., 1997; Dixon 1998).

5) When the host plant becomes unsuitable the insect moves on to a neighboring plant. In temperate regions, this category includes oligophagous and polyphagous insects that can exploit a seasonal progression of different host plant species, such as heteroecious aphids, that switch between host plants (Dixon, 1998).

6) Development may be continuous on a single host in a non-seasonal environment, with several generations per year. This is the case of many phytophagous insects of tropical forests. A further variation on this theme in seasonal environments are species that may be adapted to pass through unfavourable periods in any one of the immature or adult stage. In these cases, the life cycle also usually includes a seasonal synchronization mechanism such as diapause or photoperiodic inhibition of development (Hill and Hodkinson 1996; Gomi, 1997; Miles *et al*., 1998).

Bale *et al*. (2002) argued that temperature variations due to global warming could strongly affect growth rate and diapause requirements of insects. Moreover, increasing temperatures during the cold season will also result in a greater ability to overwinter in insect species limited by low temperatures, so favoring larger spring populations and increasing pest outbreaks. Then, consequently to these variations, temperature will also affect insect life history traits, and the dynamics and the structure of their populations (Musolin *et al*., 2010). Fast growing, non-diapausing species or those which are not dependent on low temperature to induce diapause, will respond to warming by expanding their distributions. In contrast, slow growing species which need low temperatures to induce diapause (such as boreal and mountain species in the northern hemisphere) will suffer range contractions. Thus, climate change will affect species ranges, with expansion in some species and contractions in other species, which in turn will lead to changes in regional and local diversity (Menéndez, 2007). Climate change can indirectly affect insect species, where the insect responds to climate-induced changes mediated by other factor. These factors may include interactions with other species, such as competition for shared resources, predation and parasitism. Moreover, in the case of phytophagous insects, such as aphids, climate changes could affect them through alterations of the host plant (Menéndez, 2007).

It is expected that climate change will affect the structure of existing communities because individual responses will inevitably alter species interactions, leading to changes in the composition of natural communities. Predictions inform that climate changes will determine phenological alteration (Root *et al*., 2003, Root and Hughes, 2005), distributional shifts (Andrewartha and Birch, 1954; Hughes, 2000), evolutionary changes (Thomas, 2005; Parmesan, 2006), alteration of the trophic interaction (Harrington *et al*., 1999), and, in some species, it is possible that the extinction will occur (Thomas *et al.*, 2004).

Phenological changes are well documented, and have been detected for a wide range of organisms from plants to vertebrates (Root *et al*., 2003, Root and Hughes 2005). Thermal increment may affect the relative growth rates and phenological synchrony of phytophagous insects and plants. It is well known that plant growth and seasonal availability strongly influence the life history strategy of insects associated with them, and that insect life-cycle completion in many host species requires close synchrony with host phenology (Gomi, 1997; Miles *et al.,* 1998). For instance, plant only be available for insect development for a relatively short time period during its growth stage. As climate becomes less favorable for insect development, synchrony becomes a more critical feature and particular insects become progressively less successful at completing their life cycle on specific host plants. Thermal increment may alter exploitation of the host plant by phytophagous insects, as

consequences of phenological alterations. Future climatic warming will also affect temperate annual and multivoltine species in different ways and to differing degrees. In the case of multivoltine species, such as the Aphididae, higher temperatures should allow shorter development times, probably allowing additional generations within a year (Pollard and Yates, 1993).

Empirical evidence show that under a rise in temperature insects will pass through their immature stages faster and will arrive at the adulthood earlier. This comports both an advance in the timing of adult emergence and an increase in the length of the flight period. For instance, in the case of aphids, it is expected an early adult emergence and an early arrival of migratory species on the crop (Zhou *et al*., 1995). Phenological responses are likely to be widespread in the insects at higher latitudes and elevations, where temperature has increased and is predicted to increase more than in other regions (Houghton *et al.,* 2001).

Increasing temperature also determines shift of geographic range for many insect species (Andrewartha and Birch, 1954). Also, it is possible that some species could disappear from areas that will become climatically unsuitable (Hughes, 2000). Under changing climatic conditions, spatial shifts in distribution of crops will also influence the distribution of insect pests (Walther *et al*. 2002; Elphinstone and Toth, 2008). Then, global warming could also affect the insect species infesting crop systems and determine economical losses. Distributional shifts are strictly related to evolutionary changes. In fact, colonization of new areas is possible if the expanding populations will contain genotypes that are more successful as colonizers (Haag *et al*., 2005).

Under an evolutionary point of view, global warming will alter selection pressures within populations because most populations are to a reasonable degree adapted to their local environment. Thus, traits that confer high fitness in the existing climate might not be as successful in the new climatic conditions, so evolutionary responses might take place. The potential of evolutionary changes is great in entomological species. This is due to their short generation times and high reproductive rates. These features may allow them to rapidly adjust to the environmental conditions of the new colonized area (Thomas, 2005; Parmesan, 2006).

Changes in phenological patterns and distributions of species alter species interactions within communities. Since the intensity of response differs among species, to predict the potential disruption of the equilibrium between species is difficult. Interactions that involve two or more trophic groups, such as plant-herbivore and host-parasitic interactions, are likely to suffer these changes (Harrington *et al.,* 1999). Visser and Both (2005) reviewed the phenological changes of interacting species. Some of the examples reported in their study

refer to insects, and confirm that phenological changes imposed by climate change have resulted in a mismatch between interacting species.

Distributional changes can also bring to changes in species interactions. In particular, this is due to the fact that species are expanding at different rates and expanding species will begin to overlap with others with which previous interaction may have been limited or nonexistent, as was the case of the pine processionary moth and the Andalusian Scots pine (Hódar and Zamora, 2004). It is common idea that these mismatches could have detrimental consequences on the persistence of individual species within their distribution areas and, consequently, also on the biodiversity of the ecosystems.

Under a more tragic perspective, Thomas *et al.* (2004) reported that climate change will become a major factor involved in species extinction. This will occur if thermal change will be rapid, and it will affect species that are not able to adequately adjust their biological traits.

References

Andrewartha H.G., Birch L.C., 1954. The distribution and abundance of animals. University Chicago Press, Chicago.

Angilletta M.J., 2009. Thermal adaptation. A theoretical and empirical synthesis. Oxford University press, Oxford.

Asin L., Pons X., 2001. Effect of high temperature on the growth and reproduction of corn aphid (Homptera:Aphididae) and implication for their population dynamics on the northeastern Iberian peninsula. Environmental Entomology, 30(6): 1127-1134.

Bahrndorff S., Mariën J., Loeschcke V., Ellers J., 2009. Dynamics of heat-induced thermal stress resistance and Hsp70 expression in the springtail *Orchesella cincta*. Functional Ecology, 23: 233-239.

Bakken G.S., Gates D.M., 1975. Heat-transfer analysis of animals: some implications for field ecology, physiology, and evolution. Perspectives of Biophysical Ecology: 255-290.

Bale J.S., Masters G.J., Hodkinson I.D., Awmack C., Bezemer T.M., Brown V.K., Butterfield J., Buse A., Coulson J.C., Farrar J., Good J.E.G., Harrington R., Hartley S., Jones T.H.,

Lindroth R.L., Press M.C., Symrnioudis I., Watt A.D., Whittaker J.B., 2002. Herbivory in global climate change research: direct effect of rising temperature on insect herbivores. Global Change Biology, 8: 1-16.

Ballester J., Douville H., Chauvin F., 2009. Present-day climatology and projected changes of warm and cold days in the CNRM-CM3 global climate model. Climate Dynamycs, 32: 35-54.

Barber S.B., Sutton Pringle J.W., 1966. Functional aspects of flight in belostomatid bugs (Heteroptera). Biological Science: 164(994).

Benoit J.B., Lopez-Martinez G., Patrick K.R., Phillips Z.P., Krause T.B., Denlinger D.L., 2011. Drinking a hot blood meal elicits a protective heat shock response in mosquitoes. Proceedings of the Natural Academy of Science of USA, 108: 8026-8029.

Bernays E. A., 2009. Phytophagous Insects. Encyclopedia of Insects (Second Edition), 798-900.

Brown J.H., Gillooly J.F., Allen A.P., Savage V.M., West G.B., 2004. Toward a metabolic theory of ecology. Ecology, 85: 1171-1789.

Bryant S.R., Thomas C.D., Bale J.S., 1997. Nettle-feeding nymphalid butterflies: temperature, development and distribution. Ecological Entomology, 22, 390±398.

Butterfield J., Coulson J.C., 1997. Terrestrial invertebrates and climate change: physiological and life-cycle adaptations. In: Past and Future Rapid Environmental Change (eds Huntley B, Cramer W, Morgan AV, Prentice HC, Allen JRM), Springer, Berlin, 401-412.

Chapman R.F., 1965. The behaviour of nymphs of Schistocerca gregaria (Forskål) (Orthoptera, Acrididae) in a temperature gradient, with special reference to temperature preference. Behaviour, 283-317.

Chown S.L., Nicolson S.W., 2004. Insect Physiological Ecology: mechanism and patterns. Oxford University Press.

Cloudsley-Thompson J.L. 1970: Terrestrial Invertebrates. In Whittow G.C. (ed.): Comparative Physiology of Thermoregulation. Vol. I. Academic Press, New York & London,15–77.

Cornette R, Kanamori Y, Watanabe M, Nakahara Y, Gusev O, Mitsumasu K, Kadono-Okuda K., Shimomura M., Mita K., Kikawada T., Okuda T., 2010. Identification of anhydrobiosis-related genes from an expressed sequence tag database in the cryptobiotic midge Polypedilum vanderplanki (Diptera; Chironomidae). Journal of Biology and Chemistry, 285: 35889- 35899.

Cornette R., Kikawada T., 2011. The induction of anhydrobiosis in the sleeping chironomid: current status of our knowledge. International Union of Biochemistry and Molecular Biology Life, 63: 419-429.

Cossins A.R., Bowler K., 1987. Temperature biology of animals. Chapman and Hall, London.

Coviella C.E., Trumble J.T., 1999. Effects of elevated atmospheric carbon dioxide on insect–plant interactions. Conservation Biology, 13, 700–712.

Danilevskii A.S., 1965. Photoperiodism and seasonal development of insects. Edimburg.

Dennis R.L.H., 1993. Butterfies and Climate Change. Manchester. University Press, Manchester.

Diffenbaugh N.S., Field C.B., 2013. Changes in ecologically critical terrestrial climate conditions. Science, 341(6145):486-492.

Diffenbaugh N.S., Robert J.S.P., Trapp R.J., Giorgi F., 2005. Fine-scale processes regulate the response of extreme events to global climate change. Proceedings of the National Academy of Science of the United States of America, 102(44): 15774-15778.

Dixon A.F.G., 1998. Aphid Ecology, An Optimisation Approach. 2nd ed. Chapman & Hall, Glasgow and London.

Dixon A.F.G., Kundu R., 1997. Trade-off between reproduction and length of adult life in males and mating females of aphids. European Journal of Entomology, 94: 105-109.

Elphinstone, J. and Toth, I.K., Erwinia chrysanthemi (Dikeya spp.), 2008. The facts. Potato Council, Oxford, U.K.

Flatt T., Heyland A., 2011. Mechanisms of Life History Traits. Oxford University Press.

Fraizer M.R., Huey R.B., Berrigan D., 2006. Thermodynamic constrains the evolution of insect population growth rate: "Warmer is better". American Naturalist, 168:512-20.

Gadgil M.W., Bossert. H., 1970. Life Historical Consequences of natural Selection. The American Naturalist, 104(935): 1-24.

Gomi T., 1997. Geographic variation in critical photoperiod for diapause induction and its temperature dependence in Hyphantria cunea Drury (Lepidoptera: Arctiidae). Oecologia,111, 160±165.

Gusev O., Cornette R., Kikawada T., Okuda T., 2010. Expression of heat shock protein-coding genes associated with anhydrobiosis in an African chironomid Polypedilum vanderplanki. Cell Stress Chaperones, 16: 81-90.

Harrington R., Woiwod I., Sparks T., 1999. Climate change and trophic interactions. Trends in Ecology and Evolution, 14: 146–150.

Haag, C. R., M. Saastamoinen, J. H. Marden, and I. Hanski. 2005. A candidate locus for variation in dispersal rate in a butterfly metapopulation. Proceedings of the Royal Society B-Biological Sciences 272:2449-2456

Harrison J.F., Woods A.H., Roberts S.P., 2012. Ecological and Environmental Physiology of Insects, Oxford University Press.

Harshman L.G., Zera A.J., 2007. The cost of reproduction: the devil in the details. Trends in Ecology & Evolution, 22(2):80-85.

Heinrich B., Bartholomew G.A., 1971. An analysis of pre-flight warm-up in the Sphinx Moth, Manduca Sexta. Journal of Experimental Biology, 55:223-239.

Hercus M.J., Loeschcke V., and Rattan S.I.S., 2003. Lifespan extension of Drosophila melanogaster through hormesis by repeated mild heat stress. Biogerontology. 4(3): 149-156.

Hill J.K., Hodkinson I.D., 1996. Effects of photoperiod and raised winter temperatures on egg development and timing of oviposition in the willow psyllid Cacopsylla moscovita. Entomologia Experimentalis et Applicata, 78, 143-147.

Jodar J.A., Zamora R., 2004. Herbivory and climatic warming:a Mediterranean outbreaking caterpillar attacks a relict, boreal pine species. Biodiversity and Conservation, 13, 493-500.

Hoffmann A.A., Sørensen J.G., Loeschcke V., 2003. Adaptation of Drosophila to temperature extremes: bringing together quantitative and molecular approaches. Journal of Thermal Biology 28: 175–216.

Houghton J.T., Ding Y., Griggs D.J., Noguer M., van der Linden P.J., Xiaosu D., Maskell K., Johnson C.A., 2001. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge.

Huey R.B., Kingsolver J.G., 1989. Evolution of thermal sensitivity of ectotherm performance. Trends in Ecology and Evolution, 4:131-135.

Huey R.B., Stevenson R.D., 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. American zoologist, 19(1): 357-366.

Hughes L., 2000. Biological consequences of global warming: Is the signal already apparent? Trends in Ecology and Evolution, 15: 56–61.

IPCC, 2018. Summary for Policymakers. In: Masson-Delmotte V., P. Zhai, H.O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R. Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, Maycock, M. Tignor, T. Waterfield (eds.). Global Warming of 1.5°C. World Meteorological Organization, Geneva, Switzerland, pp. 32.

Johnston I.A., Bennett A.F., 1996. Animals and temperature: phenotypic and evolutionary adaptations. Cambridge.

Kammer A.E., 1970. Thoracic temperature, shivering, and flight in the Monarch Butterfly, Danus plexippus (L.). Z- vergl. Physiologie, 68, 334-334.

Kirkwood T.B.L., 1977. Evolution of Ageing. Nature, 301-304.

Kirkwood T.B.L., Austad. S. N, 2000. Why do we age? Nature, 408, 233-238.

Kregel C.K., 2002. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. Journal of Applied Physiology, 92: 2177-2186.

Kukal O., Kevan P.G., 1987. The influence of parasitism on the life history of a high Arctic insect, Gynaephora groenlandica (Woecke) (Lepidoptera: Limantriidae). Canadian Journal of Zoology, 65, 156-163.

Lawton J.H., 2000. Community Ecology in a Changing World. Ecology Institute, Luhe, Germany.

Leston, D., Pringle, J.W.S., White, D.C.S., 1965. Muscular activity during preparation for flight in a beetle. Journal of Experimental Biology, 42, 409-414.

Ma C., B. Hau, H. Poehling, 2004. The effect of heat stress on the survival of the rose grain aphid, Metopolophium dirhodum (Hemiptera: Aphididae). European Journal of Entomology, 101:327-331.

Marengo J.A., Jones R., Alves L.M., Valverde M.N., 2009. Future change of temperature and precipitation extremes in South America as derived from the PRECIS regional climate modeling system. International Journal of Climatology, 29(15): 2241-2255.

May M.L., 1979. Insect termoregulation. Annual Reviews of Entomology, 4: 313-349.

Meehl G.A., Zwiers F., Evans J., Knutson T., Mearns L., Whetton P., 2000. Trends in extreme weather and climate events: issue related to modeling extremes in projections of future climate change. Bulletin of American Meteorological Society, 81(3):427-436.

Mehrparvari M., Hatami B., 2007. Effect of temperature on some biological parameters of an Iranian population of the Rose Aphid, Macrosiphum rosae (Hemiptera: Aphididae). European Journal of Entomology, 104: 631–634.

Menéndez R, 2007. How are insects responding to global warming? Tijdschrift voor Entomologie, 150: 355-365.

Michaud M.R., Teets N.M., Peyton J.T., Blobner B.M., Denlinger D.L., 2011. Heat shock response to hypoxia and its attenuation during recovery in the flesh fly, Sarcophaga crassipalpis. Journal of Insect Physiology, 57: 203-210.

Miles J.E., Bale J.S., Hodkinson I.D., 1998) Life cycle regulation in the heather psyllid Strophingia ericae: responses to temperature and photoperiod. Physiological Entomology, 23, 376-381.

Morimoto R. I., A. Tissieres C., 1994. The Biology of Heat Shock Proteins and Molecular Chaperones. Cold Spring Harbor Laboratory Press, New York.

Musolin D.L., Tougou D., Fujisaki K., 2010. Too hot to handle? Phenological and life-history responses to simulated climate change of the southern green stink bug Nezara viridula (Heteroptera: Pentatomidae). Global Change Biology, 16, 37–8

Neven L.G., 2000. Physiological responses of insect to heat. Postharvest Biology and technology, 21(1), 103-111.

Nguyen T.T., Michaud D., Cloutier C., 2009. A proteomic analysis of the aphid Macrosiphum euphorbiae under heat and radiation stress. Insect Biochemical and Molecular Biological, 39: 20-30.

Parmesan C., 2006. Ecological and Evolutionary responses to recent climate change. Annual Review of Ecology, Evolution and Systematics 37: 637–669.

Parmesan C., Yohe G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature, 421,37–42.

Parsell D.A., Lindquist S., 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annual Review of Genetics, 27, 437–496.

Partridge L., Harvey P.H., 1985. Evolutionary biology: costs of reproduction. Nature, 316:20,12

Pollard E., Yates T.J., 1993. Monitoring Butterfies for Ecology and Conservation. Chapman & Hall, London.

Pons X., Tatchell. G.M., 1995. Drought stress and cereal aphid performance. Annals of Applied Biology, 126: 19-31.

Porter W.P., Gates D.M., 1969. Thermodynamic equilibria of animals with environment. Ecological Monographs, 39(3):227-244.

Pounds J.A., Puschendorf R., 2004. Ecology – clouded futures. Nature, 427, 107–109.

Prince G.J., Parson P.A., 1977. Adaptative behaviour of Drosophila adults in relation to temperature and humidity. Australian Journal of Zoology, 25(2): 285-290.

Rangel D.E., Anderson A.J., Roberts D.W., 2008. Evaluating physical and nutritional stress during mycelial growth as inducers of tolerance to heat and UVB radiation in Metarhizium anisopliae conidia. Mycological Research, 112: 1362-1372.

Reznik D., 1992. Measuring the costs of reproduction. Trend in Ecology and Evolution, 7:42-45.

Ritossa F., 1962 A new puffing patter induced by temperature shock and DNP in Drosphila. Experientia, 18: 571-573.

Roff D. A., 1992. The Evolution of Life Histories. Chapman and Hall, New York.

Roff D.A., Mostowy S., Fairbairn D.J., 2002. The evolution of tradeoffs: testing predications on response to selection and environmental variation. Evolution, 56:84–95.

Root T.L., J.T. Price, K.R. Hall, S.H. Schneider, C. Rosenzweig and J.A. Pounds,2003. Fingerprints of global warming on wild animals and plants. Nature 416: 626–629.

Root, T.L., Hughes L., 2005. Present and future phenological changes in wild plants and animals. In: T. Lovejoy, L. Hannah (Eds.), Climate Change and Biodiversity: 61–69. Yale University Press, New Haven.

Shim J.K., Ha D.M., Nho S.K., Song K.S., Lee K.Y., 2008. Upregulation of heat shock protein genes by envenomation of ectoparasitoid Bracon hebetor in larval host of Indian meal moth Plodia interpunctella. Journal of Invertebrate Pathology, 97: 306-309.

Shu Y., Du Y., Wang J., 2010. Molecular characterization and expression patterns of Spodoptera litura heat shock protein 70/90, and their response to zinc stress. Comparative Biochemistry and Physiology, 158A: 102-110.

Snell T.W., King. E.C., 1977. Lifespan and fecundity patterns in rotifers: the cost of reproduction. International Journal of Organic Evolution, 882-890.

Stearns S.C., 1992. The evolution of life histories traits. Oxford University Press, Oxforf.

Stevenson R.D., 1985. The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. The American Naturalist, 126, 3.

Stower W.J., Griffiths J.F., 1966. The body temperature of the desert locust (Schistocerca gregaria). Entomologia Experimentalis et Applicata, 9(2):127-178.

Terblanche S.J., Deere J.A., Clusella-Trullas S., Janion C., Chown. S.L., 2007. Critical thermal limits depend on methodological context. Proceedings of Royal Society B, 274, 2935–2942.

Thomas C.D., 2005. Recent evolutionary effects of cli-mate change. In: T. Lovejoy, L. Hannah (Eds.), Climate Change and Biodiversity: 75–90. Yale University Press, New Haven.

Thomas, C.D., A. Cameron, R.E. Green, M. Bakkenes, L.J. Beaumont, Y.C. Collingham, B.F.N. Erasmus, M. Ferreira de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A.S. van Jaarsveld, G.F. Midg-ley, L. Miles, M.A. Ortega-Huerta, A.T. Peterson, O.L. Phillips, S.E. Williams, 2004. Extinction risk from climate change. Nature, 427: 145–148.

Vermeulen S.J., Campbell B.M., Ingram J.S.I., 2012. Climate change and food system. Annual Review of Environmental and Resources, 37:195-222

Visser M.E., Both C., 2005. Shifts in phenology due to global climate change: the need for a yardstick. – Proceedings of the Royal Society of London B 272: 2561–2569.

Walther G.R., Post E., Convey P., Menzel A., Parmesan C., Beebee T.J.C., Fromentin J.M., Hoegh-Guldberg O., Bairlein F., 2002. Ecological responses to recent climate change. Nature, 416, 389-395.

Ward S.A., Wellings P.W., Dixon F.G., 1983. The effect of reproductive mortality in aphids. Journal of Animal Ecology, 52: 305-313.

Wernegreen J.J., 2012. Mutualism meltdown in insects: bacteria constrain thermal adaptation. Current Opionion in Microboiology, 15(3), 255-262.

West-Eberhard M.J, 2003. Developmental plasticity and evolution. Oxford University Press.

Williams G.C., 1966. Naturalist selection, the cost of reproduction, and a refinement of Lack's principle. The American Naturalist, 100, 916.

Willmer P.G., 1982. Microclimate and the environmental physiology of insects. Advances in insect physiology, 16:1-57.

Wyatt I.J., White P.F., 1977. Simple estimation of intrinsic increase rates for aphids and tetranychid mites. Journal of Applied Ecology. 757-766.

Zera A.J., Harshman L.J., 2001. The Physiology of Life History Trade-Off in Animals. Annual Review of Ecology and Systematics. 32:95-126

Zhao L., Jones W.A., 2012. Expression of heat shock protein genes in insect stress responses. ISJ, 9: 93-101.

Zhou X., Carter N., 1992. Effect of temperature, feeding position and crop growth stage on the population dynamics of the rose grain aphid, Metopolophium dirhodum (Hemiptera:Aphididae). Annals of Applied Biology, 121(1), 27-37.

Zhou X., Harrington R., Woiwod I.P., Perry J.N., Bale J.S., Clark S.J., 1995. Effects of temperature on aphid phenology. Global Change Biology, 1, 303-313.

THE STUDIED INSECT SPECIES

The insect species used in my studies are the pea aphid *Acyrthosiphon pisum* (Harris), the braconid parasitic wasp *Aphidius ervi* Haliday and the aphidophagous ladybird *Hippodamia variegata* (Goeze).

Biology of aphids and of the species interacting with them

Aphid taxonomy and morphology

Taxonomically, aphids belong to the superfamily Aphidoidea (Hemiptera: Sternorrhyncha) (Blackman and Eastop, 1984). In the context of the order Hemiptera, the superfamily Aphidoidea is placed in the suborder Sternorrhyncha, together with Coccoidea and Psylloidea. There are more than 5000 species of Aphididae in the world. Of these species, about 450 were recorded from cultivated plant species (Blackman and Eastop, 19842000), but only about 100 have exploited the agricultural environment successfully to the extent that they are of significant economic importance. Aphid species having economic importance are mostly in the subfamily Aphididae, not only because this is a very ample systematic group, but also because it contains a high proportion of species feeding on cultivated plant species, above all herbaceous ones. On the contrary, other subfamilies, such as Calaphidinae and Lachninae, are principally associated to woody plants (Blackman and Eastop, 1984).

Aphids have soft and small body, pear-like shape, thin and long antennas and legs. Aphids have compound eyes and ocular tubercle behind and above each eye, made up of three lenses, also called triommatidia (Dixon, 1985). At the caudal part of the body, two tube-like structures, which are called cornicles (or siphuncules), arising from the dorsal side of the fifth or sixth abdominal segment can be present. Aphids exude droplets of a defensive fluid through cornicles. This fluid containing triacylglycerols, commonly called cornicle wax, is a tool to inform the other aphids of the colony of natural enemy presence (McGavin, 1993). Aphids are sap-feeding insects. They feed on sap using sucking mouthparts called stylets, enclosed in a sheath (*rostrum*), which are formed from modifications of the mandibles and maxilla of the insect mouthparts. Aphids passively feed on sap from phloem vessels. Occasionally they can also ingest xylem sap (Spiller *et al*., 1990). In some aphid species, the body can be covered with white waxy exudates, secreted by dermal glands. Adult aphids can be winged or wingless, a situation known as wing dimorphism. The occurrence of wings is associated with the necessity of migration to another host plant and have a relevant metabolic cost (Roff, 1986).

As above mentioned, some aphid species are considered pest. In fact, they can damage cultivated plant species in different ways. Damages may be due to sap subtraction to tissues when they feed and to saliva toxicity. Moreover, many species are able to transmit phytoviruses. Aphid plant damages are also due to honeydew excretion, a sticky substance basically composed of excess sap, to which excess sugar and waste material are added before to be expulse (Tremblay, 1995).

Aphid reproduction

In female aphids, the genital tract is formed by two ovaries. Each ovary is formed by ovarioles, and each ovariole is a chain of different follicoles with a germarium at the top, followed by a vitellarium. The germarium contains the future oocytes, and nurse the cell or trophocytes. In the Fig.1 details of the female aphid reproductive apparatus are reported.

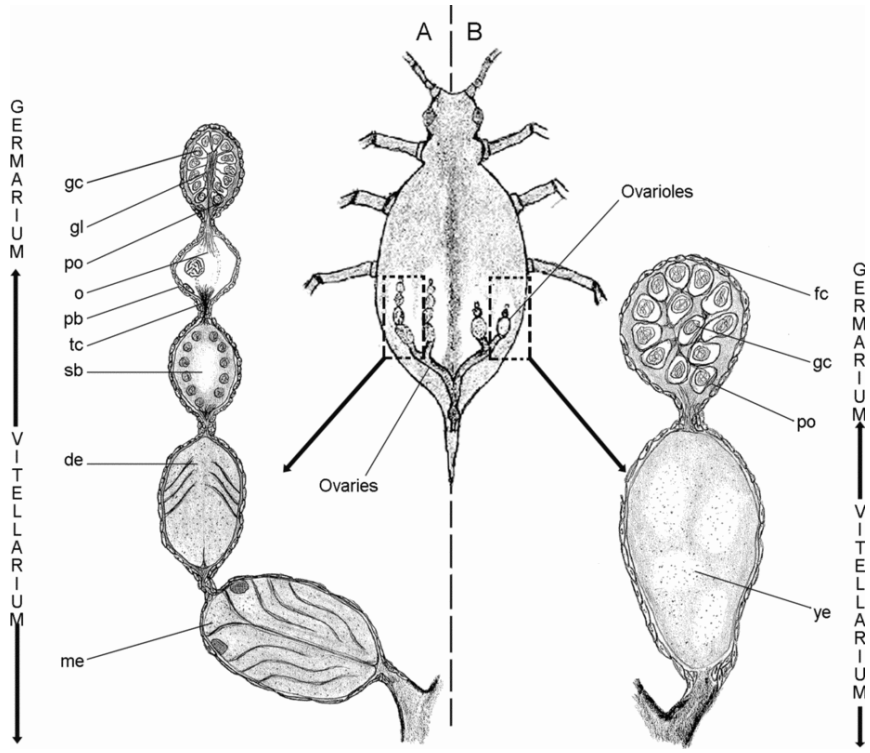

Fig.1 - Aphid reproductive apparatus: (A) ovariole of a parthenogenetic viviparous female. (B) Ovarioles of an oviparous female. (de) developing embryo, (fc) follicle cells; (gc) germarium cell; (gl) germarium lumen; (me) mature embryo; (o) oocyte; (pb) polar body; (po) preoocyte; (sb) syncytial blastoderm; (tc) trophic cord; (ye) yolky egg. Re-elaborated by Trionnaire *et al*., 2008.

One of the principal features of aphids, the parthenogenetic reproduction, sets them apart from other Hemiptera. This feature strongly influenced other aspects of their biology and ecology. Viviparous parthenogenesis reproduction is among the main factors allowing aphid populations to increase very fast (Miura *et al*., 2003; Brisson and Stern, 2006). The strategy of alternating a bisexual generation with a succession of parthenogenetic ones is a peculiarity

of aphids, allowing them to rapidly constitute abundant colonies, is an ancestral feature, probably as far back as Triassic. Generally, most aphid species alternate the reproduction type according with seasonal variations: in warm periods (spring and summer, principally) the reproduction is parthenogenetic and viviparous, and only females are produced. Generally, in autumn, a short photoperiod induces the production of males that fertilize the females and allow the production of overwintering eggs (Tremblay, 1995). The major part of aphids normally develop through four instars before reaching the adult stage (Dixon, 1998). Most Aphididae species have both winged and wingless morphs. Winged asexual female forms are produced in relation to environmental changes such as decreasing plant host quality or crowding, then aphids can colonize new host plants (Dixon, 1998; Brisson and Stern, 2006).

Aphid bacterial symbionts

Aphids can host different internal symbiotic species (Buchner, 1965; Guo *et al*., 2017). Interestingly, studies on aphid-symbionts interactions have been mostly carried out by adopting the pea aphid *A. pisum* as model species (Montllor *et al*., 2002; Skaljac *et al*., 2018). Aphids always host the bacterium *Buchnera aphidicola*, that is an obligate endocellular symbiont (Buchner, 1965). *Buchnera* provides the host with essential amino acids lacking in the aphid diet, and it is also involved in aphid thermal adaptation (Dunbar *et al*., 2007). This bacterium is particularly useful to aphids since phloem sap has low concentrations of many essential amino acids and cannot satisfy their nutritional exigencies (Douglas, 1998). It is well known that bacteria-free aphids grow poorly and produce few or no offspring, and *Buchnera* are unknown apart from aphids and apparently unculturable. *Buchnera* lives within large polyploid cells, called bacteriocytes, that are grouped into organ-like structures, called bacteriomes, located adjacent to the ovarioles. In the case of *B. aphidicola*, inheritance of bacterial symbionts is vertical and both the species mutualistically depend on each other (Douglas, 1993). When aphid embryos parthenogenetically develop from unfertilized diploid oocytes, and multiple embryos develop serially within a single ovariole (Dixon, 1985), the maternal bacteria are directly transferred to embryos at the developing blastoderm-stage through a posterior opening of the embryo (Buchner, 1965; Miura *et al*. 2003). In addition to *Buchnera*, aphids may host one or more bacterial symbionts that are not strictly necessary for their survival and reproduction but can influence aphid adaptation to specific environmental conditions (Oliver *et al*., 2010). Among the most important, there are *Hamiltonella defensa, Regiella insecticola* and *Serratia symbiotica.* In particular, *S. symbiotica* plays an important role in aphid tolerance to insecticides (Skaljac *et al*., 2018).

R. insecticola and *H. defensa* both confer resistance against the parasitization process of endoparasitic wasps (Oliver *et al*., 2008; Vorburger *et al*., 2010).

Aphid natural enemies

Aphids may be attacked by several pathogens (Hagen and Van Den Bosch, 1968). The most important pathogens, which can cause epidemics in aphid colonies, are fungi in the Entomophtorales (Barta and Cagáň, 2003). Moreover, aphids are subject to predation by several predator species (Hagen and Van Den Bosch, 1968). The most important predators are adult and larval stages of ladybird beetles (Coccinellidae), hoverfly larvae (Syrphidae), midge larvae (Cecydomyiidae), lacewing larvae (Neuroptera) and some heteropteran species such as mirid bugs (Miridae). Aphid endoparasitoids are chiefly braconids (Aphidiinae) or chalcidoids (Aphelinidae), both belonging to Hymenoptera (Hagen and Van Den Bosch, 1968; Müller and Godfray, 1999). Aphids are often parasitized by braconid and chalcidoid wasps. Parasitism is really common in insects, and many information on the host-parasite interactions are available in literature for insects (Vinson, 1990; Pennacchio *et al*., 2005). The pea aphid *A. pisum* and the braconid wasp *A. ervi* are often used as model species to study the aphid-parasitoid interaction under laboratory conditions (Digilio *et al*., 2000; Falabella *et al*., 2000, 2007, 2009).

For this particular case of aphid parasites, entomologists use the term "parasitoid" to refer to them. This term was coined by Waage and Greathea (1988) to describe a life history intermediate between that of predators and true parasites. They described parasitoid as those organisms whose females "are free-living, feed on nectar, pollen or as predator and forage actively for their arthropod hosts on plants and other substrates. Usually, on locating a host, the female lays one or more eggs in the body host, and the ensuring larvae consume the host tissues, killing the host in the process" (Eggleton and Gaston, 1990.)

By considering the physiological interaction type between host and parasitoid, it is possible to distinguish insect parasitoids in two ample categories: ideobionts and koinobionts. Idebionts are all those parasitoids that are used to paralyze, and eventually kill, the host. Koinobionts are all those parasitoids that allow the host to continue to feed and grow after parasitization for a certain/specific period of time. They have to cope with a higher degree of risks during their development, such as the host immune system (Vinson, 1990; Henter and Via, 1995). Since they live within their host, and strictly depend on it, an eventual death of the host will bring them to death. For parasitoids, the host represents the whole and unique nutritional nutrient font during the immature development. For this reason, female parasitoid evolved the ability to evaluate the host and "choice" if laying its eggs within host body or

not (Pennacchio *et al*., 1994; Larocca *et al*., 2007). Quality of the host may strongly affect many biological traits of the future adult (Vinson, 1990; Van Emden and Kifle, 2002; Colinet *et al*., 2005; Sampaio *et al*., 2008).

Aphid ecology

From an ecological point of view, many insects are considered r-strategists. Among the millions of insect species present in nature, Aphididae are a valid example of this category (Dixon, 1998). It is opportune to specify that, in ecology, species are often distinguished in two ample categories: r-strategist and k-strategist. Theory of r/k-selection refers to the selection of combinations of traits in an organism that trade-off between quantity and quality of offspring. This terminology was coined by the ecologists MacArthur and Wilson, in 1967. In particular, r-strategist species, also named r-selected species, are those that emphasize high growth rates, typically exploit less-crowded ecological niches, and produce many offspring. Among the traits that are thought to characterize r-selection are high fecundity, small body size, early maturity onset, short generation time, and the ability to disperse offspring widely. By considering these features, aphid perfectly correspond to the definitions of r-strategist. In fact, they are characterized by high offspring productivity, short lifespan and rapid arrive at the adulthood. These features confer aphid capability to rapidly form crowded colonies. On the contrary, k-strategist species include large body size, long life, and the production of fewer offspring, which often require extensive parental care until they mature (Pianka, 1970). Then, aphid population dynamic is based principally on their high reproductive potential (Rabasse and Steenis, 1999), and then also to their ability to parthenogenetically reproduce (Dixon, 1985).

The pea aphid **Acyrthosiphon pisum (Harris)**

Acyrthosiphon pisum (Harris) is the aphid species used in all the experimental studies reported in this thesis. It is Paleartic species, but now it has a worldwide distribution and is considered a pest of different legume species, such as broad bean, pea and alfalfa, vetch and red clover (Dixon, 1998). Sometimes, pea aphid races differ in coloration, they could be green and pink. Its host plants are mostly Fabaceae of the tribes Genisteae, Fabeae and Hedysareae, and also it is able to colonize a few number of plant species belonging to other tribes. *A. pisum* is a vector of more than 30 phytoviruses, including both persistent and non-persistent species affecting cultivated leguminous species, such as the red clover vein mosaic virus, bean yellow mosaic virus and pea streak virus (Dixon 1998; Brisson and Stern 2006). *A. pisum*, in cold regions is holocyclic, then it produces oviparous females and males. As in

other species of the genus *Acyrthosiphon*, there is not host alternation. In warmer regions, it overwinters without sexual phase (Dixon, 1998). In Europe and central Asia, *A. pisum* has been recognized as a complex of races and subspecies with different preference for the host plant. For instance, pea aphid populations infesting pea in Europe consists of green genotypes and have some morphological traits differing from those colonizing other leguminous species, such as alfalfa, which may be pink or green. These races produce alatae sexuparae and males in autumn, and have a sexual phase on plant of the genus *Vicia*. In some regions, the pea aphid can reach densities high enough to become a significant pest (van Emden and Harrington, 2003).

In nature, the pea aphid may be attacked by different aphidophagous species. Pons *et al*. (2009) reported that in alfalfa fields infested by the pea aphid, under low or no insecticide treatment regimes, it is possible to find more than 100 predatory species belonging to different orders, such as: Heteroptera (Anthocoridae, Nabidae, Miridae, Lygaeidae), Thysanoptera, Neuroptera (Chrysopidae, Hemerobiidae), Coleoptera (Coccinellidae, Carabidae, Staphylinidae, Cantharidae), Diptera (Syrphidae). In particular, heteropteran predators, commonly considered generalist, play an important role in the regulation of pea aphid infestations. In fact, they represent about 50–60% of the predators recorded in alfalfa fields (Pons *et al*., 2005). Moreover, several species of spiders and other Arachnida have been recorded (Núñez, 2002; Pons *et al*., 2005). Pons *et al*., (2011) reported that, in Catalogna (Spain), the parasitoid complex of *A. pisum* in alfalfa fields includes different Aphidiinae (*Praon barbatum* Mackauer*,* and *A. ervi)* and Aphelinidae (*Aphelinus abdominalis* Dalman and *Aphelinus semiflavus* Howard) species. In the above mentioned study, *A. ervi* resulted to be the most abundant among all parasitoid species, and it was considered to be a good agent for the control of the pea aphid in alfalfa.

A. pisum has been used as a model species in evolutionary, developmental and ecological researches (Snyder and Ives 2003; Brisson and Stern 2006; Shigenobu *et al.,* 2010; McLean *et al*., 2010. In particular *A. pisum* is often studied jointly with the braconid parasitoid *A. ervi* Haliday in experimental studies focusing on aphid-parasitoid interaction (Pennacchio *et al.*, 1994, 1995, 1999; Digilio *et al*., 2000; Falabella *et al*., 2000, 2007, 2009). *A. pisum* genome has been completely sequenced and published (Eisen and Eisen, 2010). The reasons that make *A. pisum* a good model species are several. It is relatively simple to rear under laboratory conditions. Moreover, it is very prolific and the number of offspring produced by the pea aphid is strongly affected by several abiotic and biotic factors, such as temperature, aphid age, and host plant quality. Since the pea aphid is very prolific, it is a valid tool to assess the effect of biotic and abiotic stresses on the life history traits related to fitness. In

literature many studies confirm the utility of the pea aphid in the investigation of the Aphidiinae thermal biology (Campbell and Mackauer, 1975; Stacey and Fellowes, 2002; Bensadia *et al*., 2006).

***The parasipotiod Aphidius ervi* Haliday**

Aphidius ervi Haliday (Hymenoptera: Braconidae) is a palaeartic oligophagous endoparasitoid. It is mainly associated with Macrosiphinae aphids, such as *A. pisum*, *Macrosiphum euphorbiae* Thomas and *Aulacorthumsolanii* (Kaltenbach) (Mackauer and Finlayson, 1967; Takada and Tada, 2000). *A. ervi* is an efficient biological control agent for the pea aphid. Moreover, it has been often used as model species together with the pea aphid to assess the effect of temperature on parasitoid behavioral and physiological traits (Campbell and Mackauer, 1975; Lagos *et al*., 2001; Stacey and Fellowes, 2002). *A. ervi* develops through three larval stages and a pupation stage (Fig. 2). When parasitoid pupation occurs, aphid dies, and after some hours, it appears mummified. The development concludes with the emergence of the adult parasitoid from the mummified aphid.

Fig. 2 - Photo of the first larval instar of *Aphidius ervi* at 96 hours after aphid parasitization. https://bugguide.net/node/view/595915/bgimage.

A. ervi is able to recreate appropriated nutritional conditions for larval development through a physiological manipulation of the host. This mainly happens through the injection of venom inside host body. *A. ervi* venom contains proteins able to disrupt host ovarioles, then aphid reproductive apparatus is disrupted, and, after some days from parasitization, the host is totally castrated (Digilio *et al*., 2000). Venom is not the unique tool to regulate host suitability. In fact, also teratocytes cells (Fig. 3) deriving from the dissociation of the embryonic serosa, play an important role. Teratocytes of *A. ervi*, in correspondence with the exponential growth phase of the parasitoid larva, de novo synthesize proteins into the

haemolymph of the host (Falabella *et al*., 2000). The most evident alteration induced by parasitism is the drastic reduction of host reproduction, which is totally suppressed when the aphid host is parasitized at the first nymph stage (Polaszek, 1986; Pennacchio *et al*., 1995). Teratocytes are responsible of the nutritional exploitation of the host by extracting nutrients from its tissues, but also redirect the metabolism of the host and its associated bacterial symbionts (Pennacchio *et al*., 1995; Falabella *et al*., 2000). In particular, in parasitized aphids, internal symbionts display an enhanced biosynthesis of essential amino acids (Pennacchio *et al*., 1999; Cloutier and Douglas, 2003), which will be assimilated by the developing parasitoids.

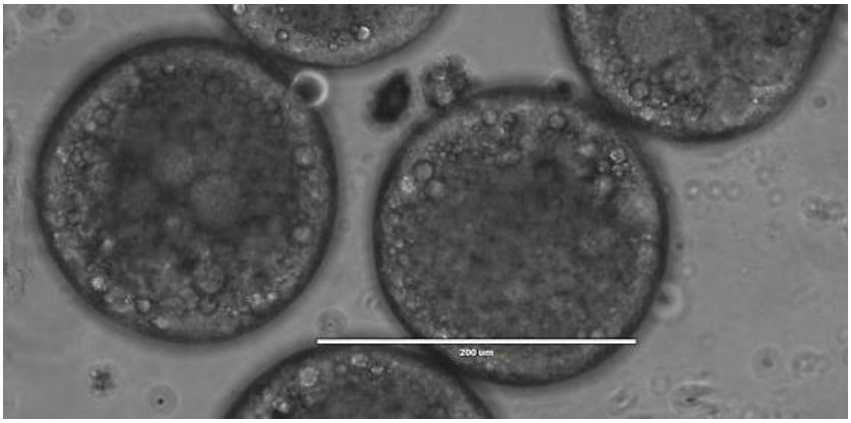

Fig. 3 - *Aphidius ervi* teratocytes at 144 hours after aphid parasitization. https://bugguide.net/node/view/595921/bgimage

For insect parasitoids, the host represents the whole nutritional and physiological environment during immature development. Thus, host quality evaluation by female parasitoids plays a key role, and host choice may result in trade-offs between lower fitness gain because of host quality and developmental requirements (Vinson, 1990; Godfray, 1994; Harvey & Strand, 2002; Beckage&Gelman, 2004). Parasitoids that paralyse or kill their host, also referred to as idiobionts, can use host size as an indicator of the potential resource for their offspring (Godfray, 1994). Endoparasitoidshave nutritional requirements similar to those of free living insects (Thompson, 1986; 1999). Some braconid wasp species, such as *A. ervi* are koinobiotic parasitoids. They have the ability to modulate host metabolism to ensure survival and development to the developing progeny within the living host. In particular, they recreate suitable nutritional conditions for their progeny during the development at the immature stages (Vinson *et al*., 2001; Beckage&Gelman, 2004; Falabella *et al*., 2000).Unlike idiobiont parasitoids, koinobiotic ones more intimately interact with their hosts and might show very specificadaptive mechanisms forfood intake and utilization

as a consequence of their close integration with the living organisms they colonize (Thompson, 1986; 1999)*Aphidius ervi*, as well as other parasitic species, injects a venom inside the host body at the oviposition. In koinobiotic wasp, venom is the principal tool to regulate host physiology (Digilio *et al*., 1998). In particular, it is known that two proteins occurring in *A. ervi* venom destroy the germarial cells of the pea aphid ovarioles(Digilio *et al*., 2000). In this way, parasitoid regulates the nutritional suitability of the host aphid to recreate suitable conditions to the developing parasitoid. After parasitization, haemolymphtitres of nutritionally important compounds, such as proteins and acylglycerols, are altered in parasitizedaphids. Inthe system *A. pisum*-*A. ervi*, these nutrients show an increase on day 5 and 6 after parasitoid oviposition (Pennacchio *et al*., 1995), in correspondence with the most intense growth activity of *A. ervi*larvae, which, at that time, substantially increase in dry mass (Sequeira&Mackauer, 1992). The alteration of the haemolymph protein titre in parasitized pea aphids substantially consists in an increase of free amino acids, which represent a suitable nutriment for the developing parasitoid (Pennacchio *et al.*,1999).

Host-parasitoid associations in insects show a high degree of specialization and provide good model systems to study such co-evolutionary relationships. Endoparasitoids and their hosts are likely to exert strong and reciprocal selection pressure during their coevolution process (Sasaki and Godfray, 1999). While the parasitoid is expected to increase its virulence, thus the capacity to successfully exploit the host, the host is selected to increase its resistance, thus its ability to block parasitoid development. In particular, it is known that resistance to parasitoid may vary among aphid clones. Virulence of a parasitoid in a given host and susceptibility of the host to the parasitoid are really complex traits, and these abilities can depend on several behavioral, physiological and cellular attributes (Oliver *et al*., 2005; Ferrari *et al*., 2007; Guay *et al*., 2009). Host-parasitoids compatibility can vary in function of environmental thermal conditions (Blumberg, 1991; Geden, 1997). Temperature could have differential impacts on *Aphidius* virulence and on the resistance of pea aphid to kill the developing parasitoid, especially because of the aphid bacterial symbionts, implicated in aphid resistance to parasitoids, are really sensible to heat stress (Ohtaka and Ishikawa, 1991; Montllor *et al*., 2002). It is known that many insect species can be cured of bacterial infection when exposed to high temperature. Results obtained by Bensadia *et al*. (2006) supported the hypothesis that pea aphid clonal resistance to *A. ervi* fails under heat stress (25-30°C), at least for all the resistant pea aphid biotypes they studied. Moreover, Thomas and Blanford (2003) found that, similar to fungal infection, high temperatures could contrast parasitoid establishment, and consequently favor host survival (behavioral fever).

Different levels of resistance of the pea aphids to *A. ervi* have been documented in different regions of the world (e.g. Henter and Via, 1995; Hufbauer and Via, 1999; Hufbauer, 2001). In these case *A. ervi* development failure in resistant aphids occurs before larval hatching. In particular, it is known that some pea aphid clones are resistant to *A. ervi*. In these cases, failure of the parasitoid at the larval stage was attributed to an incomplete deployment of teratocytes (Li *et al*., 2002; Cloutier and Douglas, 2003), which determines a scarce virulence. In particular, eggs of *Aphidius* can fail to develop and die at an early stage in resistant aphid clones (Henter and Via, 1995). In some laboratory studies, failure of the parasitoid at the larval stage was reported, and it was linked to an incomplete deployment of teratocytes (Li *et al*., 2002; Cloutier and Douglas, 2003), resulting in weak parasitoid virulence, and hence partial host resistance.

***The aphidophagous ladybird Hippodamia variegata* (Goeze)**

Many ladybirds (Colepotera: Coccinellidae) are generalist predators feeding on a wide range of prey and, due to their voracity, they play a fundamental role in reducing pest populations on crops (Obrycki, 1998; Dixon, 2000; Lanzoni *et al*., 2004). Small arthropods are the principal food for aphidophagous ladybirds, while nectar and mildew are secondary food that positively affect ladybird fitness (Bianchi and van der Werf, 2004). Since the early 20th century, ladybirds are used as a component of integrated or biological control against aleyrodids, aphids, coccids and diaspids (Obrycki and Kring, 1998), both in open field and in greenhouses and tunnels (Hodek, 1970; Oerke, 1994; Hodek and Michaud, 2008; Cabral *et al.,* 2009). Moreover, in last decades, the role of ladybirds in biological control has been recognized through conservation and enhancement techniques (Symondson *et al*., 2002; Obrycki *et al*., 2009).

Hippodamia variegata (Goeze), the species tested in this study, is an aphidophagous ladybird of Palearctic origin, currently present in different world regions (Franzmann, 2002). It has been reported, in many countries, as an efficient control agent of several aphid species, including the pea aphid *A. pisum* (Franzmann, 2002; Kavallieratos *et al*., 2004; Kontodimas and Stathas, 2005; Mandour *et al*., 2012).

References

Barta, M., Cagán, L. 2003. Entomophthoralean fungi associated with common nettle aphid (Microlophium carnosumBuckton) and a potential role of nettle patches as reservoirs for the pathogens in landscape. Journal of Pest Science, 76:6–13.

Bensadia F., Boudreault S., Guay J., Michaud D., Cloutier C., 2006. Aphid clonal resistance to a parasitoid fails under heat stress. Journal of Insect Physiology, 52(2): 146-147.

Bianchi F.J.J.A., van der Werf W., 2004. Model evaluation of the function of prey in non-crop habitats for biological control by ladybeetles in agricultural landscapes. Ecological Modelling, 171(1-2): 177-193.

Blackman R.L., Eastop V.G., 1984. Aphids on the world's crops: an identification and information guide. John Wiley and Sons, New York, New York, USA.

Blumberg D., 1991. Seasonal variations in the encapsulation of eggs of the encyrtid parasitoid *Metaphycus stanleyi* by the pyriform scale, *Protopulvinaria pyriformis*. Entomologia Experimentalis et Applicata, 58: 231-237.

Brisson J.A., Stern D.L., 2006. The pea aphid, *Acyrthosiphon pisum*: an emerging genomic model system for ecological, developmental and evolutionary studies. Bioassay, 28(7): 747-755.

Buchner P., 1965. Endosymbiosis of animals with plant microorganisms. John Wiley, New York.

Cabral S., Soares A.O., Garcia P., 2009. Predation by *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) on *Myzus persicae* Sulzer (Homoptera: Aphididae): effect of prey density. Biological Control, 50: 25-29.

Campbell A., Mackauer M., 1975. Thermal constants for development of the pea aphid (Homoptera: Aphididae) and some its parasites. The Canadian Entomologist, 107(4): 419-423.

Cloutier C., Douglas A.E., 2003. Impact of a parasitoid on the bacterial symbiosis of its aphid host. Entomologia Experimentalis et Applicata, 109, 13–19.

Colinet H.C, Salin G., Boivin G., Hance T.H., 2005. Host age and fitness-related traits in a koinobiont aphid parasitoid. Ecological Entomology, 30(4): 473-479.

Digilio M.C., Isidoro N., Tremblay E., Pennacchio F., 2000. Host castration by *Aphidius ervi* venom proteins. Journal of Insect Physiology, 46: 1041-1050.

Dixon A. F. G., 2000. Insect predator-prey dynamics. Ladybird beetles and biological control. Cambridge University Press, Cambridge, UK.

Dixon A.F.G., 1985. Aphid ecology. Glasgow: Blackie.

Dixon A.F.G., 1998. Aphid Ecology, An Optimisation Approach. 2nd ed. Chapman & Hall, Glasgow and London, 300 pp.

Douglas A.E., 1993. The nutritional quality of phloem sap utilized by natural aphid populations. Ecological Entomology, 18:31-38.

Douglas A.E., 1998. Nutritional interactions in insect-microbal symbioses: aphids and their symbiotic bacteria Buchnera. Annual Reviews of Entomology, 43:17–37.

Dunbar H.E., Wilson A.C.C., Ferguson N.R., Moran N.A., 2007. Aphid Thermal Tolerance Is Governed by a Point Mutation in Bacterial Symbionts. PLoSBiol 5(5): e96.

Eggleton and Gaston, 1990. “Parasitoid” species and assemblages: Convenient definitions or misleading compormises? Oikos, 59(3):417-421.

Eisen J.A., Eisen J.A., 2010. Genome sequence of the pea aphid Acyrthosiphon pisum. PLoS Biology 2010; 8(2): Art. e1000313.

Falabella, P., Riviello, L., Caccialupi, P., Rossodivita, T., Teresa Valente, M., Luisa De Stradis, M., Tranfaglia, A., Varricchio, P., Gigliotti, S., Graziani, F., 2007. A γ-glutamyltranspeptidase of Aphidius ervi venom induces apoptosis in the ovaries of host aphids. InsectBiochemistry and Molecular Biology, 37, 453–465.

Falabella, P., Riviello, L., De Stradis, M.L., Stigliano, C., Varricchio, P., Grimaldi, A., de Eguileor, M., Graziani, F., Gigliotti, S., Pennacchio, F., 2009. Aphidius ervi teratocytes release an extracellular enolase. Insect Biochemistry and Molecular Biology, 39, 801–813.

Falabella, P., Tremblay, E., Pennacchio, F., 2000. Host regulation by the aphid parasitoid Aphidius ervi: the role of teratocytes. Entomologia Experimentalis et Applicata, 97, 1–9.

Ferrari J., Muller C.B., Kraijeveld A.R., Godfray H.C., 2007. Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. Evolution, 55(9):1805-1814.

Franzmann BA (2002) Hippodamia variegata (Goeze) (Coleoptera: Coccinellidae), a predacious ladybird new in Australia. Australian Journal of Entomology, 41:375–377.

Geden C.J., 1997. Development models for the filth fly parasitoids Spalangiagemina, S. cameroni, and Muscidifurax raptor (Hymenoptera:Pteromalidae) under constant and variable temperatures, Biological Control, 9, 185-192.

Guay J., Broudreault S., Michaud D., Cloutier C., 2009. Impact of environmental stress on aphid clonal resistance to parasitoids: role of Hemiltonella defense bacterial symbiosis in association with a new facultative symbiont of the pea aphid Journal of Insect Physiology, 55(10):919-926.

Guo J., Hatt S., He K., Chen J., Francis F., 2017. Nine facultative endosymbionts in aphids. A review. Journal of Asia-Pacific Entomology, 20(3): 794-801.

Hagen K.S., Van den Bosh R., 1968. Impact of pathogens, parasites, and predators on aphids. Annual Review of Entomology, 13:325-384.

Henter, H. J., S. Via. 1995. The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. Evolution 49:427–438.

Hodek I, Michaud J.P., 2008. Why is Coccinella septempunctata so successful? (a point-of-view). European Journal of Entomology, 105:1–12

Hodek I., 1970. Coccinellids and the modern pest management. BioScience 20, 543–552.

Hufbauer R.A., 2001. Pea aphid-parasitoid interactions: have parasitoid adapted to different resistance? Ecology, 82(3):717-725.

Hufbauer R.A., Via S., 1999. Evolution of an aphid-interaction: variation in resistance to parasitism among aphid populations specialized on different plants. Evolution, 53(5):1435-1445.

Kavallieratos N. G., AthanassiouC. G., TomanovicŽ., Papadopoulos G. D., VayiasB. J., 2004. Seasonal abundance and effect of predators (Coleoptera, Coccinellidae) and parasitoids (Hymenoptera: Braconidae, Aphidiinae) on Myzus persicae (Hemiptera, Aphidoidea) densities on tobacco: a two-year study from Central Greece. Biologia -Section Zoology, 59 (5): 613-619.

Kontodimas D.C., Stathas, G.J., 2005. Phenology, fecundity and life table parameters of the predator Hippodamia variegata reared on Dysaphiscrataegi. Biocontrol 50, 223–233.

Lagos N.A., Fuentes-Contreras E., Bozinovic F., Niemeyer H.M., 2001. Behavioural thermoregulation in Acyrthosiphon pisum (Homoptera: Aphididae): the effect of parasitism by Aphidius ervi (Hymenoptera: Braconidae). Journal of Thermal Biology, 26(2):13-137.

Lanzoni A., Accineli G., Bazzocchia, Burgio G., 2004. Biological traits and life table of the exotic Harmonia axyridis compared with Hippodamia variegata, and Adaliabipunctata (Col, Coccinellidae), Journal of Applied Entomology, 128: 298 – 306.

Larocca A., Fanti P., Romano V.A., Marsicovetere E., Isidoro N., Romani R., Ruschioni S., Pennacchio F., Battaglia D., 2007. Functional bases of host-acceptance behaviour in the aphid parasitoid Aphidius ervi. PhysiologicalEntomology, 32, 305–312.

Li S., Falabella P., Giannantonio S., Fanti P., Battaglia D., Digilio M.C., Völkl W., Slogget J.J. Weisser W., Pennacchio F., 2002. Pea aphid clonal resistance to the endophagous parasitoid *Aphidius erv*i. Journal of Insect Physiology, 48 (10): 971-980.

MacArthur R.H., Wilson E.O., 1967. The theory of island biogeography. Princeton Univ. Press, Princeton, NJ.

Mackauer M., Finlayson T., 1967. The hymenopterous parasites (Hymenoptera: Aphidiidae et Aphelinidae) of the pea aphid in eastern North America. Canadian Entomologist, 99:1051–1082.

Mandour N.S., Sarhan A.A., El-Basha N.A., Abdel-Motaal D.S., 2012. Effect of different temperature regimes on the biology, reproduction and predation of Hippodamia variegata (Goeze) (Coleoptera: Coccinellidae). Egyptian Journal of Biological Pest Control, 21(1): 305-312.

McGavin G.C., 1993. In Bugs of the World (2nded). Facts on File, NewYork, NY, USA.

McLean A.H.C., van Asch M., Ferrari J., Godfray H.C.J., 2010. Effects of bacterial secondary symbionts on host plant use in pea aphids. Proceedings of Royal Society B., 278, 760-766.

Miura T., Braendle C., Shingleton A., Sisk G., Kambhampati S., D.L. Stern, 2003. A comparison of parthenogenetic and sexual embryogenesis of the pea aphid Acyrthosiphon pisum (Hemiptera: Aphidoidea). Molecular and Developmental Evolution, 295 Suppl B: 59–81.

Montllor C.B., Maxmen A., Purcell A.H., 2002. Facultative bacterial endosymbionts benefits pea aphids Acyrthosiphon pisum under heat stress. Ecological Entomology, 27(2):189-195.

Müller C.B., Godfray H.C.J., 1999. Predators and mutualist influence the exclusion of aphid species from natural communities. Oecologia, 119:120-125.

Obrycki J., Kring T., 1998. Predaceous coccinellidae in biological control. Annual Review of Entomology, 43: 295-321.

Obrycki J.J., Harwood J.D., Kring T.L., O'Neil R.J., 2009. Aphidophagy by Coccinellidae: Application of biological control in agroecosystem. Biological Control, 51(29): 244-254.

Obrycki, J. 1998. Predaceous Coccinellidae in biological control. Annual Review of Entomology, 43:295-321.

Oerke, E.C., 1994. Crop Production and Crop Protection: Estimated Losses in Major Food and Cash Crops. Elsevier, Amsterdam.

Ohtaka C., Ishikawa H., 1991. Effects of heat treatment on the symbiotic system of an Aphid mycetocite. Symbiosis, 11:19-30.

Oliver K.M., Campos J., Moran N.A., Hunter M.S, 2008. Population dynamics of defensive symbionts in aphids, Proceedings of Royal Society B., 275293–299.

OliverK.M., Degnan P. H., Burke G. R., Moran N. A., 2010. Facultative Symbionts in Aphids and the Horizontal Transfer of Ecologically Important Traits, Annual Review of Entomology, 55:247-266.

Oliver K.M., Moran N.A., Hunter M.S., 2005. Variation in resistance to parasitism is due to symbionts not host genotype. PNSA, 102(36): 12795-12800.

Pennacchio, F., Digilio, M.C., Tremblay, E., 1995. Biochemical and metabolic alterations in Acyrthosiphon pisum parasitized by Aphidius ervi. Archives of Insect Biochemistry and Physiology, 30, 351–367.

Pennacchio F., Digilio M.C., Tremblay E., Tranfaglia A., 1994. Host recognition and acceptance behaviour in two aphid parasitoid species: Aphidius ervi and Aphidius microlophii (Hymenoptera: braconidae). Bullettin of Entomological Research, 84, 57.

Pennacchio F., Fanti P., Falabella P., Digilio M.C., Bisaccia F., Tremblay E., 1999. Development and nutrition of the braconid wasp, Aphidius ervi in aposymbiotic host aphids. Archives of Insect Biochemistry and Physiology, 40, 53–63.

Pennacchio F., Strand M.R., 2005. Evolution of developmental strategies in parasitic Hymenoptera. Annual Reviews of Entomology, 51, 233–258.

Pianka E.R., 1970. On r- and k-selection. The American Naturalist, 104,940.

Polaszek, A., 1986. The effects of two species of hymenopterousparasitoidon the reproductive system of the pea aphid, Acyrthosiphon pisum. Entomology Experimentalis et Applicata, 40, 285–292.

Pons X., Lumbierres B., Albajes R., 2009. Heteropterans as aphid predators in inter-mountain alfalfa. European Journal of Entomology, 106:369–378.

Pons X., Lumbierres L., Antoni R., P. Stary, 2011. Parasitoid complex of alfalfa aphids in an IPM intensive crop system in northern Catalonia. Journal of Pest Science, 84: 437-445.

Pons X., Nuñez E., Lumbierres B., Albajes R., 2005. Epigeal aphidophagous predators and the role of alfalfa as a reservoir of aphid predators for arable crops. Eur J Entomol 102:519–525.

Rabasse J.M., van Steenis M.J., 1999. Biological control of aphids. Integrated Pest and Disease Management in Greenhouse Crops, 235-243.

Roff D. A.,1986. The evolution of wing dimorphism in insects. Evolution, 40:1009–1020.

Roff D.A., Mostowy S., Fairbairn D.J., 2002. The evolution of tradeoffs: testing predications on response to selection and environmental variation. Evolution, 56:84–95.

Sampaio M. V., Bueno V. H. P and De Conti B. F., 2008. The effect of the quality and size of host aphid species on the biologicalcharacteristics of Aphidius colemani (Hymenoptera: Braconidae: Aphidiinae), European Journal Entomology, 105: 489–494.

Sasaki A., Godfray H.C.J., 1999. A model for the coevolution of resistance and virulence in coupled host-parasitoid interactions. Biological Science, 266, 1418.

Shigenobu S., Richards S., Cree A.G., Morioka M., Fukatsu T., Kudo T., Miyagishima S., Gibbs R.A., Stern D.L., Nakabachi A., 2010. A full-length cDNA resource for the pea aphid, Acyrthosiphon pisum. Insect Moleculary Ecology, 19(2):23-31.

Skaljac M., Kirfel P., Grotmann A., Vilcinskas A., 2018. Fitness costs of infection with Serratia symbiotica are associated with greater susceptibility to insecticides in the pea aphid Acyrthosiphon pisum, Pest management science, 74(8): 1829-1836.

Snyder W.E., Ives A.R., 2003. Interactions between specialist and generalist natural enemies: parasitoids, predators, and pea aphid biocontrol. Ecology, 84(1):91-107.

Spiller N.J, Koenders L., Tjallingii W.F.,1990. Xylem ingestion by aphids: a strategy for maintaining water balance. EntomologiaExperimentalis et Applicata, 55,101–104

Stacey D.A., Fellowes M.D.E., 2002. Influence of temperature on pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae) resistance to natural enemy attack. Bulletin of Entomological Research, 92: 351-357.

Symondson W.O.C., Sunderland K.D., Greenstone M.H., 2002. Can generalist predators be effective biocontrol agents? Annual Review of Entomology, 47:561-594.

Takada H., Tada E., 2000. A comparison between two strains from Japan and Europe of *Aphidius ervi*. Entomologia Experimentalis et Applicata, 97: 11-20

Thomas M., Blanford S., 2003. Thermal biology in insect-parasite interactions. Trends in Ecology & Evolution,18(7):344-350.

Tremblay E, 1995. Entomologia applicata. Liguori Editore, Napoli.

Trionnaire G.L., Hardie J., Jaubert-Possamai S., Simon and D J., Tagu, 2008. Shifting from clonal to sexual reproduction in aphids: physiological and developmental aspects. Biology of the Cell, 100(8): 441-451.

van Emden H.F., Harrington R., 2003. Aphids as crop pests. CAB International, Wallingford.

van Emden H.F., Kifle A.T., 2002. Performance of the parasitoid *Aphidius colemani* when reared on *Myzus persicae* on a fully defined artificial diet. BioControl, 47: 607-616.

Vinson S.B., 1990. How parasitoids deal with the immune system of their host: an overview. Insect Biochemistry and Physiology, 13(1-2):3-27.

Vorburger C., Geher L., P. Rodriguez., 2010. A strain of the bacterial symbiont Regiella insecticola protects aphids against parasitoids. Biology Letters, Biology Letters, 6109–111.

Waage J.K., D. J. Greathead, 1988. Biological control: challanges and opportunities. 316,1189.

OBJECTIVES

This thesis focuses on the evaluation of the effects of rapid and extreme thermal variations in a tri-trophic system composed by plant-aphid-natural enemies. In particular, the experiments reported in this thesis are related with the thermal biology of the pea aphid *Acyrthosiphon pisum*, the parasitic wasp *Aphidius ervi* and the aphidophagous ladybird *Hippodamia variegata*.

In particular, in the Ch. I, I investigated the short and long-term effects of a short and sub-lethal heat stress (39°C for 30 minutes) on the longevity and fecundity of the pea aphid *A. pisum.* Then, in this study, I estimated how the cost related to heat resistance reflects the investment of pea aphid in ensuring its reproduction and the at more favorable thermal conditions, after the thermal stress, and for the rest of its life. Moreover, the possibility that survival to thermal stress altered the relationships between fecundity and longevity was also investigated.

In the Ch. II, I investigated if the pea aphids, at various stages of development, show different levels of survival after exposure to an extreme temperature. Moreover, I investigated if the parasitism of the endoparasitoid *A. ervi* has any effect on the aphid thermal sensitivity when it experiences a rapid and extreme thermal increment (39°C, for 30 minutes) in the days after the parasitization.

In the Ch. III, I investigated some aspects related to the survival of both unparasitized and parasitized aphids after exposure to a mild and to a very high temperature in the short (24 h after exposure) and in the long term (on 14 days old aphids). I also investigated the effects of a thermal hardening (mild stress) on the survival of aphids when exposed to a subsequent severe thermal stress. The trophic model system is the same described in Chapter II and consists of the endoparasitoid *A. ervi* and its aphid host, the pea aphid *A. pisum*. In Chapter II, I demonstrated that the pea aphid *A. pisum* displays a greater resistance to rapid thermal increment after being parasitized by the parasitic wasp *A. ervi*. I supposed that the stress induced by parasitization could increase aphid heat resistance through the activation of a hardening-like mechanism. In this chapter I then investigated if parasitism and a thermal hardening might or might not have any cumulative effects on aphid thermal resistance and if (and to what extent) these effects influence parasitoids survival.

In the Ch. IV, I investigated if a short and high heat exposure had any effects on the body size and the static allometries of the parasitoid *A. ervi.* For this study, parasitized aphids hosting *A. ervi* at two developmental stages (egg and 3rd larval stage) were tested at two temperatures (35°C and 39°C) for 30 minutes. In particular, tibia and wing length were

measured to assess any effect on adult size and the static allometry between these two body parts.

In the Ch. V, I investigated if an exposure of the pea aphid to a non-lethal cold temperature (0°C per 1 hour) increases the pea aphid resistance to a subsequent stronger cold stress, and if parasitization of the pea aphid by the parasitoid *A. ervi* may increase the pea aphid resistance to a subsequent stronger cold stress (-20°C per 18 minutes), as demonstrated in Chapter II in the case of heat stress. Since any interaction between parasitism and cold stress responses may be coevolved in parasitized aphid, these two kind of stress were jointly tested by exposing parasitized aphids to a cold-hardening treatment before a sub-lethal cold stress. In particular, it was investigated if in parasitized aphids the beneficial of a cold-hardening treatment further enhances the aphid cold resistance.

In the Ch. VI, I investigated if and how the predation of the ladybird *H. variegata* on *A. pisum* is affected by the experimental arena complexity, at two temperatures, and if the acclimation influences the predation rate. For the second question, I specifically tested if the rearing thermal conditions (20 and 25°C) affect aphid consumption when it preys in two different thermal environments (20 and 25°C).

CHAPTER I

Rapid thermal increment reduces longevity and nymph production in the pea aphid *Acyrthosiphon pisum*

Introduction

"Life history" is the term used to describe all the events that characterize the whole life cycle of an organism, from its birth to its death (Peters, 1983). Almost all the events of the life cycle of an organism are affected or influenced by the phenotype (Roff, 1992; Stearns 1992; Huey and Berrigan, 2001; Flatt and Heyland, 2011). Phenotypic traits affecting life history are grouped together and called "life history traits". Examples of life history traits are growth rate, developmental time, age at maturity, reproductive investment, and lifespan. Both life history and life history traits are related to fitness (Kojima and Kimura, 2003) and therefore reflect, at least in part, the relationship between an organism and its environment. Variation in these traits are related to individual fitness since each trait impacts on demographic changes. Survival, longevity and reproduction, three of the most important life history traits, represent the demographic parameters required to estimate fitness as defined by the Malthusian model, which describes population growth by considering reproductive events and survival probabilities over the individual lifetime (Stearns, 1992). A key postulate of life history theory states that life history traits are limited by factors internal to the organism (Flatt and Hayland, 2011). A life history trade-off occurs when an increased investment in one trait causes an investment reduction in another one. Energetic reserves of organism are limited, and, consequently, two or more traits cannot be optimized at the same time. Trade-off between reproduction and longevity occupies a central role in the life-history theory (Gadgil and Bossert, 1970; Stearns, 1992; Miyatake, 1997; Roff *et al.,* 2002), and it is frequently observed when a considerable investment in early reproduction leads to a low level of survival and therefore to a low investment in late reproduction (Stearns, 1992).

The life history traits architecture (trade-offs, constrains, *etc*.) could be influenced by the external environment in which an organism lives, especially if the environment is stressfull (Hoffmann and Parsons, 1991). Rapid and extreme thermal variations are considered stressful and they may be deleterious for ectothermic species (Hoffmann and Parsons 1997; Hoffmann *et al*. 2003; Mehrparvar and Hatami, 2007). If a given stress is really exceptional in its intensity, it is likely to result in a partial extinction of a population without eliciting an adaptive response. If instead some individuals survive, they are expected to show some important physiological alterations, which, in turn, could affect their biological performances.

To allow an adequate function of the organism during a thermal stress (i.e., to ensure survival), energetic resources can be differently allocated in order to prevent cellular damage, for example, through a strong up-regulation of most heat shock proteins (Hoffmann *et al*., 2003; Sørensen *et al*., 2013). Once an organism has ensured its survival, its metabolism and other physiological traits will have been inevitably damaged or, at best, altered (Hercus *et al*., 2003; Brown *et al*., 2004; Sørensen *et al.*, 2013). The remaining energetic resources will be reallocated to optimize the fitness (reviewed in Roff *et al*., 2002). After a moderate or severe thermal stress, insect longevity and reproduction could be altered and, therefore, the preexisting relationship between these two life history traits could be also altered.

Insects are very susceptible to rapid and extreme thermal changes. Environmental temperature affects insect physiology and is among the main important abiotic factors in determining mortality in their populations (Cossin and Bowler, 1987). In temperate regions, where temperatures widely fluctuate on a seasonal base, insects have to cope, and eventually to adapt, to the surrounding thermal conditions (Bale *et al*., 2002). Daily temperature variation could also affect biological performances of insects (Deutsch *et al*., 2008; Kingsolver *et al*., 2013). Current forecast models predict an increasing in the frequency of the rapid and extreme thermal events as a result of global warming. Degree, duration, and frequency of high stressful temperatures are predicted to substantially increase in the next decades (IPCC, 2018). Under these perspectives, insect species will experience more frequently lethal and sub-lethal heat stress (Marengo *et al*., 2009; Diffenbaugh and Field, 2013;).

Aphids are phloem feeder insects whose parthenogenetic reproduction and short generation time allow them to rapidly increase their population size (Dixon, 1987). Temperature is among the main abiotic factors affecting development rates, population dynamics and phenology (Dixon, 1987; Schowalter, 2000). Aphids have soft bodies, small sizes, and thin cuticle, and consequently they have a limited ability to buffer thermal changes (Dixon, 1998). Aphid immature stages have low mobility and could not easily escape from a thermal stress (Ben-Ari *et al*., 2015). All these features make aphids very sensitive to high temperatures.

For aphids, as for many other insect species, reproductive output is a valid trait used to evaluate the costs associated to stressful thermal conditions (Zhou and Carter, 1992; Mehrparvar and Hatami, 2007; Ma *et al*., 2004). Many studies on aphid thermal biology have been conducted by reproducing a gradual thermal increment for a certain exposure time (Zhou and Carter, 1992; Ma *et al*., 2004) or by rearing insect at different temperatures (Asin and Pons, 2001; Mehrparvar and Hatami, 2007). Under the perspective of the occurrence of

rapid thermal increments (IPCC, 2018), insects could be incapable to promptly and adequately acclimate to the changing environment, and the heat stress responses that ensure survival could not be promptly displayed (Sejerkilde *et al*., 2003; Zhao *et al*., 2010; Lu and Wan, 2011).

Aphids are particularly common in temperate zones, above all from the late-spring and the mid-summer. They are often exposed to thermal stresses, due to both the circadian rhythms and to the unpredictable and abnormal thermal variations caused by the global warming (Bale *et al*., 2002). Temperature is considering among the main abiotic factors affecting aphid population in open-field, both for the direct aphid mortality and the detrimental consequences at long-term on its vitality and its fitness-related traits (Tamaki *et al*., 1980; Beetge and Kruger, 2019; Zhu *et al*., 2019). It is reasonable to suppose that the aphid surviving a rapid and extreme thermal increment pay a relevant metabolic cost due to activation and maintenance of the mechanisms related to heat resistance, and that this cost may have negative consequences on the aphid fitness (Roff, 2002; Hercus *et al*., 2003; West-Eberhard, 2003).

In the present study, I investigated the short and long-term effects of a short and sub-lethal heat stress on the longevity and fecundity of the pea aphid *A. pisum*. I estimated the fitness cost related to heat resistance, *i.e*, the long-term effects on longevity and reproduction when the aphid returns to live under its optimal thermal conditions. Moreover, the possibility that survival to thermal stress has altered the relationships between fecundity and longevity was also investigated.

Materials and Methods

Insect rearing

A green clone of the pea aphid *A. pisum* was collected near Salerno, Italy and laboratory reared on broad bean plants (*Vicia faba* L.). Aphid culture started in 1995 and was maintained in a climatic chamber Binder KBF at 22±1°C and 85±5% relative humidity (RH), under an 18:6-h light/dark (L/D) photoperiod. Broad bean plants of the cultivar "Aguadulce" were grown in pots (10 cm diameter), containing commercial soil (COMPO SANA® Universal Potting Soil), in a greenhouse.

Cohorts of same-aged aphids were used in all the experiments, as both productivity and thermal resistance in aphid vary with age (Zhao *et al*., 2017). Approximately 95-100 adult females were isolated from the massive aphid culture and put on a fresh potted broad bean plant kept in a plastic box (22 × 15 × 40 cm high) for 8 hours at 22°C (18:6 L/D; 85 ± 5% RH). Females were then removed and discarded. The cohort of newborn nymphs was

maintained as a synchronous colony on a broad bean plant for 9 days at 22°C. To avoid the deterioration of the host plant and any possible effects of crowding, 90 five days old aphids (when available) were gently transferred on a new plant for another 4 days. At the end of this 9-day period, the aphids were usually at the beginning of the adult stage. Nevertheless, before their use in the experimental trials, aphids were inspected under a stereomicroscope and all individuals that were not adults, according to the morphological features as in Digilio (1995), were discarded.

Three independent replicates were generated, each consisting of three plants (that is, nine synchronous colonies), for a total of about 800 aphids.

Heat shock treatment

Within a replicate, adult aphids of each of the 3 synchronous colonies were gently removed from the plant and divided into 4 groups of about 20 aphids. Unfortunately, 4 colonies were divided into only three groups. Each replicate consisted of three groups of 20 aphids (two, in some cases) that were heat shocked, whereas the remaining group was used as control. The heat exposure was promptly carried out to reduce the starvation and dehydration stress. The heat shock treatment was performed by placing the Falcon® tubes containing the aphids in a thermostatic bath (Argolab WB 12).

Heat resistance has been assessed by measuring aphid survival following exposure to the temperature of 39.0 ± 0.1°C for an exposure time of 30 minutes, a technical method used in *Drosophila* (Hoffmann *et al*., 2003). The time interval of 30 minutes minimized the chance of any heat hardening response during the heat shock treatment (Hazell *et al*., 2010) and did not produce a significant impact of starvation and desiccation on the experimental individuals (Hoffmann *et al*., 2003; Terblanche *et al*., 2011). After the heat shock exposure, all the survived aphids of each group were transferred on a cut broad bean leaf, with the stalk inside an Eppendorf® tube filled with water and sealed with parafilm® to prevent desiccation. The leaf was then placed in a plastic cylindrical vial (150 ml) with a mesh covered ventilation hole in the screw-top. The plastic cylinders were placed in an environmental chamber at 22 °C. The control group consisted of aphids subjected to the same manipulations, except for the heat shock exposure.

Survival, productivity, and longevity experiments

Survival of heat-stressed and control aphids was recorded 24 h after the heat shock treatment. Survival was considered to occur if an aphid was able to walk or if it moved when gently touched by a soft paintbrush. This response was checked 24 h after heat exposure, as many

insects are immobilized for some time following a heat stress (Sørensen *et al*., 2001; Hazell *et al*., 2010). Nymph production during the first 24 h after the heat treatment was determined by counting the newborn nymphs inside each vial. Aphid productivity during the first 24 hours after heat treatment was estimated by dividing the number of nymphs for the number of survived aphids. After the survival and productivity check at 24 h, a subgroup of heat shocked (n = 57) and control (n = 46) aphids belonging to the three experimental replicates were individually placed on 12-day old broad bean plants. Plants were about 5-7 centimeters high, with a pair of well-developed leaves, and were placed at the same previously described rearing conditions. Mortality was daily recorded for both the control and the heat shocked aphids, starting from the second day after the heat exposure until all the aphids died. To obtain a correct estimation of the aphid productivity, after the mortality check, newborn nymphs were daily removed from the plant and counted.

Statistical analysis

Since the row data on aphid survival after heat shock have a discrete probability distribution (alive/dead), a binomial generalized linear model (GLM) with a logit link function has been considered for the analysis. This kind of analysis has greater interpretability and higher power than the analyses of percentage or percentage transformed data (Warton and Hui, 2011). Aphid survival after heat shock was therefore analyzed using a GLM with "treatment" (two levels: control and heat shocked aphids) as main effect and "replicate" (three levels) nested within treatment.

For the longevity analysis, a Cox proportional hazard regression model (Cox, 1972) was used to test for differences between control and heat shocked aphids. Longevity data were graphically represented with a Kaplan-Meier survival plot. To correctly compare the two treatments, only the productivity data from day 2 to day 23 were considered. Day 23 corresponds to the last day on which a live aphid of the heat shock group was observed. Productivity analysis first used the cumulative offspring production per aphids. The use of cumulative offspring production allows to visualize which treatment had the maximum offspring production and the time needed to reach it. The best fit for the cumulative productivity over time was given by two-degree polynomials ($R^2 \geq 0.96$ in both cases). An ANCOVA was then performed in order to identify differences in slopes between polynomials that are indicative of differences in reproductive rates between treatments (control and heat shocked aphids) and among replicates within treatment. The reproductive rates were also represented as the mean productivity per aphids over time and analyzed with a mixed model ANOVA in which "day" (22 levels) and treatment were main fixed effects and replicate was

nested within treatment. In order to detect significant differences among days and treatments, a Tukey *post-hoc* test for multiple comparisons of means was also performed. All the statistical analyses performed in this study were carried out using R.3.6.2 software (R Core Team, 2016).

Results

The aphid survival after a heat shock at 39.0±0.1 °C for 30 min was significantly different from those of the control group (Fig. 1.1).

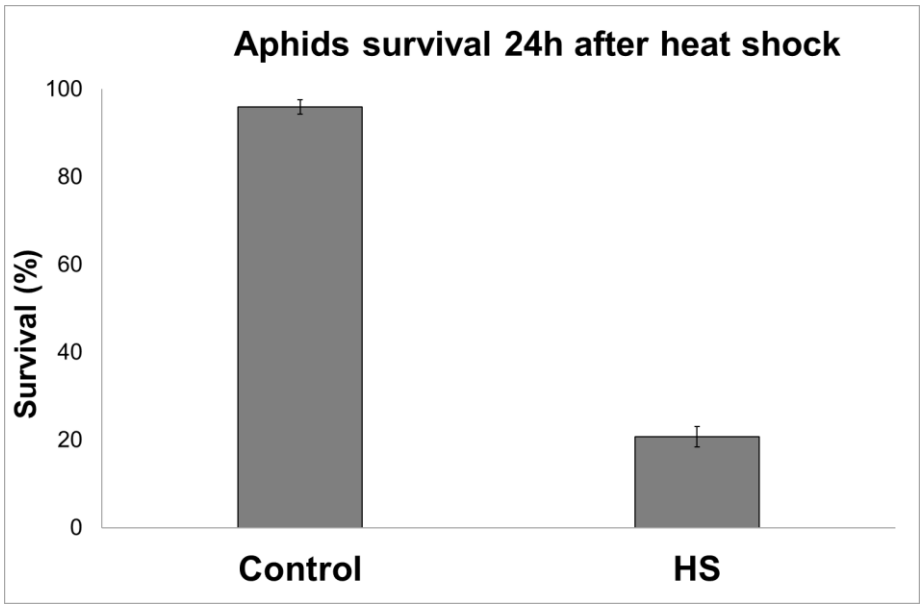

Fig. 1.1 - Mean values (±standard errors) of survival of control and aphids after a heat shock (HS) at 39,0±0.1°C for 30 min. Survival was recorded 24 hours after the heat shock treatment.

Mortality imputable to manipulation was lesser than about 4%, as shown by the control group. The binomial GLM performed on the survival data shows significant differences between treatments ($\chi^2_{(1)} = 357$, $P < 0.001$) but not among replicates within treatments ($\chi^2_{(4)} = 1.45$, $P = 0.83$). Figure 1.2 shows the mean productivity during the first 24 hours after heat shock of aphids subjected to heat shock and of control ones.

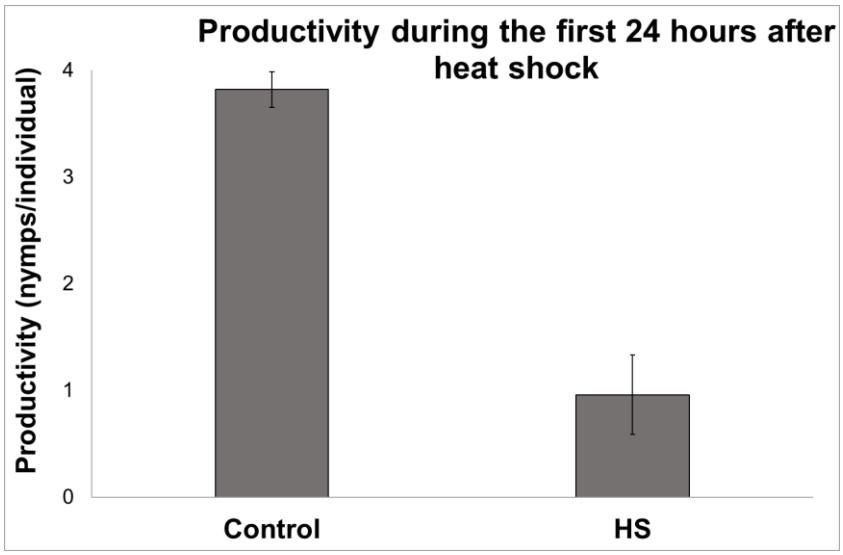

Fig. 1.2. - Mean values (± standard errors) of productivity during the first 24 hours, measured as number of nymphs produced by aphids experiencing the heat shock (HS), at 39.0±0.1°C for 30 min, and those of the un-stressed aphid group.

The aphids survived the heat shock produced less offspring than control ones during the first 24 hours ($F_{1,23} = 19.06$, $P < 0.001$), while no difference among replicates was recorded ($F_{4,23} = 0.33$, $P = 0.85$). Survival plots for control and heat shocked aphids are shown in Fig. 1.3. Significant differences between treatments ($\chi^2_{(1)} = 8.5$, $P = 0.0036$) were detected using a Cox proportional hazard model. Aphids subjected to heat shock have, on average, a lower lifespan compared to the control ones.

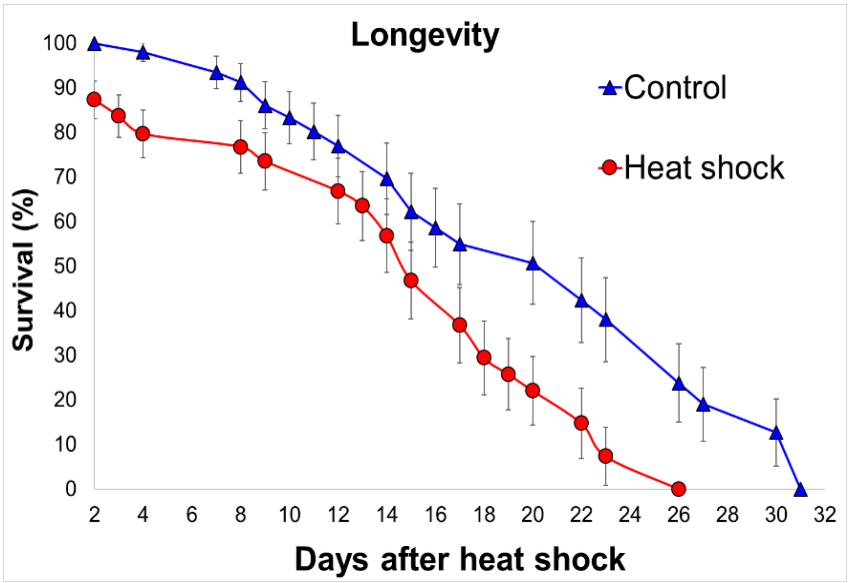

Fig. 1.3 - Kaplan-Meier survival plot based on the daily mean number (± standard errors) of aphids died after the heat treatment of heat stressed aphid group and the related aphid control group. Data are based on 46 (control) and 57 (heat shock) aphids.

The relative risk (derived from the Cox model) of aphids subjected to heat shock compared to control ones is 1.98, that is, the probability of dying of an aphid subjected to the heat treatment is 1.98 times higher than a control one. I used the cumulative offspring production as an indication of the fitness of the two aphid groups (Fig. 1.4). The best fit for the cumulative productivity over time was given by two-degree polynomials ($R^2 \geq 0.96$ in all the cases). The results of the ANCOVA confirmed the observed differences in slopes between polynomials that are indicative of differences in reproductive rates between control and heat shocked aphids (interaction between polynomial regressions and treatment: $F_{2,38} = 534$, $P < 0.001$). The mean number of nymphs produced by an individual over its whole lifespan was 75.6 for the control group and 52.8 for the heat shock one.

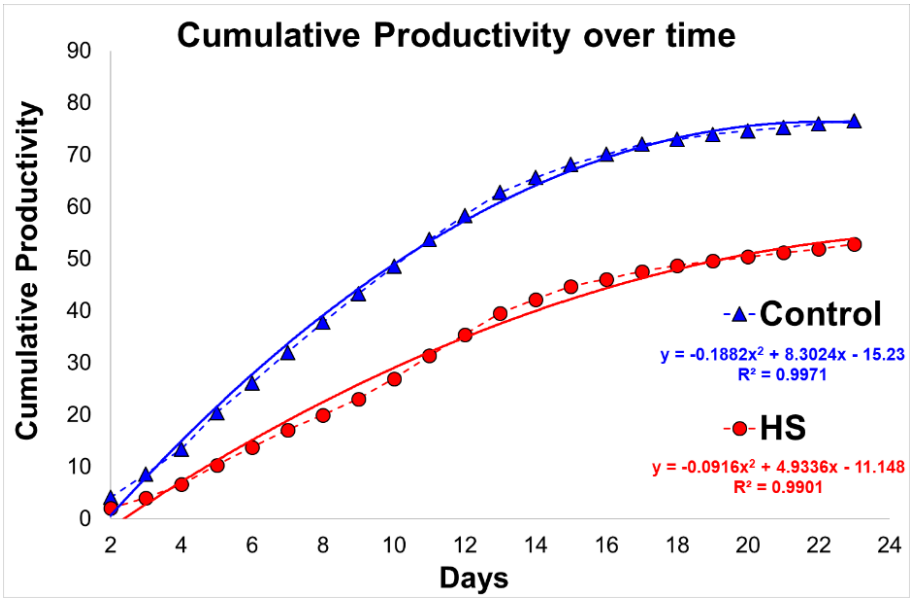

Fig. 1.4 - Cumulative number of the mean number of nymphs daily produced after the heat stress exposure for both aphid heat stressed and the unstressed control group.

For a better comparison of the two treatments, the reproductive rates were also represented as the mean productivity per aphids over time (Fig. 1.5).

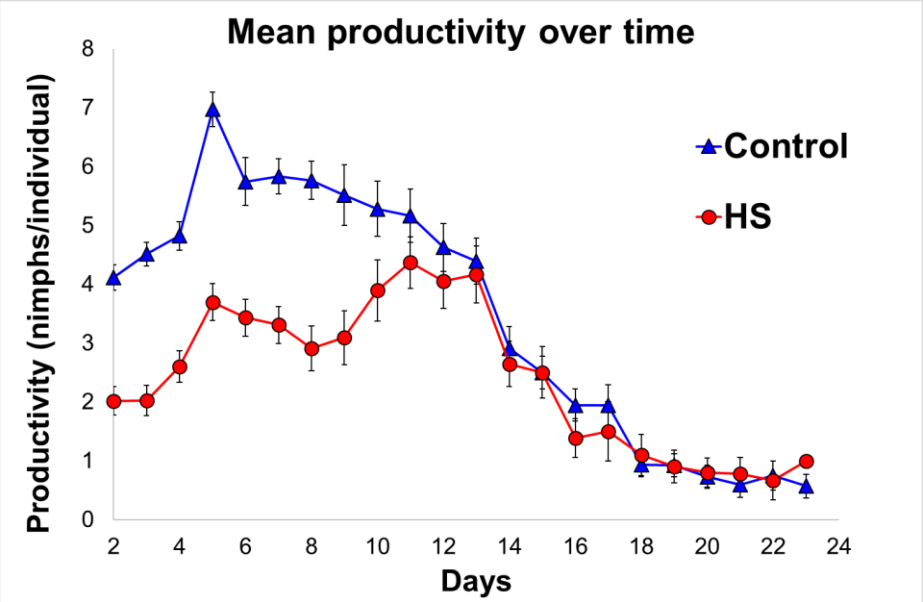

Fig. 1.5 - Mean productivity (± standard errors) per aphid over time of the two experimental groups.

The ANOVA performed on these values gave significant differences among days ($F_{21,947}$ = 25.9, $P < 0.001$), between treatments ($F_{1,947}$ = 21.6, $P < 0.001$) and, more interesting, a significant "day by treatment" interaction ($F_{21,947}$ = 4.5, $P < 0.001$). During the first nine days after the heat exposure, significant differences in daily nymph production between heat stressed and control aphids were found (Tukey's HSD *post-hoc* test, $P < 0.001$ in all cases). From the 10thuntil the 24thday, no significant differences were found in the mean number of nymphs daily produced between the two experimental aphid groups.

Discussions and Conclusions

In nature, organisms often experience gradual thermal increments. When this occurs, they have the possibility to acclimate to the stressful temperature, minimizing the detrimental effects of some well-described resistance mechanisms (Sejerkilde *et al*., 2003; Alford *et al*., 2011; Sørensen *et al*., 2016). Current predictions on global warming state that heat stresses will be more frequent in next decades and temperature peaks will be reached faster than in the past century. Under these thermal conditions, the survival as well as most of the life history traits of insects, including aphids, could be negatively affected.

In the present study, I found that after a severe heat shock, aphids have, on the long term, a reduction in both lifespan and fecundity. I also observed that, as in many organisms, aphid fecundity decreases as a function of ageing. Trade-off between reproduction and survival is very common in insects (Stearns, 1992). This trade-off is the bedrock of the evolutionary theory of ageing (Kirkwood, 1977): somatic maintenance requires a complex machinery to active and sustain many physiological mechanisms, which maintain the functionality of cells

and tissues. Trade-off between reproduction and longevity is also central in the life-history theory (Gadgil and Bossert, 1970; Stearns, 1992; Roff, 2002): a heavy investment in current reproduction leads to low levels of survival and, more important, to low levels of future reproduction. Physiological explanations for this trade-off are typically based on the assumption that these two fitness components compete for limited energy and nutrients (Reznick, 1992; Zera and Harshman, 2001; Harshman and Zera, 2007). Reproduction diverts resources away from cellular reparative mechanisms, so that survival and future fertility are subsequently lowered (Partridge and Harvey, 1985). Significant differences in the number of nymphs daily produced by the heat stressed and the control aphids were found until the 10^{th} days after the heat exposure. After this time, the offspring production of the two groups strongly decrease and the two productivity curves overlap. For both groups, the productivity decrement over time is most likely due to the effects of aging.

It is interesting to note that, compared with the control, heat stressed aphids showed a lower rate of early reproduction but the same rate of late reproduction. On these basis, lower rate of early reproduction is due to the detrimental effects of the heat stress, which will not be compensated in the future reproduction because no trade-off has been established between these two traits.

Decrement of productivity in heat stressed aphid could be both a direct and indirect consequences of the high temperatures on aphid reproductive apparatus (Silbermann and Tatar, 2000). Heat exposure could have impaired oocyte development.

The lower number of nymphs produced by the heat stressed aphid during the first 24 h after the stress, could be a consequence of the heat induced coma soon after the heat treatment. I found that much of the heat exposed aphids were observed to be in a comatose state. In particular, aphid coma period after heat exposure ranged from few minutes to some hours. In the comatose state, heat stressed insects are immobile (Sørensen *et al*., 2001; Hazell *et al*., 2010) and a temporal block of reproduction occurs, as reported by Hazell *et al*. (2010) in the case of heat stressed *Myzus persicae*. Over the long term, the lower number of offspring produced by stressed aphids could be due to a reallocation of the energetic resources that ensure survival (Hoffmann *et al.*, 2003; Sørensen *et al.*, 2013) and to a reduction of the obligate symbiont *Buchnera*. Aphids always host the bacterium *Buchnera aphidicola*, that is an obligate endocellular symbiotic species (Moran *et al*., 2008). *Buchnera* provides the host with essential amino acids lacking in the aphid diet, and it is also involved in aphid thermal adaptation (Dunbar *et al*., 2007). This bacterium is indispensable to aphids, since phloem sap of plants has low concentrations of many essential amino acids and the host plant cannot satisfy the nutritional exigencies of aphids (Douglas, 1998). It is well

known that bacteria-free aphids grow poorly and produce few offspring (Douglas, 1998). *Buchnera* is particularly sensible to heat stress: Ohtaka and Ishikawa (1991) demonstrated that aphids can be rendered infertile by heat that kills *Buchnera* cells and Montllor *et al*. (2002) reported that, in the field, temperature of 25-30°C depress the *B. aphidicola* densities within the pea aphid *A. pisum*. The lower nymph production in the heat exposed aphid could be due to a reduction of *Buchnera* population (Ohtaka and Ishikawa,1991; Montllor *et al*., 2002).

Reduction of longevity and nymph production could also due to the consequences of the heat shock proteins up-regulation process in response to heat exposure. The evolutionary and ecological role of most of the inducible heat shock proteins (HSP27, HSP70, HSC70, *etc.*) predicts that their expression is induced under environmental stressful conditions and their up-regulation may negatively affect many biological traits, such as lifespan and fecundity (Hoffmann *et al.*, 2003; Sørensen *et al*., 2003). The role of the HSP90 in the reduction of nymph production could also be very important. Unlikely other HSPs, the HSP90 is a ubiquitous constitutively expressed chaperone protein required for cell viability since supports an essential group of conserved signal transduction proteins in nearly every organism and cell type examined (Rutherford and Zucker, 1994; Rutherford and Lindquist, 1998; Richter and Buchner, 2001; Nollen and Morimoto, 2002). It is documented that the HSP90 affects the oogenesis and the embryogenesis in insects (Ding *et al*., 1993; Yue *et al*., 1999; Song *et al*., 2007; Will *et al*., 2017). To investigate the role of HSP83 (a HSP90 homologous chaperone) on complex traits in viviparous aphids, Will *et al*. (2017) used the RNA interference technique (RNAi) to attenuate its expression. They found that the RNAi-mediated depletion of HSP83 expression reduced both longevity and fecundity and suggested that this chaperone plays an evolutionarily conserved function in insects. Surprisingly, HSP83 depletion reduced the number of viviparous offspring while simultaneously increased the number of premature nymphs developing in the ovaries. They then state that HSP83 plays a central role in the aphid embryogenesis and eclosion. In particular, Will *et al*. (2017) found a lower number of early-stage embryos in the ovaries of aphids injected with hsp83dsRNA, and they presumed that this was caused by the resorption of embryos, which generally occurs under suboptimal environmental conditions. As consequence, this allowed the late-stage embryos to reach maturity (Ward and Dixon, 1982). Stress-damaged proteins deviate Hsp90 from signal transduction proteins; despite transcriptional induction of Hsp90 and other chaperones by the heat shock response, it is believed that severe stresses in nature can temporarily crush the Hsp90 chaperone system (Rutherford and Lindquist, 1998; Milton *et al*., 2006). Keeping in mind the results (and

conclusions) of all these studies (Nollen and Morimoto, 2002; Will *et al.*, 2017; Rutherford and Lindquist, 1998; Milton *et al.,* 2006; Richter and Buchner, 2001), the lower offspring production observed in the heat shocked aphids during the first 10 days could be due, at least in part, to the detrimental effects of the HSP83 sequestration in the ovaries. All the hypotheses made so far to explain the reduction in longevity and productivity (aging, *Buchnera* symbiont, detrimental effects of inducible HSP, role of the HSP90) are not mutually exclusive and further studies are necessary to better understand the effects of high temperatures on aphid population dynamics.

References

Alford L, Blackburn T.M., Bale J.S., 2011. Effect of latitude and acclimation on the lethal temperatures of the peach-potato aphid *Myzus persicae*. Agricultural and Forest Entomology, 14(1): 69-79.

Asin L., Pons X., 2001. Effect of high temperature on the growth and reproduction of corn aphid (Homptera: Aphididae) and implication for their population dynamics on the northeastern iberian peninsula. Environmental Entomology. 30(6): 1127-1134.

Bale J.S., Masters G.J., Hodkinson I.D., Awmack C., Bezemer T.M., Brown V.K., Butterfield J., Buse A., Coulson J.C., Farrar J., Good J.E.G., Harrington R., Hartley S., Jones T.H., Lindroth R.L., Press M.C., Symrnioudis I., Watt A.D., Whittaker J.B., 2002. Herbivory in global climate change research: direct effect of rising temperature on insect herbivores. Global Change Biology, 8: 1–16.

Beetge L., Kruger K., 2019. Drought and heat waves associated with climate change affect performance of the potato aphid Macrosiphum euphorbiae. Scientific Reports, 9, 3645.

Ben-Ari M, Gish M., Inbar M., 2015. Walking aphids can partake in within-field dispersal to distant plants. Basic and applied Ecology, 16(2):162-171.

Brown J.H., Gillooly J.F., Allen A.P., Savage V.M., West G.B., 2004. Toward a metabolic theory of ecology. Ecology, 85: 1171-1789.

Cossin A.R., Bowler K., 1987. Temperature biology of animals. Chapman and Hall, London, New York.

Cox D.R., 1972. Regression models and life tables. Journal of the Royal Statistical Society: Series B, 187-202.

Deutsch A., Tewksbury J.J., Huey R.B, Sheldon K.S., Ghalambor C.K., Haak D.C., Martin P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences of the Unites States of America,105 (18) 6668-6672.

Diffenbaugh N.S., Field C.B., 2013. Changes in Ecologically Critical Terrestrial Climate Conditions. Science 341, 486–492.

Digilio M.C., 1995. Identificazione rapida degli stadi giovanili di Acyrthosiphon pisum (Harris) (HomopteraAphidoidea). Bollettino di Zoologia Agrararia e di Bachicoltura 27, 111–116.

Ding D., Parkhurst S.M., Halsell S.R., Lipshitz H.D., 1993. Dynamic Hsp83RNA localization during Drosophila oogenesis and embryogenesis. Molecular and Cell Biology, 13:3773–3781.

Dixon A.F.G., 1987: Parthenogenetic reproduction and the rate of increase in aphids. In Minks A.K. and Harrewijn P. (eds): Aphids Their Biology, Natural Enemies and Control. Vol. A. Elsevier, Amsterdam, pp. 269–287.

Dixon A.F.G., 1998. Aphid ecology, An optimisation approach. 2nd ed. Chapman & Hall, Glasgow and London, 300 pp.

Douglas A.E., 1998. Nutritional interaction in insect-microbial simbioses: aphids and their symbiotic bacteria. Annual Review of Entomology, 43: 17-37.

Dunbar H. E., Wilson A. C. C, Ferguson N. R., Moran N. A., 2007. Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. PloSBiol, 5(5): e96.

Flatt T., Hyland A., 2011. Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. Oxford University Press, New York.

Gadgil M., Bossert W.H., 1970. Life historical consequences of natural selection. The American Naturalist, 104(935), 1-24, 1970.

Harshman L.G., Zera A., 2007. The cost of reproduction: The devil in the details. Trends in Ecology and Evolution, 22(2):80-6.

Hazell S.P., Neve B.P., Groutides C., Douglas A.E., Blackburn T.M., Bale J.S., 2010.Hyperthermic aphids: Insights into behaviour and mortality. Journal of Insect Physiology, 56(2): 123-131.

Hercus M.J., Loeschcke V., and Rattan S.I.S., 2003. Lifespan extension of Drosophila melanogaster through hormesis by repeated mild heat stress. Biogerontology, 4(3): 149-156.

Hoffmann A.A., Parsons P.A., 1997. Extreme environmental change and evolution. Cambridge: Cambridge University Press.

Hoffmann A.A., Parsons P.A., 1991. Evolutionary genetics and environmental stress. Oxford University Press.

Hoffmann A.A., Sørensen J.G., Loeschcke V., 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. Journal of Thermal Biology, 28: 175–216.

Huey R.B., Berrigan D., 2001. Temperature, demography, and ectotherm fitness. The American Naturalist, 158(2): 204-10.

IPCC, 2018. Global Warming of 1.5°C. Special Report Intergovernmental Panel on Climate Change.

Kingsolver J.G., Diamond S.E., Buckley L.B., 2013.Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Functional Ecology, 27(6): 1415-1423.

Kirkwood T.B.L., 1977. Evolution of ageing. Nature, 270(5635): 301-304.

Kojima K., Kimura M.T., 2003. Life history adaptations and stress tolerance of four domestic *Drosophila*. Entomological Science, 6(3), 135-142.

Lu Z.C., Wan F.H., 2011. Using double-stranded RNA to explore the role of heat shock protein genes in heat tolerance in *Bemisia tabaci* (Gennadius). Journal of Experimental Biology, 214: 764-769.

Ma C.S., Hau B., Poehling H.M., 2004. The effect of heat stress on the survival of the rose grain aphid, *Metopolophium dirhodum* (Hemiptera: Aphididae). European Journal of Entomology, 101,327-332.

Marengo J.A., Jones R., Alves L.M., Valverde M.C., 2009. Future change of temperature and precipitation extremes in South America as derived from the PRECIS regional climate modeling system. International Journal of Climatology, 29, 2241–2255.

Mehrparvari M., Hatami B., 2007. Effect of temperature on some biological parameters of an Iranian population of the Rose Aphid, Macrosiphum rosae (Hemiptera: Aphididae). European Journal of Entomology, 104: 631–634.

Milton C.C., Ulane C.M., Rutherford S., 2006. Control of canalization and evolvability by Hsp90. PLoS ONE 1.

Miyatake T., 1997. Genetic trade-off between early fecundity and longevity in Bactrocera cucurbitae (Diptera: Tephritidae). Heredity, 78 (1):93-100.

Montllor C. B., Maxman A., Purcell A. H. 2002.Facultative bacterial endosymbionts benefit pea aphids Acyrthosiphon pisum under heat stress. Ecological Entomology, 27, 189–195.

Moran N. A., J. P. McCutcheon, A. Nakabashi, 2008. Genomic and evolution of heritable bacterial symbionts. Annual Review of Genetic, 42: 165-190.

Nollen E.A., Morimoto R.I., 2002. Chaperoning signaling pathways: molecular chaperones as stress-sensing ‘heat shock’ proteins. Journal of Cell Science, 115: 2809–2816.

Ohtaka C., Ishikawa H., 1991. Effects of heat treatment on the symbiotic system of an aphid mycetocyte. Symbiosis, 11, 19–30.

Partridge L., Harvey P.H., 1985. Evolutionary biology: costs of reproduction. Nature, 316: 20-21

Peters R.H., 1983. The ecological implications of body size. Cambridge University Press, Cambridge.

Reznick D.N., 1992. Measuring the costs of reproduction. TREE, 7: 42-45.

Richter K., Buchner J., 2001. Hsp90: chaperoning signal transduction. Journal of Cell Physiology, 188: 281–290.

Roff, D. A. 1992. The Evolution of Life Histories. Chapman and Hall, New York.

Rutherford S.L., Lindquist S. 1998. Hsp90 as a capacitor for morphological evolution. Nature, 396: 336–342.

Rutherford S.L., Zuker C.S., 1994. Protein folding and the regulation of signaling pathways. Cell, 79: 1129–1132

Schowalter T.D., 2000. Insect ecology. An ecosystem approach. Elsevier, 576 pp.

Sejerkilde M., Sørensen J.G., Loeschcke V., 2003. Effect of cold- and heat hardening on thermal resistance in *Drosophila melanogaster*. Journal of Insect Physiology, 49(8): 719-726.

Silbermann R., Tatar M., 2000. Reproductive costs of heat shock protein in transgenic *Drosophila melanogaster*. Evolution, 54: 2038-2045.

Song Y., Fee L., Lee T.H., Wharton R.P., 2007. The molecular chaperone Hsp90 is required for mRNA localization in *Drosophila melanogaster* embryos. Genetics, 176:2213–2222.

Sorensen J.G., Kristensen T.N., Loeschcke V., 2003. The evolutionary and ecological role of heat shock proteins. Ecology Letters, 6:1025–1037.

Sørensen J.G., Loeschcke V., Kristensen T.N., 2013. Cellular damage as induced by high temperature is dependent on rate of temperature change - investigating consequences of

ramping rates on molecular and organismal phenotypes in *Drosophila melanogaster*. Journal of Experimental Biology, 216: 809–814.

Sørensen J.G., Dahlgaard J., Loeschcke V., 2001. Genetic variation in thermal tolerance among natural populations of Drosophila buzzatii: down regulation of Hsp70 expression and variation in heat stress resistance traits. Functional Ecology, 15, 289–296.

Sørensen J.G., T.N. Kristensen T.N., J. Overgaard J., 2016. Evolutionary and Ecological patterns of thermal acclimation capacity in Drosophila: is it important for keeping up with climate change? Current Opinion in Insect Science. 17, 98-104.

Stearns S.C. 1992. The evolution of life histories traits. Oxford University Press, Oxford.

Tamaki G., Weiss M.A., Long G. E., 1980. Impact of high temperatures on the population dynamics of the green peach aphid in field cages. Environmental Entomology, 9(3): 331-337.

Terblanche, J.S., Hoffmann, A.A., Mitchell, K.A., Rako, L., le Roux, P.C., Chown, S.L., 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. Journal of Experimental Biology, 214, 3713–3725.

Ward S.A., Dixon A.F.G., 1982. Selective resorption of aphid embryos and habitat changes relative to life-span. Journal Animal Ecology. 51:859–864.

Warton D.I. and F.K.C. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology, 92(1): 3-10.

West-Eberhard M.J, 2003. Developmental plasticity and evolution. Oxford University Press.

Will T., Schmidtberg H., Skaljae M., Vileinskas A., 2017. Heat shock protein 83 plays pleiotropic roles in embryogenesis, longevity, and fecundity of the pea aphid Acyrthosiphon pisum. Development Genes and Evolution, 227:1-9.

Yue L., Karr T.L., Nathan D.F., Swift H., Srinivasan S., Lindquist S., 1999. Genetic analysis of viable Hsp90 alleles reveals a critical role in Drosophila spermatogenesis. Genetics 151:1065-1079.

Zera A.J., Harshman L.G., 2001. The Physiology of Life History Trade-Off in Animals. Annual Review of Ecology and Systematics, 32:95-126.

Zhao F., Hoffman A.A., Xing K., Ma C., 2017. Life stage of an aphid living under similar thermal conditions differ in thermal performance. Journal of Insect Physiology. 99, 1-7.

Zhao L., Clark G.G., Linchitumk L., 2010. Expression of AeaHsp26 and AeaHsp83 in *Aedes aegypti* (Diptera: Culicidae) larvae and pupae in response to heat shock stress. Journal of Medical Entomology, 46: 490-495.

Zhou X., Carter N., 1992. Effect of temperature, feeding position and crop growth stage on the population dynamics of the rose grain aphid, *Metopolophium dirhodum* (Hemiptera:Aphididae). Annals of Applied Biology, 121(1): 27-37.

Zhu L., Wang L., Ma C.S., 2019. Sporadic short temperature events cannot be neglected in predicting impacts of climate change on small insects. Journal of Insect Physiology, 112: 48-56.

CHAPTER II

The aphid *Acyrthosiphon pisum* exhibits a greater survival after a heat shock when parasitized by the wasp *Aphidius ervi*

Introduction

An increase in the frequency of extreme thermal events is expected as a result of global warming (Kunkel *et al*., 1999; Meehl *et a*l., 2000;Vose *et al*., 2005; Ballester *et al*., 2009; Marengo *et al*., 2009; Diffenbaugh and Field, 2013; Miyan, 2015). The extreme thermal events, together with the mean global warming, may produce important effects on biodiversity since they affect species distributions, life histories, community composition, ecosystem function and biotic interactions (Hoffmann and Parsons, 1991; Bale *et al*., 2002; Johnston and Bennett, 2008; Folguera *et al*., 2009; Estay *et al.*, 2014; Miyan, 2015; Bozinovic *et al*., 2016a) . Predictions on the ability of an organism to respond to climate change and to extreme environmental conditions are considered important topics (Gilchrist *et al*., 2004; Pörtner *et al.*, 2006; Huey *et al.*, 2012; Gunderson and Stillman, 2015).

The development and survival of ectotherms are linked to environmental temperatures and the higher frequencies of extreme conditions constitute a challenge for these organisms (Folguera *et al.*, 2009; Bozinovic *et al*., 2013, 2016b). The development under sub-optimal conditions and/or the exposure to short severe stress negatively affect the life history traits of insects (Hoffmann and Hewa-Kapuge, 2000; Huey and Berrigan, 2001; Trotta *et al.*, 2006; Hance *et al*., 2007; Loeschcke and Hoffmann, 2007; Cui *et al*., 2008; Roux *et al*., 2010; Jeffs and Leather, 2014). Ideally, resistance measures should be linked to the kind of stress, defined as a condition that decreases fitness (Hoffmann and Parsons, 1991) the organisms will experience in the field. For some studies, measuring this can be important but difficult when there is incomplete information about the stress levels experienced by different species interacting in nature, including occasional stress exposures. Some laboratory procedures, like exposure to different rearing temperatures or to different heat stresses, can reflect measures of the stress experienced in nature (Hoffmann *et al*., 2003). One way of assessing heat resistance is by measuring survival following exposure to potentially lethal temperatures across a definite exposure time (Hoffmann *et al*., 2003; Cui *et al*., 2008; Hazell *et al*., 2010; Mironidis and Savopoulou-Soultani, 2010; Roux *et al*., 2010).

The biological aspects of organism vulnerability will also depend on the various stages of development (Feder and Hofmann, 1999; Bowler and Terblanche, 2008) and on how heat stress alters the interactions with competitors, predators, parasites, diseases and mutualists

(Lagos *et al*., 2001; Pincebourde and Casas, 2006; Gilman *et al*., 2010; Harley, 2011; Huey *et al*., 2012). The impact of stressful temperatures is likely even more important in higher trophic levels that depend on the capacity of the lower trophic levels to adapt to these changes. As parasitoid develop in or on hosts, a severe impact of climatic changes is expected for these organisms (Jeffs and Lewis, 2013), since they represent the third and the fourth trophic levels (Godfray, 1994). For example, koinobiont parasitoids, which maintain a strict physiological relationship with their arthropod hosts in their early larval life, must protect themselves not only against the immune system of their host, but at the same time they should avoid lethal conditions by manipulating their hosts using behavioural and/or physiological adjustments (Lagos *et al*., 2001; Hance *et al*., 2007). Exposure to extreme temperatures can then have important effects on the outcome of host-parasite interactions (Hance *et al.*, 2007; Cayetano and Vorburger, 2013) or, more generally, can have important effects on higher trophic levels (Harrington *et al*., 2001).

The model species used in this study are the pea aphid *A. pisum* and the parasitoid *A. ervi*. *A. ervi* can parasitize both the adult and the four aphid instars, even though they are not equivalent for parasitoid fitness, by laying a single egg. The larva develops inside the aphid through 3 larval stages until the formation of a mummy (the skeletonised aphid).

A. pisum displays a number of antipredator behaviours in response to predators and parasitoids, such as releasing an alarm pheromone (Bowers *et al*., 1972) and/or dropping or walking away from the threatened feeding site (Chau and Mackauer, 1997). It is also known that the antipredator behaviour of an aphid changes as a function of internal stress (Villagra *et al*., 2002) or of increased temperatures (Ma and Ma, 2012a, 2012b).

In general, when an endoparasitoid like *A. ervi* parasitizes a living host, factors of maternal origin that facilitate successful parasitism are also injected at oviposition, such as venom and ovarian proteins (Webb, 1998; Beckage, 1997; Digilio *et al*., 2000). All the parasitoid female secretions interfere both with the immune system and endocrine balance of the host (Digilio *et al*., 1998, 2000; Webb, 1998), hindering the encapsulation of the parasitoid egg, causing oxidative stress in ovarian cells and leading to the degeneration of the host germinal cells and of young sub-apical aphid embryos (Digilio *et al*., 2000; Pennacchio and Strand, 2005; Falabella *et al*., 2007). Other host regulation factors are of embryonic origin, derived from the serosal membrane or from other embryo-associated tissues (teratocytes) and are strictly linked to larval parasitoid survival by processing nutriments extracted from internal tissues of the host (Quicke, 1997; Falabella *et al*., 2000, 2009; Grossi *et al*., 2016). At the same time, the fight against parasitization begins with the activation of the host immune system or

through microbial symbiont-based defenses (Strand and Pech, 1995; Oliver *et al*., 2009; Oliver and Martinez, 2014; Martinez *et al*., 2016).

In this study, I investigate some aspects related to the survival of aphids after exposure to a very high temperature in a trophic model system consisting of the parasitoid *A. ervi* and its host *A. pisum*.

Aphids at different developmental stages vary in many traits, including size and physiology, and may experience different microhabitats.The effects of the temporal pattern and duration of temperature exposure (the "time-dependent effects", Kingsolver and Woods, 2016) as well as the long-lasting effects of thermal stress (Roitberg and Mangel, 2016) on insect performance could be very important. Incorporating these effects on the different developmental stages of an organism is essential for making more realistic predictions on ecological responses of organisms to climate change (Kingsolver *et al*., 2011; Kingsolver and Woods, 2016; Roitberg and Mangel, 2016).The response of insects with complex life cycles to heat stress depends on the thermal sensitivity of all stages, reflecting differences in thermal environments they experience (Gilchrist *et al*., 1997; Zhao *et al*., 2017). The first aim of this study was then to understand if aphids at various stages of development show different levels of survival after exposure to an extreme temperature.

Based on some preliminary experiments on the thermal tolerance of *A. pisum* it was observed that parasitized adult aphids were more resistant to a severe heat shock (exposure at 39.1°C for 30 minutes) than unparasitized aphids of the same age. As stated above, successful parasitism depends on parasitoid factors of maternal origin injected at oviposition and on factors of embryonic origin. All these factors interfere with the host physiology at different times and in different ways, necessarily causing aphid internal stress. I therefore examined whether parasitized aphids of different ages, that is, harboring parasitoids at different developmental stages and consequently subjected to different physiological modifications, showed a differential heat stress resistance compared with the unparasitized ones.

Materials and methods

Insect rearing

A. pisum colony was started in 1985 from a few hundred specimens collected in the field from alfalfa (*Medicago sativa* L.) near Salerno, Italy and laboratory reared on broad bean plants (*Vicia faba* L.). *A. ervi* parasitoids were obtained from Koppert Italia and were laboratory-reared on *A. pisum*. Aphid and parasitoid cultures were maintained in two separate climatic chambers Binder KBF at 22 ± 1°C and 75 ± 5% relative humidity (mean values ± accuracy), under an LD 18:6 h photoperiod. Broad bean plants of the Moroccan

cultivar “Aguadulce” were grown in pots (10 cm diameter) containing commercial soil (COMPO SANA® Universal Potting Soil) in a greenhouse. Since all the experiments required same-aged aphids, approximately 120 adult virginoparae females were isolated from the mass rearing colony and put on a fresh potted broad bean plant kept in a plastic box (22 x 15 cm x 40 cm height) for 6 h at 22 ± 1°C, 75 ± 5% RH and 18:6 LD photoperiod. Adult females were then removed and discarded. The newborn nymphs were maintained as a synchronous colony on a broad bean plant for 72 h, roughly corresponding, at this rearing temperature, to the beginning of the third nymphal instar. Nevertheless, before their use in the experimental trials, aphids were inspected under a stereo-microscope and all nymphs that were not in the appropriate stage, according to the morphological features as in Digilio (1995) were discarded. Twenty-five independent synchronous colonies were generated.

The parasitoid females used in the experiment were between 24 and 48 h old. Before the experiment, each newborn female was left for 24 h with two males and fed on water and honey. Different aphid instars, even though all accepted as hosts, are not equivalent in terms of parasitoid successful development. Aphids at the beginning of the third nymphal instar were used in this experiment since they allow a successful elevated parasitoid development (Trotta *et al*., 2014) and their experimental handling implies a lower mortality than in younger instars. Each synchronous colony of three day old aphids was subsequently split into two groups and maintained on two separate plants (about 50 aphids per plant): at different times, the aphids of one group were parasitized by *A. ervi* whereas the other group was composed of unparasitized aphids used as controls.

To avoid possible effects of the plant on the growth of aphids (Guldemond *et al*., 1998), young vegetative plants (3 weeks after sowing seeds) were used in all the experiments.

The time of the aphid transition from the first nymphal instar to adult (I, II, III, IV, adult) was independently recorded for control and parasitized aphids. Aphids were visually inspected twice a day and the time of nymphal instar transition was recorded.

Parasitization experimental procedure

The parasitized aphids used in these experiments were observed to be stung once by a parasitoid female. In *A. ervi,* the decision to accept or reject a host follows the insertion of the ovipositor (Pennacchio *et al.*, 1994; Larocca *et al.*, 2007). In a small number of instances there could be no insertion of egg in the host, and there is no way of knowing for sure if an aphid hosts a parasitoid egg without dissecting it. For this reason, we estimated the parasitization rate using a control group with 15 replicates of about 20 parasitized aphids that were dissected under a stereomicroscope five days after *A. ervi* oviposition.

The parasitized aphids, as well as the control ones, were maintained on plants in an environmental chamber at 22 ± 1°C, 75 ± 5% RH and 18:6 LD to continue their development until they were used in the heat shock treatments. Preliminary experiments showed these manipulations caused negligible aphid mortality (less than 2%). Five experimental treatments of parasitized and unparasitized aphids were successively established according to which day after parasitization (DaP) the heat shock treatment was applied, that is, aphids heat shocked one, two, three, four or five days after parasitization (Fig. 2.1).

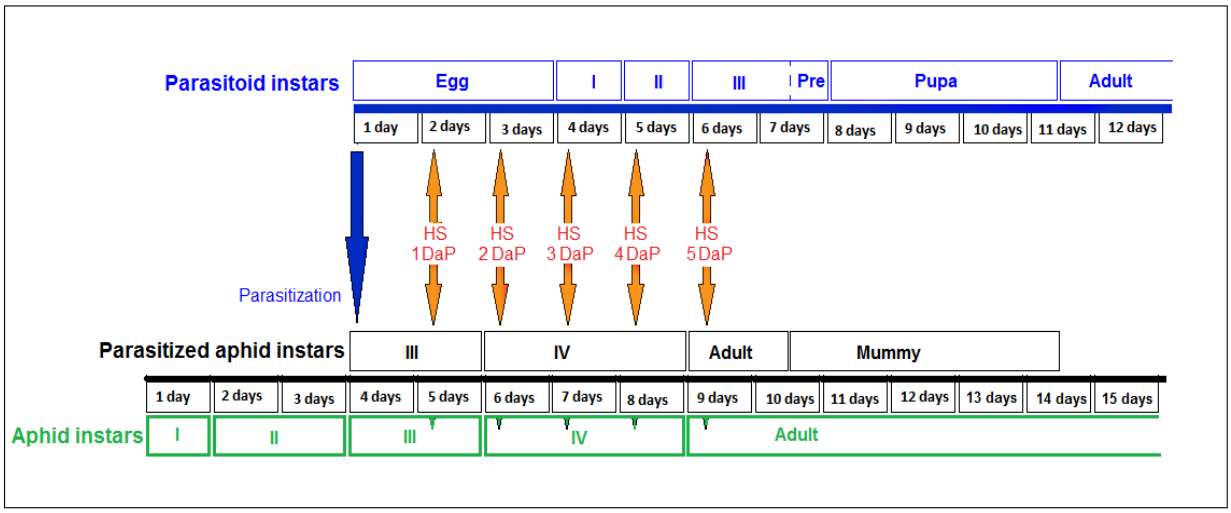

Fig.2.1 - Schematic representation of the experimental procedure. Timing of the experimental procedure in relation to the developmental stages of the aphids (parasitized and unparasitized) and the parasitoids at 22°C. DaP is the day after parasitization in which the heat shock treatment was applied (red arrows). The control of each treatment consisted of aphids of the same age but unparasitized. As a result of this experimental design, five experimental (1-DaP, 2-DaP, 3-DaP, 4-DaP, 5-DaP) and five control treatments were obtained. Each treatment consisted of five replicates (five independent aphid synchronous colonies) and a replicate was composed of three (or four, when available) separate groups each one of about ten aphids, for a total of 150-160 aphids per treatment. The parasitized aphids from each of the five treatments were dissected 24 hours after heat shock to record the time of the parasitoid egg / nymphal instar transition as in Pennacchio *et al*. (1999).

Heat shock experiments on parasitized and control aphids

The ten aphids belonging to each experimental group were gently moved from the plant and placed in a 50 ml Falcon® tube with a mesh covered ventilation hole in the screw-top within a water bath at 39.0 ± 0.1°C. Heat resistance has been assessed by measuring survival of unparasitized (control) and parasitized aphids following exposure to the potentially lethal temperature of 39 ± 0.1°C for an exposure time of 30 minutes, a technical method commonly used in *Drosophila* (Hoffmann *et al*., 2003). The choice of the stress temperature is based on preliminary results on heat shock experiments on unparasitized adult aphids at different temperatures, since at 38.5 ± 0.1°C the survival rate was about 80% whereas at 39.5 ± 0.1°C

the survival rate was close to 0%. Lethality is, however, a function of both temperature and time (Hoffmann *et al*., 2003).

An exposure of 30 minutes was chosen since this time interval, compared with shorter exposure times, caused significant changes in the survival of aphids (personal observation). In addition, this time interval minimised the chance of any heat hardening response during the heat shock (Hazell *et al*., 2010) and, in general, did not produce a significant impact of starvation and/or desiccation on the experimental individuals, effects that should be considered when longer exposure times are used (Terblanche *et al*., 2011).

The aphids were transferred after the heat shock treatment on a cut broad bean leaf, with the stalk inside an Eppendorf® tube filled with water and sealed with parafilm® to prevent desiccation, placed in a plastic cylinder (150 ml) with a mesh covered ventilation hole in the screw-top. The plastic cylinders were placed in an environmental chamber at 22 ± 1°C (mean values ± accuracy) and the survival was recorded after 24 h. Survival was considered to occur if the aphids were able to walk or if they moved when gently touched by a brush. This response was checked 24 h after heat exposure, as many insects are immobilized for some time following a heat stress (Hazell *et al*., 2010; Sørensen *et al*., 2001). Survival of the parasitized aphids from each treatment was then compared with the appropriate control.

Statistical analysis

A linear mixed model has been considered as a possible model for analysis of the percentage and of the percentage after a square root arcsine transformation of aphid survival after heat shock given that the sample size is quite similar for all the experimental groups. However, the raw data have a discrete probability distribution (alive/dead) and a binomial generalized linear model with a logit link function has also been considered for the identification of the best model. We finally chose the linear model performed on the percentage of aphid survival after a square root arcsine transformation because it has the lowest Akaike Information Criterion (AIC). Therefore, this model best approximates the process that generated the observed data, minimizing the lack of model fit to the observed data (Johnson and Omland, 2004). The arcsine transformation also improved the error distribution and amended the heterogeneity of variances (Levene's tests: $F_{9, 145} = 1.54$, $P = 0.14$).

Data on aphid survival after heat shock were therefore analyzed using a mixed linear model ANOVA in which "treatment" (two levels: parasitized and unparasitized aphids) and "DaP" (five levels: 1-DaP, 2-DaP, 3-DaP, 4-DaP, 5-DaP) were the main fixed effects; "replicate" (five levels) was nested within treatment and DaP. This analysis was used to test for treatment and DaP differences, as well as for their interaction, using the variation among replicates as

the error term. In order to detect significant differences between parasitized and unparasitized aphids, a Tukey *post-hoc* test for multiple comparisons of means was also performed. All the analyses in this study were carried out using R.3.2.4 revisited software (R Core Team, 2016).

Results

Parasitoids and aphid nymphal instars

In the present experiment, parasitized and unparasitized aphids from the third nymphal instar to adult and parasitoids from the egg stage to the fourth instar larvae were heat shocked. Under our experimental conditions, the parasitization rate (assessed by dissecting 15 replicates of about 20 parasitized aphids) was 86.7% ± 0.14 (mean ± S.E.).

The mean duration of each of the nymphal instars of the aphids (parasitized and unparasitized) and parasitoids are shown in Fig. 2.2. A specific nymphal stage was considered completed when 90% of the specimens showed the morphological traits of the next one. No differences were observed between parasitized and unparasitized aphids in the mean duration of each of the nymphal instars.

Aphid survival after heat shock

The survival of parasitized and unparasitized aphids after a heat shock at 39.0 ± 0.1°C for 30 min was significantly different (Fig. 2.2).

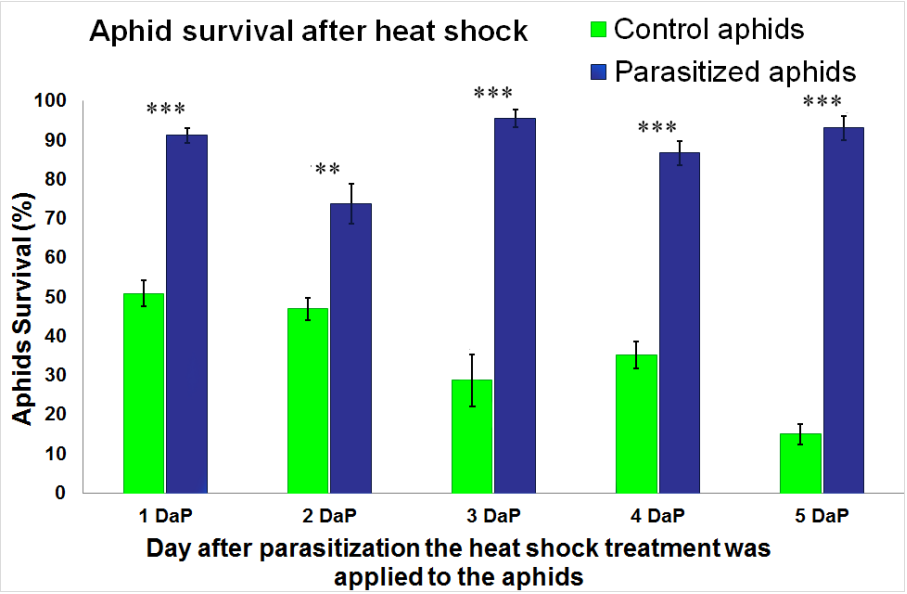

Fig. 2.2. - Mean values (± standard errors) of survival of control and parasitized aphids after a heat shock at 39.0 ± 0.1°C for 30 min. DaPs are the days after parasitization the heat shock treatment was applied to the aphids. Asterisks on bars indicate significant differences between control and parasitized aphids (Tukey's test, **: $P<0.01$ and ***: $P<0.001$).

The linear mixed model ANOVA performed on the survival data after arcsine transformation (Table 1) shows significant differences among replicates nested within DaP and treatment, indicating a certain level of heterogeneity among aphids in response to heat shock treatment, independently of the experimental groups identity.

Table 1 - Results of the mixed model ANOVAa on the survival of aphids after heat shock. "Treatment "(two levels: parasitized and unparasitized aphids) and "DaP" (day after parasitization aphids were heat shocked; five levels: 1-DaP, 2-DaP, 3-DaP, 4-DaP, 5-DaP) are fixed effects; "replicate" (five levels) is nested within "treatment" and "DaP". The variation among replicates was used as error term in the F tests for "treatment", "DaP" and their interaction.

Source of variation	*df*	MS	*F*
DaP	4	0.149	1.53 n.s.
Treatment	1	19.75	202.7***
DaP X treatment	4	0.720	7.39***
Replicate within DaP and treatment	40	0.097	3.47***
Residuals	105	0.028	

n.s., not significant; *df*, degrees of freedom; MS, mean square; *F*, variance ratio.
[a] Model: $X_{ijke} = \mu + D_i + T_j + DT_{ij} + R(D(T))_{k(i(j))} + E_{e(ijk)}$.
*** $P < 0.001$.

n.s., not significant; df, degrees of freedom; MS, mean square; F, variance ratio. a Model: Xijke = μ + Di + Tj + DTij + R(D(T))k(i(j)) + Ee(ijk). *** P<0.001.

This heterogeneity is particularly evident for the control treatment at 3-DaP and for the parasitized treatment at 2-DaP. The effects of treatment, DaP and their interaction were then compared with the replicates effect. The DaP effect was not significant, while the effects of treatment (parasitized *vs* unparasitized aphids) and the interaction "DaP X treatment" were found highly significant. Aphid survival after heat shock was significantly higher for the parasitized individuals than for the unparasitized ones. This result is independent of the number of days elapsing between the parasitization and the application of the heat shock treatment and therefore, independent of the aphid age.

It is also interesting to note that the survival of parasitized aphids is basically constant in the different DaPs. Survival of control (unparasitized) aphids after heat shock decreases with the increase of the aphid age; this trend is particularly evident for adult aphids (5-DaP control) that significantly differ from the other groups ($P< 0.001$) except for 3-DaP control. These trends implicate that survival differences between parasitized and unparasitized aphids increase when older aphids were heat shocked (significant interaction "DaP X treatment",

Tab.2.2). We should also underline that the higher thermal resistance in the parasitized groups is probably slightly underestimated because the real "parasitization rate" was about 86% (see Results 3.1).

Discussions and Conclusions

Climate change could affect population dynamics of insects producing important impacts in trophic interactions since thermal fluctuation may be an important selective factor in nature (Folguera *et al*., 2009; Bozinovic *et al*., 2013, 2016a, 2016b; Jeffs and Leather, 2014).This study considers the consequences of a severe heat shock for a short exposure time in a trophic model system consisting of the parasitoid *A. ervi* and its host *A. pisum*. We found that, after a thermal stress, parasitized aphids show a significantly greater survival than unparasitized ones.

As stated in the Introduction, successful parasitism of *A. ervi* strongly depends on factors of maternal origin such as venom and ovarian proteins injected at oviposition as well as on host regulation factors of embryonic origin (Beckage, 1997; Quicke, 1997; Webb, 1998; Digilio *et al*., 2000; Pennacchio and Strand, 2005; Falabella *et al*., 2000, 2007).

In the aphid *Macrosiphum euphorbiae*, it has been shown that the resistance to *A. ervi* parasitism is mediated, among other things, by an upregulation of several aphid and symbiont proteins (Nguyen *et al.*, 2008), including the early increase of the Hsp60 chaperone. When the ectoparasitoid *Bracon hebetor* envenomizes its host *Plodia interpunctella*, small hsp and the heat shock cognate 70 genes are upregulated 48 – 96 h after envenomation (Shim *et al.*, 2008). Other heat shock genes (*hsp23* and *hsp70*) expression are also upregulated in the flesh fly *Sarcophaga crassipalpis* 13 hours after envenomation by the ectoparasitic wasp *Nasonia vitripennis* (Rinehart *et al.*, 2002).

Our results clearly show that the increased survival after the heat shock of parasitized aphids occurs already at an early stage of parasitization (1-DaP, 24 h after parasitization), that is, before the hatching of the parasitoid egg. On this basis, we can exclude that the aphid heat resistance is linked to the presence of the parasitoid larva or to the appearance of factors of embryonic origin such as the teratocytes. The increased survival displayed by parasitized aphids could be linked in a direct way to the parasitoid egg insertion and to the action of venom and ovarian proteins; all these factors cause host stress (Digilio *et al*., 2000; Pennacchio and Strand, 2005; Falabella *et al*., 2007). The results of Shim *et al*. (2008) and Rinehart *et al*., (2002) suggest a link between the parasitoid venom and HSPs.

Another explanation can be an indirect effect through the activation of the aphid immune system (Strand and Pech, 1995; Strand, 2008; Oliver *et al*., 2009; Oliver and Martinez, 2014;

Martinez *et al*., 2016). It is also interesting to note that the increased survival displayed by parasitized aphids after heat shock seems to be independent of the infection with the facultative bacterial symbionts (Heyworth and Ferrari, 2016). Other kinds of experiments are needed to assess the mechanisms behind the increased survival of the parasitized aphids.

Aphids at various stages of development vary in size, morphology, physiology, behaviour and, based on the results of this experiment, they also exhibit different survival after exposure to an extreme temperature. Specifically, aphid survival decreases with the age at which the heat shock treatment was applied. The lowest survival was displayed by the unparasitized adult aphids. Similar results were observed in the aphid *Sitobion avenae* (Fabricius), where a lower basal tolerance was found in younger nymphs, increasing in older nymphs (3rd and 4th-instars) and then decreasing again in adults (Zhao *et al*., 2017). It is known that apterous aphids are able to move in response to extrinsic perturbations (Irwin *et al*., 2007) and that apterous *A. pisum* adults move, on average, farther and cover longer distances than nymphs (Ben-Ari *et al.*, 2015). The greater survival found in the younger nymphs could be a consequence of an adaptation to the microclimatic conditions these aphid life stages experience since nymphal instars of *A. pisum* are more sedentary and then more exposed to extreme temperatures than adults. The lower survival found in the adult stage might also reflect a trade-off between heat tolerance and reproductive output (Zhao *et al*., 2017). When aphids are parasitized by *A. ervi*, all the survival differences after heat shock among the different stages of development disappear as a consequence of the strong internal stress caused directly or indirectly by the parasitization.

Lagos *et al*. (2001) observed a different thermal sensitivity in *A. pisum* parasitized by *A. ervi* mediated by a different behaviour response, that is, a different walking activity. In the present study, we show evidence that parasitism in the same system can also affect the host thermal sensitivity through a physiological mechanism whose exact nature needs to be further investigated. To our knowledge, this is one of the first papers that has reported a greater survival after a heat shock due to a metazoan parasite. High temperatures and drought are known to induce many changes in hosts and parasitoids and can generate a disjunction between their population growth rates, affecting the role of parasitoids in pest control (Romo and Tylianakis, 2013). To avoid high temperatures, aphids may also drop off host plants, reducing the efficiency of parasitoid attacks (Ma and Ma, 2012a, 2012b).

References

Bale J.S., Masters G.J., Hodkinson I.D., Awmack C., Bezemer T.M., Brown V.K., Butterfield J., Buse A., Coulson J.C., Farrar J., Good J.E.G., Harrington R., Hartley S., Jones T.H., Lindroth R.L., Press M.C., Symrnioudis I., Watt A.D., Whittaker J.B., 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biology, 8: 1-16.

Ballester J., Giorgi F., Rodò X., 2009. Changes in European temperature extremes can be predicted from changes in PDF central statistics. Climate Change, 98: 277-284.

Beckage N.E., 1997. The parasitic wasp's secret weapon. Scientific American, 277: 82-87.

Ben-Ari M., Gish M., Inbar M., 2015. Walking aphids can partake in within-field dispersal to distant plants. Basic and Applied Ecology, 16: 162-171.

Bowers W.S., Nault L.R., Webb R.E., Dutky S.R., 1972. Aphid alarm pheromone: isolation, identification, synthesis. Science, 177: 1121-1122.

Bowler K., Terblanche J.S., 2008. Insect thermal tolerance: What is the role of ontogeny, ageing and senescence? Biological Review, 83, 339–355.

Bozinovic F., Catalan T.P., Estay S.A., Sabat P., 2013. Acclimation to daily thermal variability drives the metabolic performance curve. Evolutionary Ecology Research, 15, 579–587.

Bozinovic F., Medina N.R., Alruiz J.M., Cavieres G., Sabat P., 2016a. Thermal tolerance and survival responses to scenarios of experimental climatic change: changing thermal variability reduces the heat and cold tolerance in a fly. Journal of Comparative Physiology. B Biochem. Syst. Environ. Physiol. 186, 581–587.

Bozinovic F., Sabat P., Rezende E.L., Canals M., 2016b. Temperature variability and thermal performance in ectotherms: Acclimation, behaviour, and experimental considerations. Evolutionary Ecology Research, 7, 111–124.

Cayetano L., Vorburger C., 2013. Effects of heat shock on resistance to parasitoids and on life history traits in an aphid/endosymbiont system. PLoS One 8(10): e75966.

Chau A., Mackauer M., 1997. Dropping of pea aphids from feeding site: A consequence of parasitism by the wasp, Monoctonus paulensis. Entomologia Experimentalis Applicata, 83, 247–252.

Cui X., Wan F., Xie M., Liu T., 2008. Effects of heat shock on survival and reproduction of two whitefly species, Trialeurodes vaporariorum and Bemisia tabaci Biotype B. Journal Insect Science, 8, 1–10.

Diffenbaugh N.S., Field C.B., 2013. Changes in Ecologically Critical Terrestrial Climate Conditions. Science 341, 486–492.

Digilio M.., Pennacchio F., Tremblay E., 1998. Host regulation effects of ovary fluid and venom of Aphidius ervi (Hymenoptera: Braconidae). Jounral of Insect Physiology, 44, 779–784.

Digilio M.C., 1995. Identificazione rapida degli stadi giovanili di Acyrthosiphon pisum (Harris) (Homoptera Aphidoidea). Bollettino di Zoologia Agraria e di Bachicultura, 27, 111–116.

Digilio M.C., Isidoro N., Tremblay E., Pennacchio, F., 2000. Host castration by Aphidius ervi venom proteins. Journal of Insect Physiology, 46, 1041–1050.

Estay S.A., Lima M., Bozinovic F., 2014. The role of temperature variability on insect performance and population dynamics in a warming world. Oikos 123, 131–140.

Falabella P., Riviello L., Caccialupi P., Rossodivita T., Teresa Valente M., De Stradis, L.M., Tranfaglia A., Varricchio P., Gigliotti S., Graziani F., 2007. A γ-glutamyl transpeptidase of Aphidius ervi venom induces apoptosis in the ovaries of host aphids. Insect Biochemistry and Molecular Biology, 37, 453–465.

Falabella P., Riviello L., De Stradis M.L., Stigliano C., Varricchio P., Grimaldi A., de Eguileor M., Graziani F., Gigliotti S., Pennacchio F., 2009. Aphidius ervi teratocytes release an extracellular enolase. Insect Biochemistry and Molecular Biology, 39, 801–813.

Falabella P., Tremblay E., Pennacchio F., 2000. Host regulation by the aphid parasitoid Aphidius ervi: the role of teratocytes. Entomologia Experimentalis et Applicata, 97, 1–9.

Feder M.E., Hofmann G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual Review Physiology, 61, 243–282.

Folguera G., Bastías D.A., Bozinovic F., 2009. Impact of experimental thermal amplitude on ectotherm performance: Adaptation to climate change variability? Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, 154, 389–393.

Gilchrist G.W., Huey R.B., Balanya J., Pascual M., Serra L., 2004. A time series of evolution in action: a latitudinal cline in wing size in south american Drosophila subobscura. Evolution 58, 768–780.

Gilchrist G.W., Huey R.B., Partridge L., 1997. Thermal sensitivity of Drosophila melanogaster: Evolutionary responses of adults and eggs to laboratory natural selection at different temperatures. Physiological Zoology, 70, 403–414.

Gilman S.E., Urban M.C., Tewksbury J., Gilchrist G.W., Holt R.D., 2010. A framework for community interactions under climate change. Trends in Ecology and Evolution, 25, 325–331.

Godfray H.C.J., 1994. Parasitoids: Behavioral and evolutionary ecology, Monographs in behavior and ecology. Princeton University Press.

Grossi G., Grimaldi A., Cardone R.A., Monné M., Reshkin S.J., Girardello R., Greco M.R., Coviello E., Laurino S., Falabella P., 2016. Extracellular matrix degradation via enolase/plasminogen interaction: Evidence for a mechanism conserved in Metazoa. Biology of the Cell, 108, 161–178.

Guldemond J.A., Brink W.J., Belder E., 1998. Methods of assessing population increase in aphids and the effect of growth stage of the host plant on population growth rates. Entomologia Experimentalis et Applicata, 86, 163–173.

Gunderson A.R., Stillman J.H., 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. Proceedings of the Royal Society B: Biological Sciences, 282, 20150401–20150401.

Hance T., van Baaren J., Vernon P., Boivin, G., 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. Annual Review Entomology, 52, 107–126.

Harley C.D.G., 2011. Climate change, keystone predation, and biodiversity loss. Science 334, 1124–1127.

Harrington R., Fleming R.A., Woiwod I.P., 2001. Climate change impacts on insect management and conservation in temperate regions: can they be predicted? Agricultural and Forest Entomology, 3, 233–240.

Hazell S.P., Neve B.P., Groutides C., Douglas A.E., Blackburn T.M., Bale J.S., 2010. Hyperthermic aphids: Insights into behaviour and mortality. Journal of Insect Physiology, 56, 123–131.

Heyworth E.R., Ferrari J., 2016. Heat stress affects facultative symbiont-mediated protection from a parasitoid wasp. PLoS One 11, 1–11.

Hoffmann A.A., Hewa-Kapuge S., 2000. Acclimation for heat resistance in Trichogramma nr. brassicae: Can it occur without costs? Functional Ecology, 14, 55–60.

Hoffmann A.A., Parsons P.A., 1991. Evolutionary genetics and environmental stress, Oxford Science Publications. Oxford University Press.

Hoffmann A.A., Sørensen J.G., Loeschcke V., 2003. Adaptation of Drosophila to temperature extremes: bringing together quantitative and molecular approaches. Journal of Thermal Biology, 28, 175–216.

Huey, R.B., Berrigan, D., 2001. Temperature, demography, and ectotherm fitness. American Naturalist, 158, 204–210.

Huey R.B., Kearney M.R., Krockenberger A., Holtum, J.A.M., Jess M., Williams S.E., 2012. Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. Philosiological Transactions of the Royal Socociety B: Biological Sciences, 367, 1665–1679.

Irwin M., Kampmeier G., Wolfgang W., 2007. Aphid movement: process and consequences, in: van Hemden, H.F., Harrington R. (Eds.), Aphids as Crop Pests. CABI, pp. 153–186.

Jeffs, C.T., Leather, S.R., 2014. Effects of extreme, fluctuating temperature events on life history traits of the grain aphid, Sitobion avenae. Entomologia Experimentalis et Applicata, 150, 240–249.

Jeffs C.T., Lewis O.T., 2013. Effects of climate warming on host-parasitoid interactions. Ecological Entomolody, 38(3): 209-218.

Johnson J.B., Omland K.S., 2004. Model selection in ecology and evolution. Trends in Ecology and Evolution, 19, 101–108.

Johnston I.A., Bennett, A.F., 2008. Animals and temperature. Phenotypic and evolutionary adaptation, Society for Experimental Biology Seminar Series. Cambridge University Press.

Kingsolver J.G., Arthur Woods, H., Buckley L.B., Potter K.A., MacLean H.J., Higgins, J.K., 2011. Complex life cycles and the responses of insects to climate change. Integrative and Comparative Biology, 51, 719–732.

Kingsolver J.G., Woods H.A., 2016. Beyond thermal performance curves: Modeling time-dependent effects of thermal stress on ectotherm growth rates. American Naturalist 187, 283–294.

Kunkel K.E., Pielke R.A., Changnon S.A., 1999. Temporal fluctuations in weather and climate extremes that cause economic and human health impacts: A review. Bullettin of the American Meteorological Society, 80, 1077–1098.

Lagos N.A., Fuentes-Contreras E., Bozinovic F., Niemeyer H.M., 2001. Behavioural thermoregulation in Acyrthosiphon pisum (Homoptera: Aphididae): The effect of parasitism by Aphidius ervi (Hymenoptera: Braconidae). Journal of Thermal Biology, 26, 133–137.

Larocca A., Fanti P., Romano V.A., Marsicovetere E., Isidoro N., Romani R., Ruschioni S., Pennacchio F., Battaglia D., 2007. Functional bases of host-acceptance behaviour in the aphid parasitoid Aphidius ervi. Physiological Entomology, 32, 305–312.

Loeschcke V., Hoffmann A.A., 2007. Consequences of heat hardening on a field fitness component in Drosophila depend on environmental temperature. American Naturalist, 169, 175–183.

Ma G., Ma C.-S., 2012a. Effect of acclimation on heat-escape temperatures of two aphid species: Implications for estimating behavioral response of insects to climate warming. Journal of Insect Physiology, 58, 303–309.

Ma G., Ma C.-S., 2012b. Climate warming may increase aphids' dropping probabilities in response to high temperatures. Journal of Insect Physiology, 58, 1456–1462.

Marengo J.A., Jones R., Alves L.M., Valverde M.C., 2009. Future change of temperature and precipitation extremes in South America as derived from the PRECIS regional climate modeling system. International Journal of Climatology, 29, 2241–2255.

Martinez A.J., Kim K.L., Harmon J.P., Oliver K.M., 2016. Specificity of multi-modal aphid defenses against two rival parasitoids. PLoS One 11, 1–17.

Meehl G.A., Karl T., Easterling D.R., Changnon S., Pielke R., Changnon D., Evans J., Groisman P.Y., Knutson T.R., Kunkel K.E., Mearns L.O., Parmesan C., Pulwarty R., Root T., Sylves R.T., Whetton P., Zwiers F., 2000. An introduction to trends in extreme weather and climate events: Observations, socioeconomic impacts, terrestrial ecological impacts, and model projections. Bull. Am. Meteorol. Soc. 81, 413–416.

Mironidis G.K., Savopoulou-Soultani M., 2010. Effects of heat shock on survival and reproduction of Helicoverpa armigera (Lepidoptera: Noctuidae) adults. Journal of Thermal Biology, 35, 59–69.

Miyan M.A., 2015. Droughts in Asian Least Developed Countries: Vulnerability and sustainability. Weather Climatic Extreme, 7, 8–23.

Nguyen T.T.A., Boudreault S., Michaud D., Cloutier C., 2008. Proteomes of the aphid *Macrosiphum euphorbiae* in its resistance and susceptibility responses to differently compatible parasitoids. Insect Biochemistry and Molecular Biology, 38, 730–739.

Oliver K.M., Degnan P.H., Hunter M.S., Moran N.A., 2009. Bacteriophages encode factors required for protection in a symbiotic mutualism. Science 325, 992–994.

Oliver K.M., Martinez A.J., 2014. How resident microbes modulate ecologically-important traits of insects. Current Opinion in Insect Science 4, 1–7.

Pennacchio F., Digilio M.C., Tremblay E., Tranfaglia A., 1994. Host recognition and acceptance behaviour in two aphid parasitoid species: Aphidius ervi and Aphidius microlophii (Hymenoptera: Braconidae). Bullettin of Entomological Research, 84, 57.

Pennacchio F., Fanti P., Falabella P., Digilio M.C., Bisaccia F., Tremblay E., 1999. Development and nutrition of the braconid wasp, Aphidius ervi in aposymbiotic host aphids. Archives of Insect Biochemistry and Physiology, 40, 53–63.

Pennacchio F., Strand M.R., 2005. Evolution of developmental strategies in parasitic Hymenoptera. Annual Reviews of Entomology, 51, 233–258.

Pincebourde S., Casas J., 2006. Multitrophic biophysical budgets: Thermal ecology of an intimate herbivore insect-plant interaction. Ecolgical Monographs, 76, 175–194.

Pörtner H.O., Bennett A.F., Bozinovic F., Clarke A., Lardies M.A., Lucassen M., Pelster B., Schiemer F., Stillman J.H., 2006. Trade-offs in thermal adaptation: The need for a molecular to ecological integration. Physiological and Biochemical Zoology, 79, 295–313.

Quicke D.L.J., 1997. Parasitic wasps. Chapman & Hall Ltd, London.

R Core Team, 2016. R: A language and environment for statistical computing.

Rinehart J.P., Denlinger D.L., Rivers D.B., 2002. Upregulation of transcripts encoding select heat shock proteins in the flesh fly Sarcophaga crassipalpis in response to venom from the ectoparasitoid wasp Nasonia vitripennis. Journal of Invertebrate Pathology, 79, 62–63.

Roitberg B.D., Mangel M., 2016. Cold snaps, heatwaves, and arthropod growth. Ecol. Entomol. 41, 653–659.

Romo C.M., Tylianakis J.M., 2013. Elevated temperature and drought interact to reduce parasitoid effectiveness in suppressing hosts. PLoS One 8.

Roux O., Le Lann C., van Alphen J.J.M., van Baaren J., 2010. How does heat shock affect the life history traits of adults and progeny of the aphid parasitoid Aphidius avenae (Hymenoptera: Aphidiidae)? Bullettin of Entomological Research, 100, 543–549.

Shim J.K., Ha D.M., Nho S.K., Song K.S., Lee K.Y., 2008. Upregulation of heat shock protein genes by envenomation of ectoparasitoid *Bracon hebetor* in larval host of Indian meal moth Plodia interpunctella. Journal of Invertebrate Pathology, 97, 306–309.

Sørensen J.G., Dahlgaard J., Loeschcke V., 2001. Genetic variation in thermal tolerance among natural populations of Drosophila buzzatii: down regulation of Hsp70 expression and variation in heat stress resistance traits. Functional Ecology, 15, 289–296.

Strand M.R., 2008. The insect cellular immune response. Insect Science 15, 1–14.

Strand M.R., Pech L.L., 1995. Immunological basis for compatibility in parasitoid-host relationships. Annual Reviews of Entomology, 40, 31–56.

Terblanche J.S., Hoffmann A.A., Mitchell K.A., Rako L., le Roux P.C., Chown S.L., 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. Journal of Experimental Biology, 214, 3713–3725.

Trotta V., Calboli F.C.F., Ziosi M., Guerra D., Pezzoli M.C., David J.R., Cavicchi S., 2006. Thermal plasticity in Drosophila melanogaster: a comparison of geographic populations. BMC Evolutionary Biology, 6, 67.

Trotta V., Duran Prieto J., Battaglia D., Fanti P., 2014. Plastic responses of some life history traits and cellular components of body size in Aphidius ervi as related to the age of its host Acyrthosiphon pisum. Biological Journal of Linnean Society, 113, 439–454.

Villagra C.A., Ramírez C.C., Niemeyer, H.M., 2002. Antipredator responses of aphids to parasitoids change as a function of aphid physiological state. Animal Behavioral, 64, 677–683.

Vose R.S., Easterling D.R., Gleason, B., 2005. Maximum and minimum temperature trends for the globe: An update through 2004. Geophysical Research Letters, 32, 1–5.

Webb B.A., 1998. Polydnavirus biology, genome structure, and evolution, in: Miller, L.K., Ball, L.A. (Eds.), The Insect Viruses. Springer US, Boston, MA, pp. 105–139.

Zhao F., Hoffmann A. a, Xing K., Ma C.-S., 2017. Life stages of an aphid living under similar thermal conditions differ in thermal performance. Journal of Insect Physiology, 99, 1–7.

CHAPTER III

Effects of two heat stresses and their combination on the survival of the pea aphid *Acyrthosiphon pisum* and its parasitoid *Aphidius ervi*

Introduction

Sporadic short Extreme Heat Events (EHEs) are common to occur in temperate zones in spring and during mild periods in summer (Zhu *et al*., 2019). EHEs may have irrelevant effects on species with long lifespan, but they can strongly affect biological performances of short lifespan species, probably because such EHEs account for a relatively larger proportion of their whole short lifespan (Vincenzi *et al*., 2012). Extreme temperatures can determine cellular injuries, causing deleterious effects on the metabolism and the physiology of an organism (Yocum and Denlinger, 1998; Tomanek, 2010). However, the majority of organisms have well-developed reparative mechanisms to counteract the detrimental effects of high temperatures (Bowler and Kashmeery 1979, Malmendal *et al*. 2006, Rodriguez-Trelles *et al*., 2013). When temperature rapidly increases, and a critical threshold is reached in few time, if the heat stress responsive mechanisms ensuring survival are not promptly activated, an organism could die. In insects, a single extreme heat exposure could quickly elevate the body temperature to lethal levels causing mortality; it could also reduce the fitness in the survived individuals (Zhang *et al*., 2015; Zhao *et al*., 2017).

Survival under stressful thermal conditions is influenced by different factors, such as the rate and duration of temperature variation (Powell and Bale, 2006; Terblanche *et al*., 2007; Mitchell and Hoffmann, 2010), the acclimation and the short pre-exposure to sub-lethal temperatures (cold and heat-hardening) (Sejerkilde *et al*., 2003; Sinclair and Roberts, 2005; Sørensen *et al*., 2016). Bowler (2005) described the acclimation as all the "physiological and behavioral changes occurring within an organism, which reduce the strain or enhances endurance of strain caused by experimentally induced stressful changes in particular climatic factors" and the hardening as "a quick, transitory adaptation to an extreme temperature that followed brief exposure at a sub-lethal temperature". Heat acclimation and hardening represent examples of thermal plasticity, an important strategy that allow many insect species to cope with thermal stress (Mitchell *et al*., 2011). Exposure to mild heat stress could activate some physiological heat-induced mechanisms that ensure a greater survival to a subsequent higher thermal stress. The beneficial effects of acclimation and of heat-pre-hardening have been recorded in different insect taxa, such as Diptera (Sejerkilde *et al*., 2003; Krebs and Loeschcke, 1994; Malmendal *et al*. 2006; Loeschcke and Hoffman, 2007; Sgro *et al*., 2010),

Hemiptera (Chanthy *et al*., 2012; Cao *et al*., 2018), Hymenoptera (Scott *et al*., 1997; Mutamiswa *et al*., 2018) and Lepidoptera (Manjunatha *et al*., 2010; Chidawanyika and Terblanche, 2011; Mutamiswa *et al.,* 2019). The extent of the response to hardening and acclimation can vary among species, among populations and among the individuals of a population, and it can also depend on the life stage involved (Slabber *et al*., 2007; Hoffmann *et al*., 2003). Among insects, the thermal biology (*i.e*, the effects of heat stress, acclimation and hardening) of *Drosophila melanogaster* is well known. Krebs and Loeschcke (1994) reported that a heat exposure to 36°C increases survival of *D. melanogaster* to a later exposure to 39°C for 100 minutes.

In nature, temperatures fluctuate in repeated irregular waves, with multiple extraordinary high temperature spells during a given time period. As a consequence, organisms are exposed to several heat waves rather than a single heat event (Bailey and van de Pol, 2016). When repeated thermal stresses occur, the ability to survive to a given stress strongly depends on the effects of the previously ones, since the possibility to display some heat-induced resistance mechanisms could be enhanced (hardening) or suppressed (irreversible cellular damages). Heat-induced recovery mechanisms are generally effective only when the recovery occurs within the optimal thermal range of a species (Bowler and Kashmeery 1979, Malmendal *et al*., 2006, Rodriguez-Trelles *et al*., 2013). For instance, the heat shock proteins (HSP), a group of proteins commonly induced under stressful temperatures, can require a certain period of time (minutes to hours) for their up-regulation under unfavorable conditions (Sørensen *et al*., 2005). HSPs play a fundamental role in the cellular reparation processes, caused by both abiotic and biotic stressors (Zhao and Jones, 2012). In literature it is reported that these polyfunctional proteins are basilar in the heat and cold hardening process in insects (Nielsen *et al*., 2005. Huang *et al*., 2007; King and MacRace, 2015). Thus, the up-regulation during a mild thermal stress could substantially increase the probability to survive a subsequent severe thermal stress.

Aphid and endoparasitoid form a complex symbiotic relationship. This is principally due to the strict physiological interaction among them. If aphid dies, for any reason other than parasitization, also the developing endoparasitoid will die too (Vinson, 1990). Moreover, any change in aphid physiology, for example changes due to thermal stress, could also affect the features of the adult parasitoid, such as the life history traits related to its fitness. The study of aphid-endoparasitoid relationship is also complicated for the presence of different bacterial symbiont species in aphids. Some of this species, such as *Buchnera aphidicola*, an obligate species playing a fundamental role in the synthesis of amino acids in aphid, is also involved in aphid thermal adaptation (Dunbar *et al*., 2007). *Buchnera* is

particularly sensible to heat stress (Ohtaka and Ishikawa, 1991; Montllor *et al*., 2002). Ohtaka and Ishikawa (1991) demonstrated that if a heat exposure kills *Buchnera* cells, aphids are infertile. Montllor *et al*. (2002) reported that, in the field, temperatures of 25-30°C depress the *B. aphidicola* densities within the pea aphid *A. pisum.* From a more complex point of view, it is supposable that when thermal stress affects *B. aphidicola*, even the stressed aphids and the developing parasitoids indirectly suffer consequences (abnormal development, delayed growth, *etc.*). On the other hand, given the strict physiological relationship between aphids and endoparasitoids, it is also supposable that aphid exposure to mild heat temperature could enhance thermal tolerance of the developing parasitoids.

High temperature above the critical thresholds could determine different cellular injuries in stressed insects. These injuries vary in function of several factors, such as: the previous experienced thermal environment, the conditions at which the thermal stress occur (humidity, for instance), the thermal variation rate, *etc*. These factors could determine in the stressed insect different types of injury, which affect the probability to survive the stress. Entomologists are used to distinguish the mortality due to the direct effect of the heat stress from the indirect mortality occurring a few days after the stress. Insects could appear immobile some hours after the heat stress as a consequence of the muscles injuries. For this reason, mortality due to the direct effect of stressful temperature is generally recorded 24 hours after stress occurrence (Hazell and Bale, 2010; Sørensen *et al*., 2001). Mortality in the days after the stress is mainly related to the interaction of different factors, such as the ineffectiveness of certain cellular reparative mechanisms and of physiological adjustments; it could also be related to the metabolic cost of the previously heat-induced mechanisms. If the mortality rate measured 24 hours after the heat stress is useful to evaluate the ability to promptly cope with the stress, the mortality recorded in the days after the stress is useful to evaluate the effectiveness at long-term scale of the heat-induced reparative mechanisms.

In this study I investigate some aspects related to the survival of both unparasitized and parasitized aphids after exposure to a mild and to a very high temperature in the short (24 h after exposure) and in the long term (on 13 days old aphids). I also investigate the effects of a thermal hardening (mild stress) on the survival of aphids when exposed to a subsequent severe thermal stress. The trophic model system is the same described in Ch. II and consists of the endoparasitoid *A. ervi* and its aphid host, the pea aphid *A. pisum*. In Ch. II, I demonstrated that the pea aphid *A. pisum* displays a greater resistance to rapid thermal increment after being parasitized by the parasitic wasp *A. ervi*. I suppose that the stress induced by parasitization could increase aphid heat resistance through the activation of a

hardening-like mechanism. In this chapter, I investigate if parasitism and a thermal hardening may or may not have any cumulative effects on aphid thermal resistance and if (and to what extent) these effects influence parasitoids survival.

Materials and Methods

Insect rearing

Both aphid and parasitoid were reared as previously described in the Ch. II. Aphids at the beginning of the third nymphal instar were used in this experiment. Parasitoids were removed from the culture at the mummy stage and enclosed in a separate holding container until adult emergence to ensure that experimental wasps were of similar age and had no prior oviposition experience. After emergence and before the experiments, adult parasitoid females were left for 24 h with two males and were provided with water and honey ad libitum. All the parasitoid females used in bioassays were between two and three days post-eclosion and were assumed to be mated. For this study, four independent replicates, each consisting of 10 synchronous colonies (i.e., different plants, for a total of 40 experimental colonies) were generated with an interval of 14 days from each other.

Parasitization procedure

Four replicates were performed in this study. All the four-day old aphids of the 10 synchronous colonies were gently removed from the plants and placed together in a plastic box (27 x 19 x 8 cm) with a mesh covered ventilation hole in the screw-top (about 1200-1300 aphids per replicate). Twenty aphids were randomly transferred from the box into a plastic cylinder (150 ml) with a mesh covered ventilation hole in the screw-top, for a total of 60 plastic cylinders (unfortunately, only 52 cylinders were generated for one replicate). Aphids in the plastic cylinders were subsequently split into two groups: the aphids of one group were parasitized by *A. ervi* whereas the other group was composed of unparasitized aphids. According to a method similar to that described by Oliver *et al.* (2005), parasitoid female were given oviposition experience by exposing them to two aphids in a Petri dish before the experiment. Females with oviposition experience (that is, observed to oviposit in an aphid within 2-3 min after introduction in the Petri dish) were randomly assigned to the plastic cylinders (one parasitoid per cylinder) and removed after 3 h.

In *A. ervi,* the decision to accept or reject a host follows the insertion of the ovipositor (Pennacchio *et al.*, 1994; Larocca *et al.*, 2007) and there is no way of knowing for sure if an aphid hosts a parasitoid egg without dissecting it. Preliminary experiments showed that under our experimental conditions the parasitization rate (assessed by dissecting 140

parasitized aphids after five days) was about 78%, lower but comparable to the parasitization rate described in the Chapter II, obtained with aphids observed to be stung once by a parasitoid female. The aphids after the parasitization procedure were merged to form one homogeneous group with the same percentage of parasitization. Groups of 55-60 aphids were then randomly transferred from the box into plastic cylinders (150 ml). The same protocol was used for the unparasitized aphids. Twenty plastic cylinders were generated for the replicates 1 and 2, 18 cylinders for replicates 3 and 4 (half cylinders with parasitized aphids and half with unparasitized ones). Preliminary experiments showed that these manipulations cause negligible aphid mortality (less than 2%).

Heat shock treatments

The first thermal treatment consisted of a mild heat shock (Mild-HS) at 35.0 ± 0.1°C (mean values ± accuracy) for an exposure time of 30 minutes and was performed on 3 days old aphids (3rd instar), i.e., 3 hours after parasitization in the case of aphid parasitized groups. The second thermal treatment consisted of a heat shock at the sub-lethal temperature (SubLet-HS) of 39.1 ± 0.1°C for an exposure time of 30 minutes and was performed on 8 days old aphids, i.e., 5 days after parasitization (adult stage). The two heat treatments were applied alone or in combinations, generating 3 experimental groups plus a control one: Mild-HS, SubL-HS, Mild+SubL-HS and NoHS. These different thermal treatments were both applied to parasitized and unparasitized aphids generating a total of 8 experimental groups. Each experimental group consisted of 55-60 aphids. During the heat shock treatments, the not treated control groups were placed in the climatic chamber. Heat exposure were carried out as previously described in the Ch.II. The unparasitized aphids of each treatment consisted of aphids of the same age but unparasitized. Heat resistance has been assessed by measuring survival of unparasitized and parasitized aphids following exposure to the heat treatment. The choice of the stress temperature of 35°C for 30 min produces small effects on survival in *A. pisum* (survival rate of adult aphids was about 95%, preliminary results) and therefore can be considered mild. The stress temperature of 39.1°C caused instead a greater mortality in aphid experimental group, as reported in the Ch. II.

Mortality check

After the heat shock treatments, aphids of each Falcon® tube were transferred on a Petri dish lid and placed at the base of a fresh broad bean plant (13-15 cm tall) within a plastic box. The plants were then placed in an environmental chamber at 22 ± 1°C. Survival was considered to occur if the aphids were able to walk away from the Petri dish lid and climb

the plant. Aphid survival was checked 24 h after the thermal treatment, as many insects are immobilized after a heat stress (Hazell and Bale, 2010). After the survival check, the Petri dish lid and the dead aphids were moved from the plants. To investigate the possible long term effects of the heat shock treatments on aphids, survival was also checked at the end of the experiment on 13 days old aphids. For the parasitized groups, the number of mummies was added to the count of aphids surviving the heat treatments, as a live parasitized aphid after 10-11 days gives rise to a mummy. Survival of the parasitized and unparasitized aphids was then compared one with each other and with the appropriate control (aphids of the same age but untreated).

Parasitoid survival.

To determine the parasitoids survival after the heat shock treatments, the numbers of surviving aphids and mummies in the groups exposed to the parasitoids were counted. Mummies are almost found within 8 days after parasitization. Mummified aphids were removed from the plants, counted and placed in plastic vials. After emerging, adult parasitoids were counted.

Statistical analysis.

Binomial generalized linear models (GLM) with a logit link function have been considered for the analysis of the aphid survival after heat shock since the row data have a discrete probability distribution (alive/dead). This kind of analysis has greater interpretability and higher power than the analyses of percentage or percentage transformed data (Warton and Hui, 2011) and best approximate the process that generated the observed data, minimizing the lack of model fit to the observed data (Johnson and Omland, 2004). Aphid survival was analyzed 24 h after heat shock and at the end of the experiment, i.e. on 14 days old aphids. Aphid survival 24 h after heat shock was independently analyzed for the mild and for the sub-lethal heat shock since aphids of different ages were considered (3rd nymphal instar for Mild-HS and adults for SubL-HS). In both cases, survival was analyzed using a GLM in which “parasitization” (two levels: parasitized and unparasitized aphids) and “treatment” were the main fixed effects; “replicate” (four levels) was nested within “parasitization” and “treatment”. The levels of “treatment” were two for the “mild” analysis (No-HS and Mild-HS) and three for the “sub-lethal” one (No-HS, SubL-HS and Mild+SubL-HS). Since at the end of the experiment all the aphids are of the same age (14-days-old), survival in this case was analyzed with the same GLM but including all the four treatments (No-HS, Mild-HS, SubL-HS and Mild+SubL-HS). The number of mummies observed after the heat shock (an

estimate of parasitoid survival) and the number of emerging adult parasitoids were analyzed using GLMs with "treatment" (four levels: No-HS, Mild-HS, SubL-HS and Mild+SubL-HS) as the main fixed effects and "replicate" (four levels) nested within "treatment". The binomial variable was composed of the number of mummies/number of aphids at the beginning of the experiment minus the number of mummies. All the statistical analyses performed in this study were carried out using R.3.6.2 software (R Core Team, 2016).

Results

Aphid survival 24 hours after the heat treatments

- Mild-HS treatment

Figure 3.1 shows the mean values of aphid survival recorded 24h after the first mild heat shock treatment at 35°C (mild-HS) for an exposure time of 30 minutes for both parasitized and unparasitized aphids. The mild-HS was performed on 4-days-old aphids (3rd nymphal instar); for the parasitized groups, the parasitization occurred 3 hours before the mild heat shock. The Mild-HS treatment slightly reduced aphid survival for both parasitized and unparasitized aphids. For parasitized aphids, survival recorded at 24 h after the mild heat shock treatment was 86.78% for the group experienced the mild heat stress, and 92.41% for control group not exposed to the mild temperature. For unparasitized aphids, survival recorded at 24 h after the mild heat shock treatment was 88.76% for the group experienced the mild heat stress and 93.18% for the control group not exposed to the mild temperature.

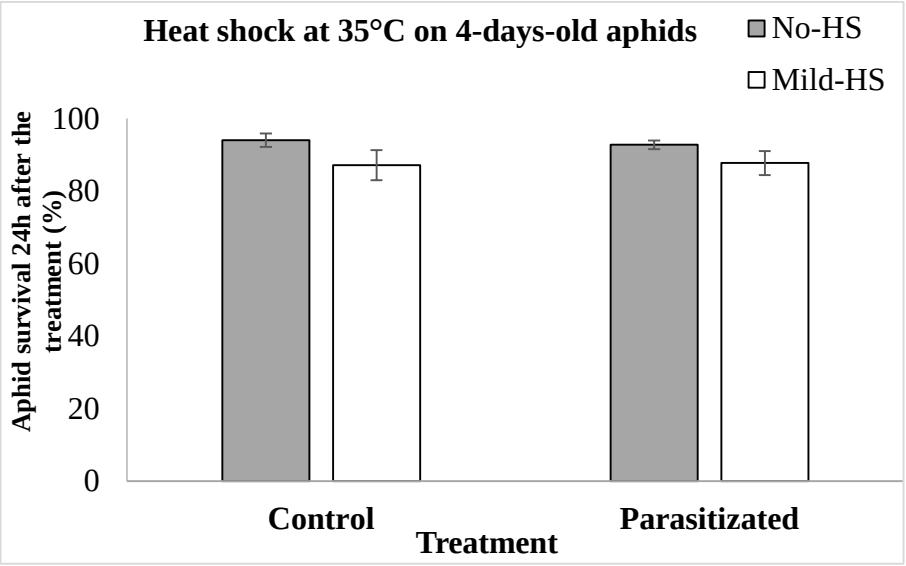

Fig. 3.1 - Mean values (± standard errors) of aphid survival 24h after the mild heat shock at 35°C (Mild-HS) for an exposure time of 30 minutes. No-HS unparasitized: N=395; No-HS parasitized: N= 350; Mild-HS unparasitized: N=350; Mild-HS parasitized: N=215.

A GLM with a binomial error distribution and a logit link function (Table 2) gave significant differences as related to treatments (No-HS *vs* Mild-HS: $\chi^2_{(1)}$= 10.51, P < 0.01) but not between parasitization ($\chi^2_{(1)}$= 0.034, P = 0.854) nor for the "parasitization by treatment" interaction ($\chi^2_{(1)}$= 0.409, P = 0.522). A significant difference between replicates was found ($\chi^2_{(12)}$= 35.434 P < 0.001). Even if an heat shock at 35°C for an exposure time of 30 minutes could be considered mild (see Ch.II), the Mild-HS reduces aphids survival for both parasitized (-5.6%) and unparasitized control aphids (-6.9%) compared to the untreated aphids.

Table 2. Results of the GLM with a binomial error distribution and a logit link function on aphid survival 24h after the mild heat shock at 35°C (Mild-HS).

Source of variation	*Df*	*Deviance*	*Pr(Chi)*
Parasitization	1	0.034	0.854
Treatment	1	10.513	0.00118
Parasitization X treatment	1	0.409	0.522
Replicate within treatment and parasitization	12	35.434	0.000399

Parasitization and treatment (No-HS *vs* Mild-HS) are main fixed effects, replicates is nested within parasitization and treatment. Df, degrees of freedom; Pr(Chi), Chi-square probability.

- SubL-HS treatment

Figure 3.2 shows the mean values of aphid survival recorded 24h after the sub-lethal heat shock treatment at 39°C for an exposure time of 30 minutes for both parasitized and unparasitized aphids. The SubL-HS was performed on 9 days old adult aphids; for the parasitized groups, the parasitization occurred 5 days before the heat shock treatment. The SubL-HS was applied alone or in combination with the Mild-HS (Mild+SubL-HS).

The sub-lethal heat shock treatment determined a greater mortality, in all aphid experimental groups, than the mild heat shock treatment. For parasitized aphids, survival recorded 24 h after the sub-lethal heat shock treatment, was 56.3% for the group experiencing this treatment and it was 58.5% for aphid group that experienced both the mild and the sub-lethal

shock treatment. For unparasitized aphids, survival recorded at 24 h after the sub-lethal heat shock treatment was 3.1% for the group experienced this treatment, and it was 31.4% for aphid group that experienced both the mild and the sub-lethal shock treatment. In the control groups, consisting of parasitized and unparasitized aphids that were not exposed to these thermal stress, mortality was lesser than 2%, and it was attributed to aphid manipulation.

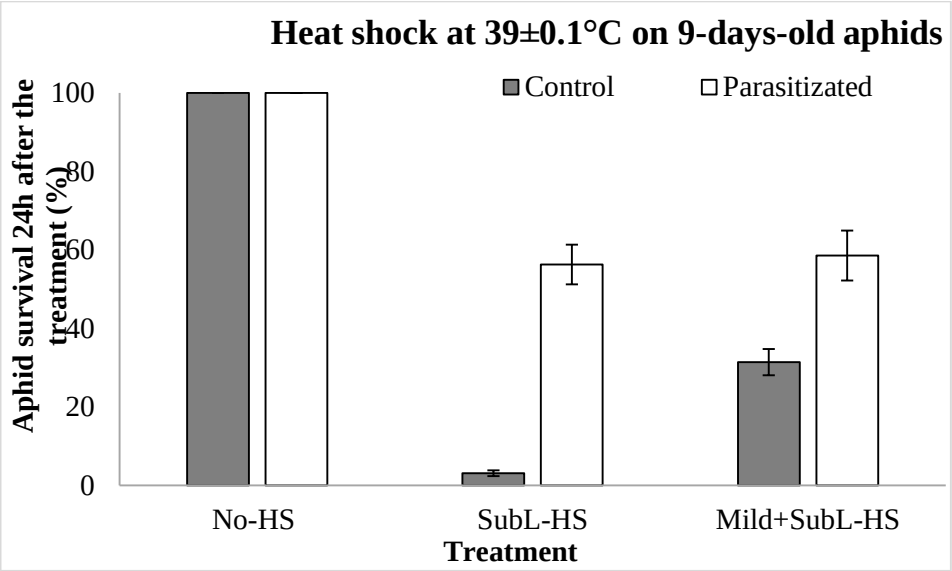

Fig. 3.2 - Mean values (± standard errors) of aphid survival 24h after the Sub Lethal heat shock at 39°C for an exposure time of 30 minutes. No-HS unparasitized: N=353; No-HS parasitized: N= 299; SubL-HS unparasitized: N=260; SubL-HS parasitized: N=282; Mild+ SubL-HS unparasitized: N=214; Mild+ SubL-HS parasitized: N=145.

A GLM with a binomial error distribution and a logit link function (Table 3) gave significant differences as related to treatments ($\chi^2_{(2)}$= 998.19, P < 0.001), between parasitization ($\chi^2_{(1)}$= 195.43, P < 0.001) as well as for the "parasitization by treatment" interaction ($\chi^2_{(2)}$= 44.91, P < 0.001). No significant difference between replicates were found ($\chi^2_{(16)}$= 21.73, P = 0.15).

Table 3. Results of the GLM with a binomial error distribution and a logit link function on aphid survival 24h after the Sub-Lethal heat shock at 39°C.

Source of variation	*Df*	*Deviance*	*Pr(Chi)*
Parasitization	1	195.43	< 2.2e-16
Treatment	2	998.19	< 2.2e-16
Parasitization X treatment	2	44.91	1.77e-10
Replicate within treatment and parasitization	16	21.73	0.15

Parasitization and treatment (No-HS, SubL-HS and Mild+SubL-HS) are main fixed effects, replicates is nested within parasitization and treatment. Df, degrees of freedom; Pr(Chi), Chi-square probability.

Survival of 14-days-old aphids

In order to investigate possible long term effects of the heat shock treatments on aphids, survival was also checked at the end of the experiment on 14-days-old aphids. For the parasitized groups, the number of mummies was added to the count of aphids surviving the heat treatments, as a live parasitized aphid after 10-11 days gives rise to a mummy. Aphid survival was then estimated as the ratio between the number of aphids counted at the end of the experiment and the number of aphids at the beginning of the experiment.

Figure 3.3 shows the mean values of aphid survival of the experimental groups recorded at the end of the experiment (14-days-old aphids).

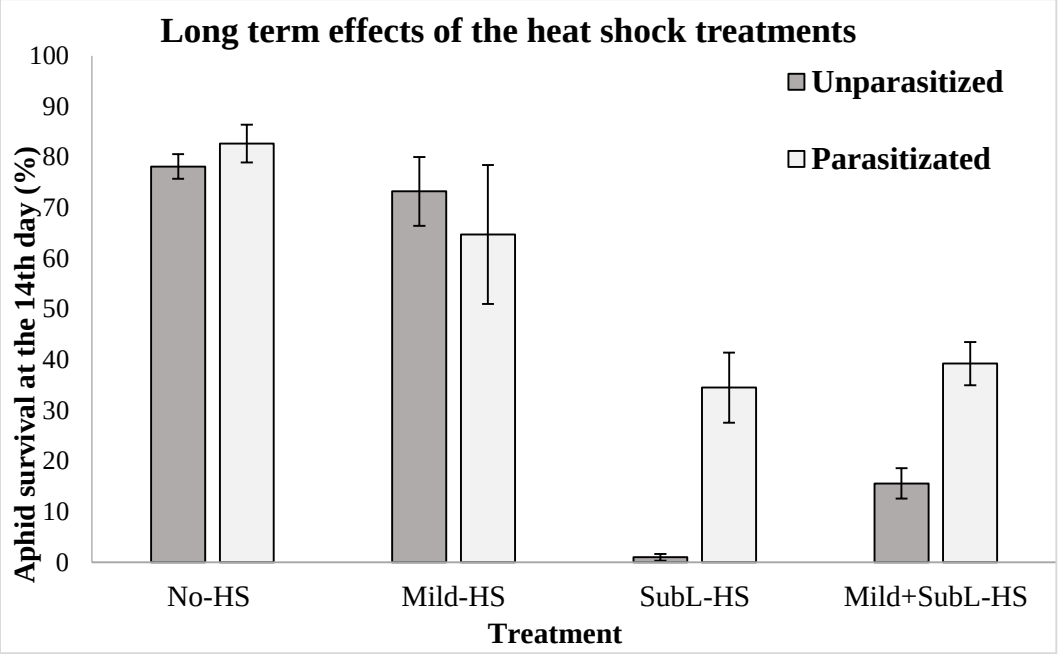

Fig. 3.3 - Mean values (± standard errors) of aphid survival at the end of the experiment on 14 days old aphids. Aphid survival was estimated as the number of aphids counted at the end of the experiment / the number of aphids at the beginning of the experiment. No-HS unparasitized: N=395; No-HS parasitized: N=350; Mild-HS unparasitized: N=230; Mild-HS parasitized: N=215; SubL-HS unparasitized: N=290; SubL-HS parasitized: N=335; Mild+SubL-HS unparasitized: N=238; Mild+SubL-HS parasitized: N=170.

In general, the sub-lethal heat shock treatment had a long term effects on aphid survival, particularly evident for the unparasitized aphids (1%) compared to the parasitized ones (34.48%). When just the mild heat stress was applied, aphid survival was 73.22% in the case

of unparasitized aphid group and 64.72% in parasitized aphid group. In this case, aphid survival was not significantly different from that of the related control group (NO-HS), for both parasitized and unparasitized aphid groups, that were 82.7 and 78.1%, respectively. When both the mild and the sub-lethal heat shock treatment were applied, aphid survival was 15.56% for the of unparasitized aphid group and 39.24% for the parasitized aphid group.

A GLM with a binomial variable composed of live (the number of aphids counted at the end of the experiment plus the number of mummies) and dead aphids (number of aphids at the beginning of the experiment minus the number live aphids) gave significant differences as related to treatments ($\chi^2_{(3)}$= 763.25, P < 0.001), between parasitization ($\chi^2_{(1)}$= 65.62, P < 0.001) as well as for the "parasitization by treatment" interaction ($\chi^2_{(3)}$= 113, P < 0.001). A significant difference between replicates was also found ($\chi^2_{(22)}$= 125.39, P < 0.001).

Table 4. Results of the GLM with a binomial error distribution and a logit link function on aphid survival at the end of the experiment on 14-days-old aphids Parasitization and treatment (No-HS, Mild-HS, SubL-HS and Mild+SubL-HS) are main fixed effects, replicates is nested within parasitization and treatment. Df, degrees of freedom; Pr(Chi), Chi-square probability.

Source of variation	*Df*	*Deviance*	*Pr(Chi)*
Parasitization	1	65.62	5.463e-16
Treatment	3	763.25	< 2.2e-16
Parasitization X treatment	3	113.00	< 2.2e-16
Replicate within treatment and parasitization	22	125.39	< 2.2e-16

Parasitoid survival

In this experiment, the parasitoid survival to a heat shock was estimated as the number of mummies observed at the end of the experiment, i.e.,10 days after the parasitization and as the number of emerging adult parasitoids. The mummification rates and the percentage of emerging parasitoids (Fig. 3.4) were calculated as the ratio between the number of observed mummies/parasitoids on the initial number of parasitized aphids.

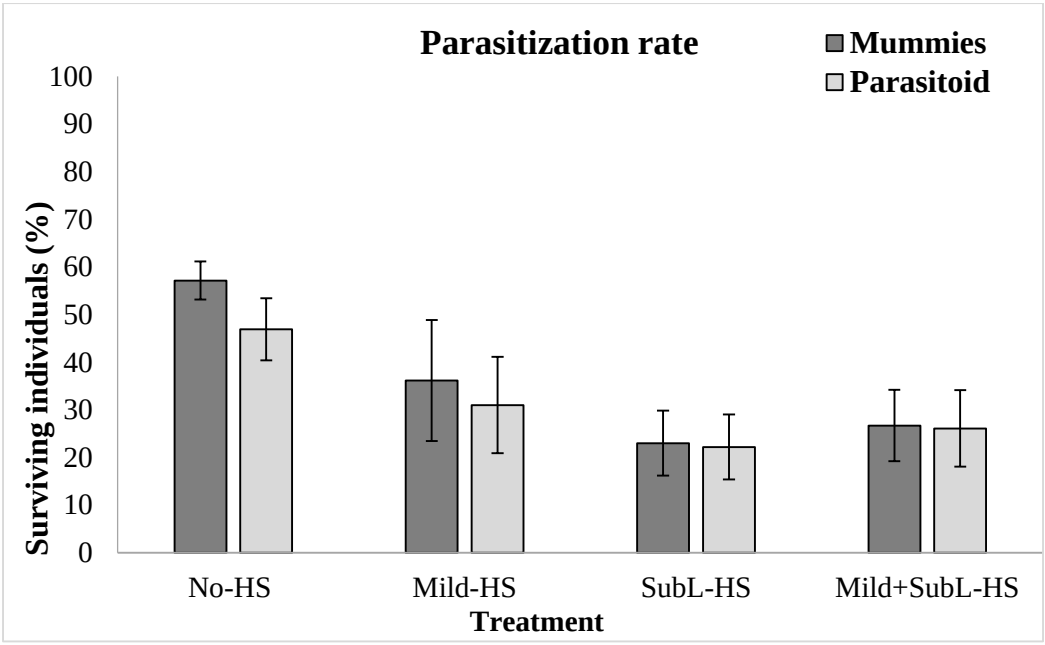

Fig. 3.4 - Mean values (± standard errors) of the parasitoid survival after the heat shock treatments. The parasitization rate was measured as the number of mummies and as the number of emerging parasitoid on the starting number of parasitized aphids. Starting number of aphids: No-HS, N = 350; Mild-HS, N = 215; SubL-HS, N = 335; Mild+SubL-HS, N = 170.

Survival rates differed among the aphid groups that just experienced the mild heat stress, the sub-lethal one and both of them in combination. On the contrary, for these three experimental groups, the mummification rate and the adult parasitoid hatching rate were not significantly different among them. The mummification rate was 57.12% for the control group, and it was lower for all the experimental group that experienced the heat treatments. It was 36.15% for the aphid group that experienced just the mild heat exposure, 23% for the aphid group that experienced just the sub-lethal stress, and 26.7% for the aphid group that experienced both the mild and the sub-lethal heat exposure. Although the mummification rate of the aphid group that experienced the mild heat exposure was higher than those of the other two heat treated groups, no significant differences were found among them. The parasitoid hatching rate was 46.93% for the control group, and it was lower for all the experimental group that experienced the heat treatments. It was 31.0% for the aphid group that experienced just the mild heat exposure, 22.2% for the aphid group that experienced just the sub-lethal stress, and 26.10% for the aphid group that experienced both the mild and the sub-lethal heat exposure. For both the number of mummies and adult parasitoids, the GLMs gave significant differences as related to treatments ($\chi^2_{(3)}$= 94.7 and $\chi^2_{(3)}$= 52.8 respectively, P < 0.001 in both cases) and among replicates within treatment ($\chi^2_{(11)}$= 99.7 and $\chi^2_{(3)}$= 93.8 respectively, P < 0.001 in both cases).

Discussions and Conclusions

In the present study, two different thermal treatments and their combination (mild, sub-lethal and mild followed by the sub-lethal) were applied to parasitized and unparasitized aphids.

When the mild heat exposure (35°C for 30 minutes) was applied alone, a slightly reduction of the 24h survival in both unparasitized and parasitized aphids has been observed (-7% and -5% respectively). In the short term, the mild-HS do not lead to a significant survival reduction, as reported in the literature for other organisms (Scott *et al*., 1997; Huang *et al*., 2007). When the long-term effects of the mild heat exposure were considered (survival measured 10 days after the treatment), the parasitized aphids showed a decreased survival compared both with the parasitized but untreated (-18%) and with the unparasitized and treated aphids (-8.5%). It is known that parasitized aphids show a reduced survival as a consequence of the physiological alterations caused by the parasitoid, as well as by the aphid suicide behaviour (McAllister and Roitberg, 1987; McAllister *et al*., 1990). The mild HS is in every way an additional source of stress in an already compromised environment (the parasitized aphids) which consequently could cause an increased aphid mortality, even if not statistically significant.

When the sub-lethal heat exposure (39°C per 30 minutes) has been applied alone, parasitized aphids had a greater survival than the unparasitized ones, both on the short and long terms. This result has been widely discussed in Ch. II for the short term survival, but it is still valid for the long term survival. Briefly, parasitism could affect the host thermal sensitivity through a physiological mechanism whose exact nature needs to be further investigated, but it is reasonable to suppose that it is mediated by the activation of the HSP or by the activation of other stress proteins. In fact, in literature is well documented that the up-regulation of HSP genes play an important role in the host-parasite interaction in insects (Rinehart *et al*., 2002; Shim *et al*., 2008).

It is interesting to note that the mild heat exposure increased aphid survival after the sub-lethal HS only in the unparasitized group, whereas the parasitized aphid survival is the same regardless of the mild heat exposure. This result is consistent when the survival is measured over both short and long terms. A possible explanation is that the heat stress and the parasitization stress may trigger the same physiological mechanisms, such as the heat shock proteins. In the case of parasitized aphids, the maximum expression level of HSPs genes (*plateau*) was reached as a consequence of the parasitization stress, independently from the exposure to the hardening treatment (see Didomenico *et al*., 1982). In other words, the pre-hardening treatment does not determine any benefic additive effects (in terms of survival) in

the parasitized aphids since the same mechanism is involved in response to the two stresses and it is already activated.

In the parasitized groups, no significant differences between the mummification and the parasitoid adult hatching rates were found, regardless of the heat treatment. For this reason, both mummification and the parasitoid adult hatching rates can be used as an estimate of the parasitoid survival.

All the parasitized aphid groups exposed to the thermal treatments (35°C and 39°C, both alone and in their combination) showed a lower parasitoid survival than the untreated groups. This additional mortality in the parasitized aphids could be attributed to an interaction between the consequences of the physiological response to the heat and the physiological response to the parasitism. In particular, the higher parasitoid mortality in the heat treated groups could be related to the metabolic costs over the long-term of the heat-induced physiological mechanisms (Williams *et al*., 2008; Sorvari *et al*., 2011). The thermal treated parasitized aphids could be considered "weaker" since they suffer from the sum of the negative effects of two stresses: the parasitization and the heat.

Interestingly, in the parasitized aphid groups exposed to all the three thermal exposures, a similar parasitoid survival was observed. The mild exposure did not play any heat hardened effect on the development of the parasitoid *A. ervi*. The mild heat exposure had instead a long-term stressfull effect on parasitized aphids since it causes an increase in mortality during the 5 days that pass between the mild and the sub-lethal heat stresses. When the sub-lethal stress was performed, it is reasonable to suppose that only the "stronger" parasitized aphids survive. Based on these considerations, I suppose that the parasitoids survival in the three heat stressed groups was the same among them because of the mild exposure had a selective effect on aphids, which is comparable to the mortality due to sub-lethal HS.

In conclusion, I found that *A. ervi* determines a variation in the heat resistance of its host. This finding is coherent with those of the Ch. II. In this Chapter, I found that the heat tolerance of the host-parasite system could vary in function of the temperature experienced before a sub-lethal stress. Moreover, the heat exposures applied in this study caused different mortality rates both in parasitized and unparasitized aphids; it also caused a certain mortality in the developing parasitoid. The different thermal sensitivity of parasitized and unparasitized aphids could affect both the population dynamic of the aphids and of the endoparasitoids in nature. In particular, these results could help to clarify the changes in the population dynamics that commonly occurred in mid-summer for these insect kinds (Karley *et al*., 2004). Moreover, these results are a valid contribute for predicting how the rapid

thermal increment due to global warming will affect these species in next decades, when thermal variations rate will be higher and more frequent than now.

References

Baily L.D., van de Pol M., 2016. Climwin: An R toolbox for climate window analysis. PLoS ONE 11(12): e0167980.

Bowler K., 2005. Acclimation, heat shock and hardening. Journal of Thermal Biology, 30: 125-130.

Bowler K., Kashmeery A.M.S., 1979. Recovery from heat injuries in the blowfly, *Calliphora erythrocephala*. Journal of Thermal Biology, 4(3): 197-202.

Cao J., Xing K., Liu H., Zhao F., 2018. Effects of developmental acclimation on fitness costs differ between two aphid species. Journal of Thermal Biology, 78: 58-64.

Chanty P., Martin R.J., Gunning R.V., Andrew N., 2012. The effect of thermal acclimation on lethal temperatures and critical thermal limits in the green vegetable bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae). Frontiers in Phisiology, 3: 465.

Chidawanyika F., Terblanche J.S., 2011. Costs and benefits of thermal for codling moth, *Cydia pomonella* (Lepidoptera:Tortricidae): implications for pest control and the sterile insect release. Evolutionary Applications, 4(4): 534-544.

Yocum G.D., Denlinger D.L., 1998. Physiology of heat sensitivity. CRC Press, 7-53.

Didomenico B.J., Bugaisky G.E., Lindquist S., 1982. The heat shock response is self-regulated at both the transcriptional and posttranscriptional levels. Cell, 32: 593-603.

Dunbar H.E., Wilson A.C.C., Ferguson N.R., Moran N.A., 2007. Aphid Thermal Tolerance Is Governed by a Point Mutation in Bacterial Symbionts. PLoS Biol 5(5): e96.

Hazell S.P., Bale J.S., 2011. Low temperature thresholds: are chill coma and CT_{min} synonymous? Journal of Insect Physiology, 57(8):1085-1089.

Hoffmann A.A., Sørensen J.G., Loeschcke V., 2003. Adaptation of Drosophila to temperature extremes: bringing together quantitative and molecular approaches. Journal of Thermal Biology 28: 175–216.

Huang L.-H., Chen B., Kang L., 2007. Impact of mild temperature hardening on thermotolerance, fecundity, and Hsp gene expression in Liriomyza huidobrensis. Journal of Insect Physiology, 53(12): 1199-1205.

Johnson J.B., Omland K.S., 2004. Model selection in ecology and evolution. Trends in Ecology & Evolution, 19(2):101-108.

Karley A.J., Parker W.E., Pitchford J.W., Douglas A.E., 2004. The mid-summer crash in aphid populations: Why and how does it occurs? Ecological Entomology, 29(4): 383-388.

King A.M., MacRace T.H., 2015. Insect heat shock proteins during stress and diapause. Annual Review of Entomology, 60:59-75.

Krebs R.A., Loeschcke V.,1994. Costs and benefits of activation of the heat-shock response in Drosophila melanogaster. Functional Ecology, 8: 730–737.

Larocca, A., Fanti, P., Romano, V.A., Marsicovetere, E., Isidoro, N., Romani, R., Ruschioni, S., Pennacchio, F., Battaglia, D., 2007. Functional bases of host-acceptance behaviour in the aphid parasitoid Aphidius ervi. Physiological Entomology, 32, 305–312.

Loeschcke V., Hoffmann A.A., 2007. Consequences of hardening on a field fitness component in Drosophila depend on environmental temperature. The American Naturalist, 192(2):175-183.

Malmendal A., Overgaard J., Bundy J.G., Sørensen J.G., Nielson N.C., Loeschke V., Holmstrup M., 2006. Metabolic profiling of heat stress: hardening and recovery of homeostasis in *Drosophila.* American Journal of Physiology-Regulatory, 291(1), R205-R212.

Manjunatha H.B., Rajesh R.K., Aparna H.S., 2010. Silkworm thermal biology: a review of heat shock response, heat shock proteins and heat acclimation in the domesticated silkworm, Bombyx mori. Journal of Insect Science, 10(1), 204.

McAllister M.K., Roitberg B.D, 1987. Adaptative suicidal behaviour in pea aphids. Nature, 326, 797-799.

McAllister M.K., RoitbergB.D., Weldone K.L., 1990. Adaptative suicide in pea aphids: decisions are cost sensitive. Animal Behaviour, 40(1): 167-175.

Mitchell K., Hoffman A.A., 2010. Thermal ramping rate influences evolutionary potential and species differences for upper thermal limits in Drosophila. Functional Ecology, 24(3):694-700.

Mitchell K.A., Sgrò C.M., Hoffman A.A., 2011. Phenotypic plasticity in upper thermal limits is weakly related to Drosophila species distributions. Functional Ecology, 25(3):661-670.

Montllor C.B., Maxmen A., Purcell A.H., 2002. Facultative bacterial endosymbionts benefits pea aphids Acyrthosiphon pisum under heat stress. Ecological Entomology, 27(2):189-195.

Mutamiswa R., Chidawanyika F., Nyamukondiwa C., 2018. Thermal plasticity potentially mediates the interaction between host partellus swinhoe (Lepidoptera:Crambidae) and the endoparasitoid Cotesia flavipes Cameron (Hymenoptera:Braconidae) in rapidly changing environments. Pest Management Science, 74,6.

Mutamiswa R., Machejano H., Chidawanyika F., Nyamukondiwa C., 2019. Life-stage related responses to combined effects of acclimation temperature and humidity on the thermal tolerance of Chilo partellus (Swinhoe) (Lepidoptera:Crambidae). Journal of Thermal Biology, 79,85-94.

Nielsen M.M., Overgaard J.O., Sørensen J.G., Holmstrup M., Justen J., Loeschke V., 2005. Role of HSF activation for resistance to heat, cold and high-temperature knock-down. Journal of Insect Physiology, 51:1320-1329.

Ohtaka C., Ishikawa H., 1991. Effects of heat treatment on the symbiotic system of an Aphid mycetocite. Symbiosis, 11:19-30.

Oliver K.M., Moran N.A., Hunter M.S., 2005. Variation in resistance to parasitism is due to symbionts not host genotype. PNSA, 102(36): 12795-12800.

Pennacchio, F., Digilio, M.C., Tremblay, E., Tranfaglia, A., 1994. Host recognition and acceptance behaviour in two aphid parasitoid species: Aphidius ervi and Aphidius microlophii (Hymenoptera: braconidae). Bullettin of Entomological Research, 84, 57.

Powell S.J., Bale J.S., 2006. Effect of long-term and rapid cold hardening on the cold torpor temperature of an aphid. Physiological Entomology, 31(4), 348-352.

Rinehart J.P., Denlinger D.L., Rivers D.B., 2002. Upregulation of transcripts encoding select heat shock proteins in the flesh fly Sarcophaga crassipalpis in response to venom from the ectoparasitoid wasp Nasonia vitripennis. Journal of Invertebrate Pathology,79: 62–63.

Rodriguez-Trelles F., Tarrio R., Santos M., 2013. Genome-wide evolutionary response to a heat wave in. Biology Letters, 9.

Scott M., Berrigan D., Hoffman A.A., 1997. Costs and benefits of acclimation to elevated temperature in Trichogramma carverae. Ebtomologia Experimentalis et Applicata, 85(3): 211-219.

Sejerkilde M., Sørensen J.G., Loeschcke V., 2003. Effect of cold- and heat hardening on thermal resistance in *Drosophila melanogaster*. Journal of Insect Physiology, 49(8): 719-726.

Sgro C.M., Overgaard J., Kristensen T.N., Mitchell K.A., Cockerell F.E., Hoffman A.A., 2010. A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in population of Drosophila melanogaster form eastern Australia. Journal of Evolutionary Biology, 23(11):2484-2493.

Shim J.K., Ha D.M., Nho S.K., Song K.S., Lee K.Y., 2008. Upregulation of heat shock protein genes by envenomation of ectoparasitoid Bracon hebetor in larval host of Indian meal moth Plodia interpunctella. Journal of Invertebrate Pathology, 97, 306–309.

Sinclair B.J., Roberts S.P., 2007. Acclimation, shock and hardening in the cold. Journal of Thermal Biology, 30(8):557-562.

Slabber S., Worland M.R., Petter H.L., Chown S.L., 2007. Acclimation effects on thermal tolerance of springtails from sub-Antarctic Marion Island: indigenous and invasive species. Journal of Insect Physiology, 53(2),113-125.

Sørensen J.G., Dahlgaard J., Loeschcke V., 2001. Genetic variation in thermal tolerance among natural populations of *Drosophila buzzatii*: down regulation of Hsp70 expression and variation in heat stress resistance traits. Functional Ecology, 15, 289–296.

Sørensen J.G., Kristensen T.N., Overgaard J., 2016. Evolutionary and ecological patterns of thermal acclimation capacity in Drosophila: is it important for keeping up with climate change? Current Opinion in Insect Science, 17,98-104.

Sørensen J.G., Nielsen M.M., Kruhøffer M., J. Justesen, V. Loeschke, 2005. Full genome gene expression analysis of the heat stress response in Drosophila melanogaster. *Cell stress & chaperones,* 10: 312–328.

Sorvari J., Haatanen M.-K., Vesterlund S.-R., 2011. Combined effects of overwintering temperature and habitat degradation on the survival of boreal wood ant. Journal of Insect Conservation,15, 727–731.

Terblanche, J.S., Hoffmann, A.A., Mitchell, K.A., Rako, L., le Roux, P.C., Chown, S.L., 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. Journal of Experimental Biology, 214, 3713–3725.

Tomanek L., 2010.Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. Journal of Experimental Biology, 213(6):971-979.

Vincenzi S., De Leo G.A., Bellingeri M., 2012. Consequences of extreme events on population persistence and evolution of a quantitative trait. Ecological Informatics, 8:20-28.

Vinson S.B., 1990. How parasitoids deal with the immune system of their host: an overview. Insect Biochemistry and Physiology, 13(1-2):3-27.

Warton D.I., Hui F.K.C., 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology, 92(1):3-10.

Williams S.E., Shoo L.P., Isaa, J.L., Hoffman A.A., Langham G., 2008. Towards an integrated framework for assessing the vulnerability of species to climate change. PLoS Biology,6, 2621–2626.

Zhang W., Chang x.Q., Hoffmann A.A., Zhang S., Ma C.S., 2015. Impact of hot events at different development stages of a moth: the closer to adult stage, the less reproductive output. Science Reproduction, 5,10436.

Zhao F., Zhang W., Hoffmann A.A., Ma C.S., 2017. Life stages of an aphid living under similar thermal conditions differ in thermal performance. Journal of Insect Physiology, 99,1-7.

Zhao L., Jones W.A., 2012. Expression of heat shock protein genes in insect stress responses. ISJ, 9: 93-101.

Zhu L., Wang L., Ma C., 2019. Sporadic short temperature events cannot be neglected in predicting impacts of climate change on small insects. Journal of Insect Physiology, 112:48-56.

CHAPTER IV

Heat stress experienced at the immature stages affects body size of the adult parasitoid *Aphidius ervi*

Introduction

The relationships between environmental temperature and adult body size in animals have intrigued biologists for over a century. In particular, an increasing interest in last decades has been fueled by the discovery of specific patterns related to body size in different insect taxa (Angilletta *et al*., 2004). Relationships between environmental temperature and life history in ectotherms have puzzled biologists because of the effects of temperature on growth rate and size: lower temperatures determine a slower growth rate, but, at the mature age, body size is larger (Berrigan and Charnov,1994). It is well known that different populations of the same species, when distributed over broad geographic thermal ranges (Bergman rule), often show different body size, with the majority of species exhibiting larger adult size in colder environments (Partridge and French, 1996; Ashton, 2004). Moreover, laboratory studies have shown that a reduction in environmental temperature causes an increase in adult size in many ectotherms (Atkinson, 1994, 1995; Atkinson *et al*., 2003; Trotta *et al*., 2006).

Some life history traits of insects, such as developmental time, fecundity and sex ratio are directly affected by body size. Body size has then relevant effects on individual fitness (Roff, 2002; Kingsolver and Huey 2008), and it is a parameter often used in quality control assessment of mass reared arthropods used in agriculture as pest control agents (van Lenteren *et al.*, 2003), such as the parasitoid species used in this study. Moreover, the correlations between body size and some life history traits under selection is well documented (Reeve *et al*., 2000; Hayes and Shonkwiler, 2006).

In the case of body size, 'bigger' is not always 'better'. In life history theory, the basic idea of optimization assumes that a balance of costs and benefits determines the value of a trait within the range of available variation, so the adult size of an organism is optimized, and not maximized (Stearns, 1992). There are costs associated with a larger body size that decrease the overall fitness, such as an increased juvenile mortality and a prolonged developmental time, which increases the offspring exposure to a high predation risk (Sequeira and Mackauer, 1992; Harvey *et al*.,1994).

In insects, adult body size is influenced by numerous physiological factors such as growth rates, duration of juvenile growth, and rate and duration of cell proliferation (Nijhout, 2003; Emlen and Allen, 2004; Edgar, 2006). The definition of body (or organ) size and shape is a

process that requires tight coordination of different cell dynamics, like cell proliferation, apoptosis, cell allocation and mitotic orientation (Baena-López *et al.*, 2005; Dworkin and Gibson, 2006). Developmental and physiological processes influencing the growth and final size of adult body parts in insects have been mainly studied in *Drosophila* (Emlen and Allen, 2004). In holometabolous insects, larvae are very different from adults, they are specialized for feeding and growing, and some of the body organs present at the adulthood are not present during the immature stages. The cells that will form the adult body are already present during the immature stage as imaginal discs and histoblast nest. The imaginal discs contain the cells that will origin the different organs of the adult body, such as wings, legs, and others. The imaginal disc growth occurs primarily by cell proliferation. Each of the different imaginal discs behaves as a relatively autonomous developmental unit and the final dimensions of the morphological structures will depend on how fast the cells in each imaginal disc proliferate and how long imaginal cell proliferation continues (Emlen and Allen, 2004). Histoblasts undergo mitosis and during adult development they replace the larval cells and form the adult epidermis. Histological studies demonstrated that histoblasts during the entire larval development do not increase in number (Madhavan and Schneiderman, 1977). All these cells undergo a series of temporally regulated transitions during which neither cell size nor division rate is constant (Nivon *et al*., 2009). Imaginal discs and histoblasts may be affected by different kind of stress during the organism development, such as the thermal ones, which can interfere with the normal development and growth of the organism and affecting different body features (Wake and Hall, 1999; Truman and Riddiford, 2002)

To ensure survival, many species have to adjust their physiology since they live in an ever-changing environment. According to the environmental conditions experienced by insect populations, individuals of different sizes and shapes can be generated as a results of phenotypic plasticity (Partridge *et al.*, 1994; Partridge and French, 1996; Trotta *et al.*, 2006). The environmental differences are translated, during development, into phenotypic differences (the phenomenon called phenotypic plasticity). In insects, two particularly important environmental and ecological variables induce body size plasticity: the diet (quantity and quality) and the environmental temperature (Davidowitz *et al*., 2004; Trotta *et al.*, 2006, 2010, 2014; Stillwell *et al.*, 2007).

Small random local perturbations in metabolic processes, such as the developmental noise resulting after the exposure to a thermal stress, can produce deviations from the normal developmental pattern (Rutherford and Lindquist, 1998). A thermal stress can produce different and independent effects on the body of an organism and, unless compensatory

mechanisms are present, it can alter the final size. As development is an integrated process, perturbations occurring in specific regions (or organs) can also affect the development of other body structures. The wing of some insects is regarded as an excellent model system to investigate body and organ size variation. Wing size is positively correlated with body size as a whole and it can be measured accurately (Robertson and Reeve, 1952; Partridge *et al.*, 1999; Huey *et al.*, 2000; David *et al.*, 2005, 2006; Trotta *et al.*, 2006, 2011). Wing length was also used as a measure of body size for many parasitoid wasp species (40 species in 13 families; Jervis *et al*., 2003).

Animal species bear a specific relationship between the size of their organs and the body size (Conlon and Raff, 1999). It is evident that within a species, individuals with larger bodies have larger constituent parts. The relationship between the sizes of individual traits and the whole body size is named allometry (Shingleton *et al.*, 2007). As overall body size varies, some organs may grow in strict proportion with it ("isometry"), whereas other organs may disproportionally increase (hyperallometry) or decrease (hypoallometry) in size. This variable size relationship, that reflects the regulation grow sensitivity that varies from organ to organ, is a named morphological allometry. These phenomena are very evident in the living world (Texada *et al*., 2020).

Allometry describes how the characteristics of an organism scale with each other and with body size. When conspecific individuals at the same life stage are considered, the allometry is named "static allometry". Static allometry reveal how the sizes of a trait scale with the size of the other traits and with the whole body size. This kind of allometry, typical of holometabolous insects, depends on the genetic and physiological mechanisms regulating both organ and body size in response to environmental (nutrition, developing temperature, *etc.*) variations (Shingleton *et al*., 2007).

Changes in nutrition affect the size of some organs like the wings and legs in isometric proportion to body size (Shingleton, 2005; Shingleton *et al*., 2009). The correlation between wing and tibia length is often used to detect allometry (Shingleton 2005; Shingleton *et al*., 2009; Trotta *et al*., 2010), as done in this study. In holometabolous insects, such as *Drosophila*, other organs such as the central nervous system and the genitalia are less sensitive to changes in nutrition and develop to an approximately similar size, irrespective of increased body size (Shingleton, 2005; Cheng *et al*., 2011; Dreyer and Shingleton 2011; Tang *et al*., 2011). This allows tissues whose function is highly size-dependent to compensate for the effects of nutritional input (Shingleton, 2010; Koyama *et al.*, 2013).

From an evolutionary point of view, adaptations and the emergence of novel phenotypes would require some degree of developmental independence among body parts (Cowley and

Atchley, 1990). Since linkage between body parts exists, different morphological traits cannot be genetically completely independent (Carreira *et al*., 2009). Successful development is the outcome of multiple coordinated programs of gene expressions. Environmental stresses such as heat, which can disrupt this coordination in developing organisms, can damage the body parts of surviving adults. Environmental allometries arise because both the body and the different organs do not respond in similar ways to the environmental factors affecting the rate and duration of cell growth and division. This developmental alteration due to the stressful environmental conditions could be considered a form of phenotypic plasticity (Stern and Emlen, 1999). For this reason, variation of the static allometry is an important component of phenotypic diversity (Shingleton *et al*., 2007). Endoparasitoids are holometabolous insects living within the host body during the immature stages. For several days after parasitization, both host and parasitoid continue to grow and develop (Lin and Ives, 2003; Wyckhuys *et al*., 2008). Endoparasitoid development is highly influenced by the features of the host, since it represents the only nutrient font during the immature stages (Vinson, 1990). Any stress affecting host could potentially affect the biological features of the adult parasitoid (Nicol and Mackauer, 1999), and if a stress kills the host after parasitization, also the developing parasitoid die (Vison, 1990).

The braconid wasp *A. ervi* prefers to parasitize the younger *A. pisum* nymphal stages. This is principally due to the ease of handling the host during parasitization (He *et al*., 2011). When *A. ervi* parasitizes younger nymph stages, the aphid continues its development until parasitoid pupation but its physiology, behaviour and reproduction are finely de-regulated to meet nutritional and physiological requirements of parasitoid larvae (Digilio *et al*., 2000; Falabella *et al*., 2000; Rahbé *et al*., 2002; Pennacchio and Strand, 2006).

Many biological features of the endoparasitoids are affected by the thermal conditions at which the host live and stressful conditions could potentially affect its development and its body size. Many studies conducted at different constant rearing temperature report that body size of adult insects is affected by temperature (Davidowits *et al*., 2003; Trotta *et al*., 2006, 2014; Winterhalter and Mosseau, 2008; Horne *et al.,* 2017), but it is not clear if and how a short and strong heat stress could affect insect body size. Parasitoid correct development depends on its physiology and on the interactions with the host. A thermal stress could interfere both with the host and parasitoid physiology at different times and in different ways, necessarily causing internal stress.

The aim of this chapter is to understand if parasitoids at various stages of development show differences in body size and in their static allometry after exposure to stressful temperatures. Body size was estimated considering leg and wing length. Parasitized aphids were subjected

to a thermal treatment at three different developmental stage for an exposure time of 30 minutes, that are the same of those reported in Ch.II. Moreover, the effects of the different thermal treatment and its combination on aphid survival 24 hours after the cold exposure was also investigated.

Materials and Methods

Experimental treatments

Aphids and parasitoids were obtained as described in the Ch. II. Aphids were parasitized at the 3rd nymphal stage (3-days-old), and then subjected to two thermal treatments. The first thermal treatment consisted of a heat exposure at 35.0 ± 0.1°C (mean values ± accuracy) for 30 minutes. The second thermal treatment consisted of a heat exposure at 39.0 ± 0.1°C for 30 minutes. Both thermal treatments were applied at two different developmental stages of the parasitoid: egg and 3rd larval stage (L3), that respectively corresponds to 24 hours and 120 hours after parasitization. The control group consisted of parasitized aphids that did not experienced the thermal stress (No-HS). Each thermal treatment was composed of about 36 aphids. Heat stress was performed as described in the previous chapters. For all the experimental groups, aphid survival was recorded 24 hours after the heat stress.

Body size measurement

Wing and tibia length were used to estimate parasitoid body size (Dmitriew and Blanckenhorn, 2012; Trotta *et al*., 2010, 2011). The fore left wings and the hind legs were removed from each parasitoid and fixed under cover slips on microscope slides. Images of the wings and tibiae were recorded with a Nikon video camera connected to a PC and mounted on a Nikon microscope. As stated above, the images were then used to record the fore wing and tibia length of the hind leg (Fig.4.1) using the Image J 1.31 software (http://rsb.info.nih.gov/ij/). All the data were subsequently transformed in millimeters using an image of a micrometer. Since the number of males was very low, just females were subjected to body measurement.

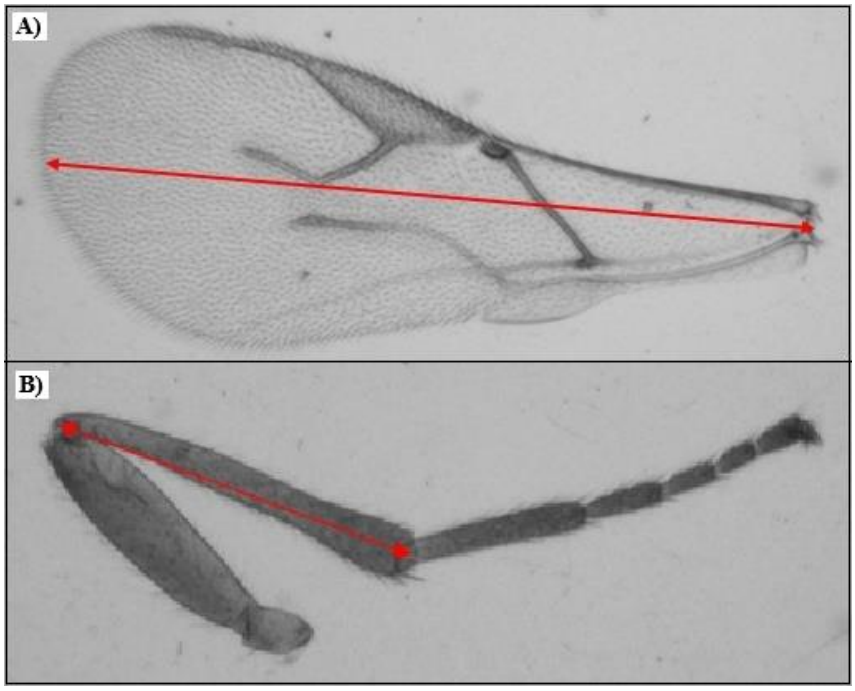

Fig. 4.1 - *A. ervi* wing (A) and hind leg (B). The red arrows indicate the standard region used for length measures.

Statistical analyses

To test for differences in survival, in wing and tibia size among experimental groups, three independent linear model ANOVAs, with 'treatment' as main effects, were used. Tukey post-hoc tests for multiple comparisons of means were also performed to detect significant differences among the treatments in the ANOVAs. The correlation between wing and tibia size was also tested for all the experimental groups.

Results

Aphid survival rate was statistically different among the experimental groups ($F_{4,95}$= 3.36, P< 0.05). The statistical significance was only due to the differences between the control (No-HS = 98.5%) and the other experimental groups, that are: 35°C/Egg, 39°C/Egg, 35°C/L3 and 39°C/L3 groups (35°C/Egg=87.9%; 35°C/L3=90.0%; 39°C/Egg=88.9%; 39°C=87.0%; see Fig. 4.2).

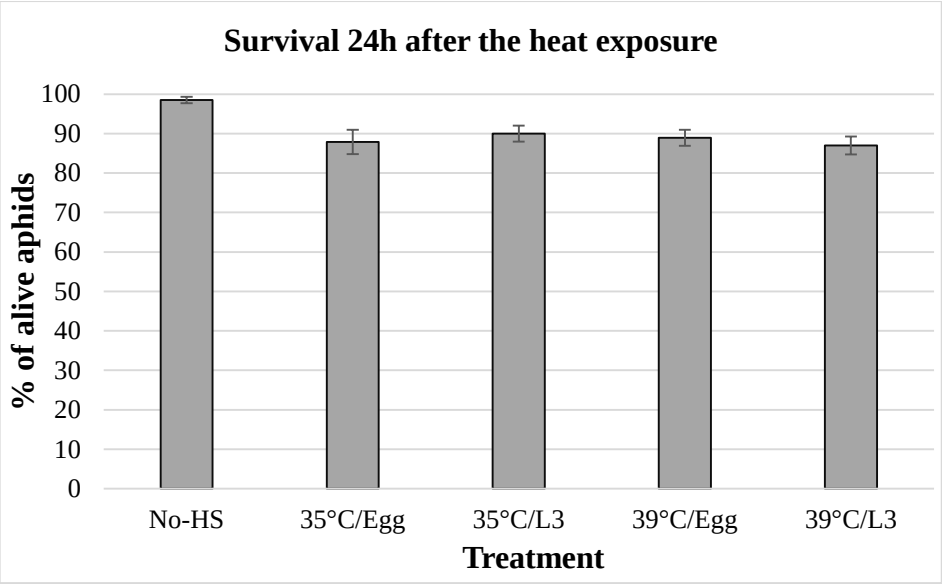

Fig. 4.2 – Mean values (± standard errors) of aphid survival 24 hours after the heat exposures.

Mean values of parasitoid wing and tibia length of the four experimental groups are shown in Fig. 4.3 and Fig. 4.4. Significant differences in wing length among the four parasitoid groups ($F_{4,161}$= 12.3, P< 0.001), as well as significant differences in tibia length ($F_{4,161}$= 8.18, P< 0.001) were found. Compared with the control, the parasitoid body size decreases when parasitized aphids were heat shocked at 39°C, independently if the heat stress has been experienced by the egg or by the 3rd larval stage (P< 0.001 in all cases). At 35°C, no differences in wing and tibia size were found when compared with the control.

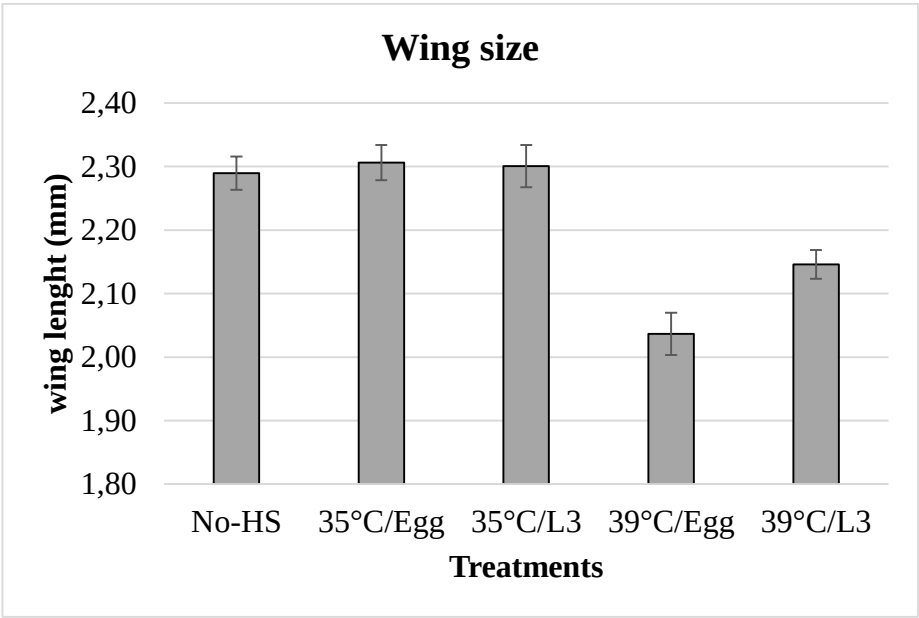

Fig. 4.3 - Mean values (± standard errors) of *A. ervi* wing length of the four experimental groups.

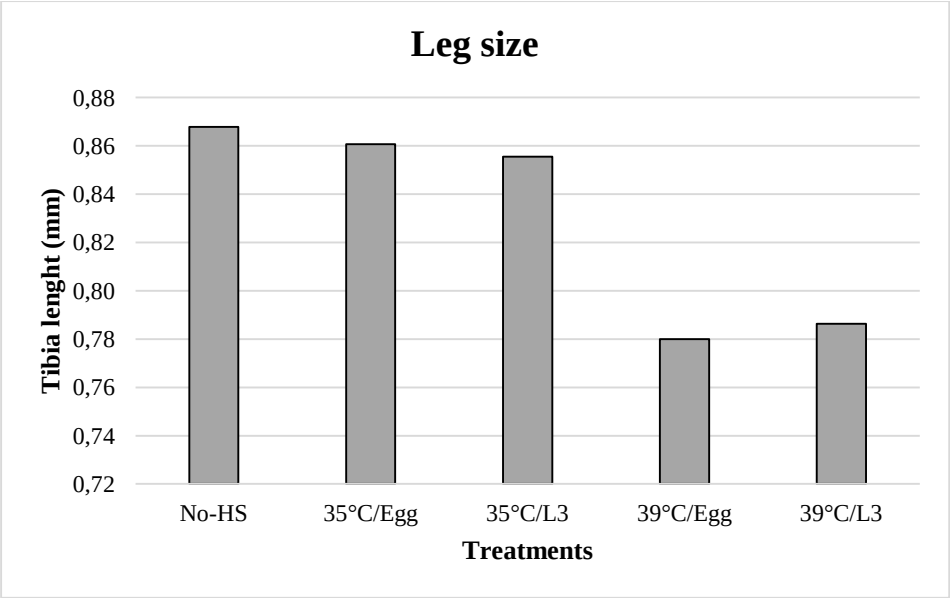

Fig. 4.4 - Mean values (±standard errors) of *A. ervi* tibia length of the four experimental groups.

Considering together all the experimental groups, wing length of parasitoids was highly correlated with tibia length (r_{162} = 0.55, P < 0.001). When the individual treatments were considered, the relationship between wing and tibia length was always significant except for the 39°C/Egg (Fig. 4.5).

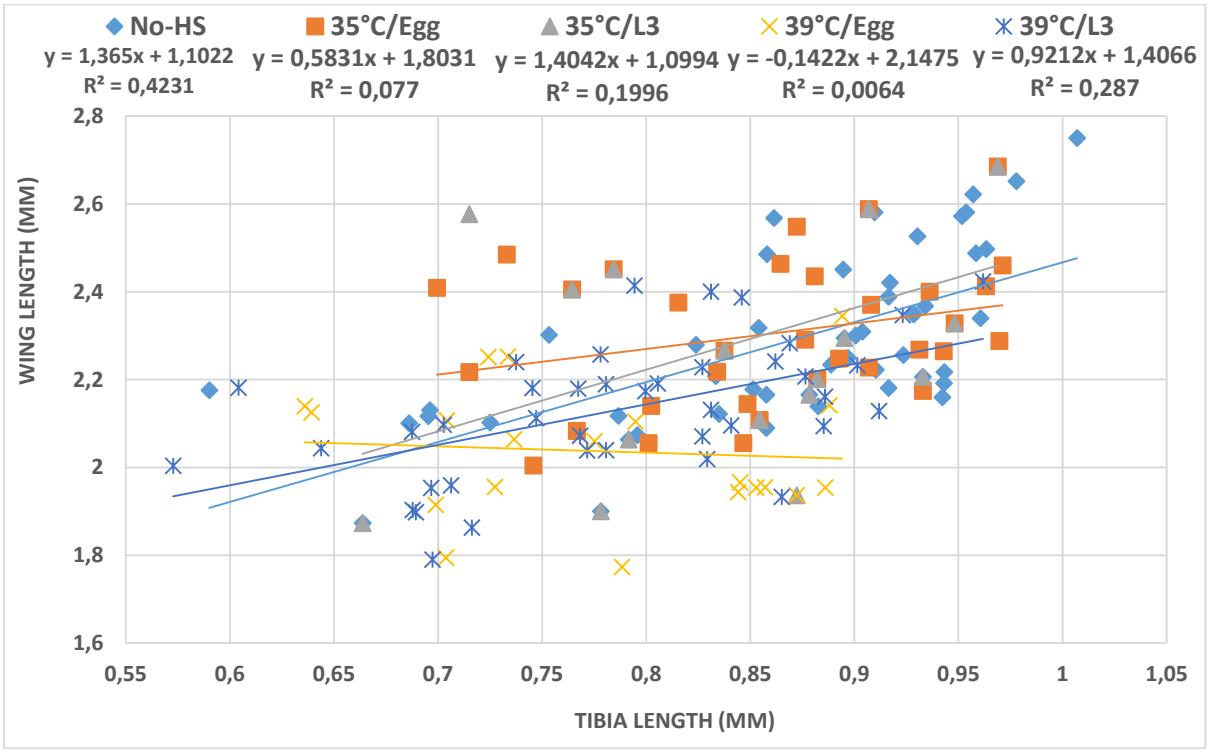

Fig. 4.5 - Relationship between wing and tibia size. Individual relationship between wing length and tibia length in *A. ervi* developed in parasitized aphids subjected to different thermal treatments.

Discussion and Conclusions

In this study, I investigate the effect of two different stressfull temperatures applied at two immature developmental stages (egg and 3rd larval stage) of the aphid endoparasitoid *A. ervi*. Since *A. ervi* is an endoparasitoid of *A. pisum*, the heat exposures were performed on parasitized aphids that host a parasitoid egg or a 3rd larval stage. I found that the heat exposure at 39°C determine a significant body size reduction in adult *A. ervi*., both when it experienced the heat stress at the egg and 3rd larval stage.

In the case of the endoparasitoid that experienced the strong heat stress at the 3rd larval stage, the wasp body size reduction could be independent of the host size (the aphid has reached its final dimensions) but directly related to the effect of the heat stressful temperature on the parasitoid. The size reduction of adult parasitoid could be due to a trade-off between the heat resistance mechanisms and body organ formation. The activation and the maintenance of heat-induced reparative mechanisms, both those ensuring survival during stress occurrence and those implicated in the following cellular injuries reparation, may have a relevant cost. Consequently, the amount of energy directed to the soma could be reduced and the parasitoids reached a smaller size.

Moreover, although the endoparasitoid lives within the host and could appear "protected", it can be assumed that the wasp perceives any thermal stress and it could be directly affected by the stress. Heat stress can also indirectly affect the developing endoparasitoid throughout any physiological change in the host aphid. In fact, the aphid represents both the physical space in which endoparasitoid develops as well as the unique nutriment fount for the developing endoparasitoid (Vinson, 1990).

It is supposable that a reduction of the nutritional quality of the aphid host, due to the heat exposure, could also be a cause of the smaller parasitoid size observed in this study. This hypothesis is supported by the fact that heat stressed aphid appeared paler (yellowish) than those of the control group. In these green clone of the pea aphid, a low nutritive valor is generally associated with paler body color (Libbrecht *et al*., 2007). Any changes in aphid physiology could produce nutritional changes for the parasitoid and can potentially influence its life history traits such as the adult body size. It is known that the heat stress depresses the population of the aphid symbionts (Montllor *et al*., 2002) that, in turn, leads to a reduction of the synthesis of essential amino acids in aphids. In the case of the parasitoids experiencing the strong heat stress at the egg stage (when the aphid was at the 3rd nymphal stage), the parasitoid body size reduction could be also attributed to a body size reduction of the aphid host. Under this hypothesis, a reduction of *Buchnera aphidicola* population in aphid may have negatively affected the aphid size, also reducing its nutritional quality and leading to

smaller adult parasitoids. It could be interesting to investigate if and how the heat affects this obligate aphid symbiont, and if the heat stress induces any physiological alterations in the symbiont-aphid-parasitoid system and the repercussions aphid and endoparasitoid body size and its fitness.

As for the measure of body size, wing and tibia length were also used to assess any allometry change in the adult parasitoid due to heat exposure. Relevant allometry variations were found in the adult parasitoids that experienced the heat stress at the egg stage, both at 35°C and at 39°C. The higher allometry variation was found in the parasitoids that experienced the heat stress at 39°C during the egg stage, since the relationship between the two traits disappear. This lack of correlation could be attributed to the high sensibility of the egg to this stressful temperature. On the contrary, allometry change was lesser significant in the parasitoids that experienced the heat exposure at the 3rd larval stage, at both temperatures. Compared with the 3rd larval stage, the 39°C heat stress experienced at the egg stage had higher negative effects on the harmonic development of *A. ervi*.

The variations of the adult parasitoid body size and allometry, exposed at the heat temperature during the egg and larval stage, could be due to the direct detrimental effect of the heat on imaginal disc cells. At the last larval stage, wing imaginal discs are well-formed and the characteristic of the adult are less alterable. On the contrary, a heat stress at an early developmental stage strongly affected the cellular dynamics which cannot be adjusted in the later developmental phases, determining more visible body alterations. This could explain why the allometry change and the body size reduction were particularly evident in the parasitoids that experienced the heat stress at the egg stage.

Imaginal discs of insects contain intrinsic information on body organs size and form. Growth of imaginal discs can be altered by heat stress, causing alterations on the whole harmonic body growth (Stern and Emlen, 1999). In literature it is well reported that some organs of *Drosophila* adults also show different scaling relationships to the body size in response to environmental stressfull conditions. In particular, changes in nutrition affect the size of some organs like the wings and legs in isometric proportion to body size (Shingleton, 2005; Shingleton *et al.*, 2009). This proportional scaling is mainly mediated by nutrition-dependent insulin/TOR signaling. In contrast, other organs such as the arrival at the adulthood, the females of koinobiotic parasitoids already have fertile eggs within their reproductive apparatus. It is basically for this reason that the reproductive success of females is primarily affected by egg production (Godfray, 1994).

By considering the body size reduction of adult parasitoid due to heat stress find in this study, and considering the positive relationship between adult parasitoid size and egg number

(Visser, 1994; Pandey and Singh, 1999), it will be interesting to investigate if, and how, the heat stress affects the parasitoid reproduction. For instance, the hypothesis that in heat stressed parasitoid the egg number may be reduced could be investigated. If central nervous system and the genitalia are less sensitive to nutritional changes and develop to an approximately similar size, irrespective of increased body size (Shingleton, 2005; Cheng *et al*., 2011; Dreyer and Shingleton 2011; Tang *et al*., 2011). This allows tissues whose function is highly size-dependent to compensate for the effects of nutritional input (Shingleton, 2010; Koyama *et al.*, 2013).

It is supposable that heat stress also affected the features of the reproductive apparatus (Tang *et al*., 2011). It is important to consider that in many hymenopteran endoparasitoids, as in *A. ervi*, the reproductive success of the females is primarily affected by egg production (both the egg number and the egg size) (Godfray, 1994). This parasitoid species usually shows arrhenotoky, where fertilized eggs produce diploid females and unfertilized eggs produce haploid males. Virgin females only produce sons while inseminated females produce both sons and daughters (Quicke, 1997). Then, the decrease in fitness, associated with reduces size, will be confirmed by subsequent studies, it will be reasonable to suppose that extreme heat events may strongly impact parasitoid population in nature through both direct and also indirect effects. Being able to make predictions on the variation of the population structure and dynamic following a heat stress will be very important for a correct use of parasitoids in biological control strategies.

References

Angilletta M. J. Jr., Todd D., Steury T.D., Michael W., Sears, 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle, Integrative and Comparative Biology, 44(6): 498–509.

Ashton, K.G., 2004. Sensitivity of intraspecific latitudinal clines of body size for tetrapods to sampling, latitude and body size. Integrative and Comparative Biology, 44: 403–412.

Atkinson D., 1994. Temperature and organism size a biological law for ectotherms? Advances in Ecological Research, 25: 1–58.

Atkinson D., 1995. Effects of temperature on the size of aquatic ectotherms: Exceptions to the general rule. Journal of Thermal Biology, 20: 61–74.

Atkinson D., Ciotti B. J., and Montagnes D. J. S., 2003. Protists decrease in size linearly with temperature: ca. 2.5% 8C21. Proceedings of the Royal Society of London, 270:2605–2611.

Baena-López L.A., Baonza A., García-Bellido A., 2005. The orientation of cell divisions determines the shape of Drosophila organs. Current Biology 15: 1640–1644.

Berrigan D., Charnov E. L., 1994. Reaction norms for age and size at maturity in response to temperature: A puzzle for life historians. Oikos, 70:474–478.

Carreira V.P., Mensch J., Fanara J.J., 2009. Body size in *Drosophila*: genetic architecture, allometries and sexual dimorphism. Heredity, 102,246-256.

Cheng, L. Y., A. P. Bailey, S. J. Leevers, T. J. Ragan, P. C. Driscoll, A.P. Gould., 2011 Anaplastic lymphoma kinase spare organ growth during nutrient restriction in Drosophila. Cell 146: 435–447.

Conlon I., Raff M., 1999. Size control in animal development. Cell, 96,235–244.

Cowley D.E., Atchley W.R., 1990. Development and quantitative genetics of correlation structure among body parts of *Drosophila melanogaster*. The American Naturalist, 135,2.

David J.R, Araripe L.O., Bitner-Mathé B.C., Capy P, Goñi B, Klaczko LB, Legout H, Martins MB, Vouidibio J, Yassin A, Moreteau B. 2006. Quantitative trait analysis and geographic variability of natural populations of Zaprionus indianus, a recent invader in Brazil. Heredity,96: 53–62.

David J.R., Gibert P., Pétavy G., Capy P., Moreteau B., 2005. Isofemale lines in Drosophila: an empirical approach to quantitative trait analysis in natural populations. Heredity,94: 3–12.

Davidowitz G., D'Amico L.J, Nijhout HF., 2004. The effects of environmental variation on a mechanism that controls insect body size. Evolutionary Ecology Research 6: 49– 62.

Davidowitz G., D'Amico L.J., Nijhout H.F., 2003. Critical weight in the development of insect body size. Evolution & Development, 5(2):188-197.

Digilio M.C., Isidoro N., Tremblay E., Pennacchio F., 2000. Host castration by *Aphidius ervi* venom proteins. Journal of Insect Physiology, 46: 1041–1050.

Dmitriew C., Blanckenhorn W.U., 2012. The Role of Sexual Selection and Conflict in Mediating Among-Population Variation in Mating Strategies and Sexually Dimorphic Traits in *Sepsis punctum*. PLoS ONE, 7(12): e49511.

Dreyer A. P., A. W. Shingleton, 2011 The effect of genetic and environmental variation on genital size in male Drosophila: canalized but developmentally unstable. PLoS One 6: e28278.

Dworkin I., Gibson G., 2006. Epidermal growth factor receptor and transforming growth factor signaling contributes to variation for wing shape in Drosophila melanogaster. Genetics,173: 1417–1431.

Edgar B.A., 2006. How flies get their size: genetics and physiology. Nature Reviews Genetics 7: 907–916.

Emlen D.J., Allen C.E., 2004. Genotype to phenotype: physiological control of trait size and scaling in insects. Integrative and Comparative Biology,43: 617–634.

Falabella P., Tremblay E., Pennacchio F., 2000.Host regulation by the aphid parasitoid Aphidius ervi: the role of teratocytes. Entomologia Experimentalis et Applicata, 97: 1–9.

Godfray H.C.J., 1994. Parasitoids: behavioural and evolutionary ecology. Princeton, NJ: Princeton University Press.

Harvey J.A., Harvey I.F., Thompson D.J., 1994.Flexiblelarval growth allows use of a range of host sizes by a parasitoid wasp. Ecology, 75:1420–1428.

Hayes J.P., Shonkwiler J.S., 2006.Allometry, antilog transformations, and the perils of prediction on the original scale. Physiological and Biochemical Zoology,79: 665–674.

He X.Z., Wang Q., Teulon D.A.J., 2011. Host age preference in Aphidius ervi Haliday. Journal of Insect Behavior, 24:447-455.

Horne C.R., Hirst A.G., Atkinson D., 2017. Insect temperature-body size trends common to laboratory, latitudinal and seasonal gradients are not found across altitudes. Functional Ecology, 32(4):948-057.

Huey R.B., Gilchrist G.W., Carlson M.L., Berrigan D., Serra L., 2000. Rapid evolution of a geographic cline in size in an introduced fly. Science,287: 308–309.

Jervis M.A., Ferns P.N., Heimpel G.E., 2003.Body size and the timing of egg production in parasitoid wasps: a comparative analysis. Functional Ecology,17: 375–383.

Kingsolver J.G., Huey R.B., 2008. Size, temperature, and fitness: three rules. Evolutionary Ecological Research, 10:251–268.

Koyama, T., C. C. Mendes, C. K. Mirth, 2013 Mechanisms regulating nutrition-dependent developmental plasticity through organ-specific effects in insects. Frontiers of Physiology, 4: 263.

Libbrecht R., Gwynn D.M., Fellowes M.D.E., 2007- Aphidius ervi preferentially attacks the green morph pf the pea aphid, Acyrthosiphon pisum. Journal of Insect Behaviour, 20, 1.

L.A., Ives A.R., 2003. The effect of parasitoid host-size preference on host population growth rates: an example of Aphidius colemani and Aphis glycines. Ecological Entomology,28, 5: 542-550.

Madhavn M. M., Schneiderman H. A., 1977. Histological analysis of the dynamics of growth of imaginal discs and histoblast nests during the larval development of Drosophila melanogaster. Wilhelm Roux' Arch, Developmental Biology. 183, 269-305.

Montllor C.B., Maxmen A., Purcell A.H., 2002. Facultative bacterial endosymbionts benefits pea aphids Acyrthosiphon pisum under heat stress. Ecological Entomology, 27(2):189-195.

Nicol C.M.Y., Mackauer M., 1999. The scaling of body size and mass in a host–parasitoid association: influence of host species and stage. Entomologia Experimentalis et Applicata,90: 83–92.

Nijhout H.F., 2003.The control of body size in insects. Developmental Biology, 261:1–9.

Nivon N., Manjon C., Martin-Blanco E., 2009. Dynamic control of cell cycle and growth coupling by ecdysone, EGFR, and PI3K signaling in Drosophila Histoblasts. PLOSBiology, 7(4): e1000079.

Pandey S., Singh R., 1999. Host size induced variation in progeny sex ratio of an aphid parasitoid *Lysiphlebia mirzai*. Entomologia Experimentalis et Applicata, 90: 61-67.

Partridge L. French V., 1996. Thermal evolution of ectotherm body size: Why get big in the cold?InI. A. Johnston and A.F. Bennett (eds.), Animals and temperature: Phenotypic and evolutionary adaptation. 265–292. Cambridge UniversityPress, Cambridge.

Partridge L., Barrie B., Fowler K., French V., 1994. Evolution and development of body size and cell size in Drosophila melanogaster inresponse to temperature. Evolution, 48:1269-1276.

Partridge L., Langelan R., Fowler K., Zwaan B., French V., 1999.Correlated responses to selection on body size in Drosophila melanogaster. Genetical Research,74: 43–54.

Partridge, L. French V., 1996. Thermal evolution of ectotherm body size: Why get big in the cold? In I. A. Johnston and A. F. Bennett (eds.), Animals and temperature: Phenotypic and evolutionary adaptation, pp. 265–292. Cambridge UniversityPress, Cambridge.

Pennacchio F., Strand M.R., 2006. Evolution of developmental strategies in parasitic Hymenoptera. Annual Reviews of Entomology,51: 233–258.

Quicke D.L.J., 1997. Parasitic Wasps. Chapman and Hall, London

Rahbé Y., Digilio M.C., Febvay G., Guillaud J., Fanti P., Pennacchio F., 2002. Metabolic and symbiotic interactions in amino acid pools of the pea aphid, Acyrthosiphon pisum, parasitized by the braconid Aphidius ervi. Journal of Insect Physiology,48: 507–516.

Reeve M.W., Fowler K., Partridge L., 2000. Increased body size confers greater fitness at lower experimental temperature in male Drosophila melanogaster. Journal of Evolutionary Biology,13: 836–844.

Robertson F.W., Reeve E.C.R., 1952. Studies in quantitative inheritance. I. The effects of selection of wing and thorax length in Drosophila melanogaster. Journal of Genetics,50: 414–448.

Roff D. A., 2002. Life history evolution. Sinauer Associates, Inc., Sunderland, Massachusetts.

Rutherford S., Linquist S., 1998. Hsp90 as a capacitor for morphological evolution. Nature, 396:336-342.

Sequeira R., Mackauer M., 1992. Nutritional ecology of an insect host–parasitoid association: the pea aphid – Aphidius ervi system. Ecology,73: 183–189.

Shingleton A. W., 2005 Body-size regulation: combining genetics and physiology. Curr Biol. 15: R825–R827.

Shingleton A. W., 2010 The regulation of organ size in Drosophila: physiology, plasticity, patterning and physical force. Organogenesis 6: 76–87.

Shingleton A. W., C. M. Estep, M. V. Driscoll, I. Dworkin, 2009 Many ways to be small: different environmental regulators of size generate distinct scaling relationships in Drosophila melanogaster. Procedure in Biology Sciences, 276: 2625–2633.

Shingleton A.W., Frankino W.A., Flatt T., Nijhout H.F., Emlen D.J., 2007. Size and shape: the development regulation of static allometry in insects. BioEssays, 29(6):536-548.

Stearns S. C., 1992. The evolution of life histories. Oxford University Press, Oxford.

Stern D.L., Emlen D.J., 1999.The development basis for allometry in insects. Development, 126:1091-1101.

Stillwell R.C., Wallin W.G., Hitchcock L.J., Fox C.W., 2007. Phenotypic plasticity in a complex world: interactive effects of food and temperature on fitness components of a seed beetle. Oecologia,153: 309–321.

Tang, H. Y., M. S. Smith-Caldas, M. V. Driscoll, S. Salhadar, A. W. Shingleton, 2011. FOXO regulates organ-specific phenotypic plasticity in Drosophila. PLoS Genet. 7: e1002373.

Texada M.J., T. Koyama, K. Rewitz, 2020. REgulation of body size and growth control. Development and Growth, 216, 269–313.

Trotta V., Calboli F.C.F., Ziosi M, Guerra D., Pezzoli M.C., David J.R., Cavicchi S., 2006. Thermal plasticity in Drosophila melanogaster: a comparison of geographic populations. BMC Evolutionary Biology,6: 67.

Trotta V., Cavicchi S., Guerra D., Andersen D.H., Babbitt G.A., Kristensen T.N., Pedersen K.S., Loeschcke V., Pertoldi C., 2011. Allometric and non-allometric consequences of inbreeding on *Drosophila melanogaster* wings. Biological Journal of the Linnean Society,102: 626–634.

Trotta V., Duran Prieto J., Battaglia D., Fanti P., 2014. Plastic responses of some life history traits and cellular components of body size in *Aphidius ervi* as related to the age of its host *Acyrthosiphon pisum*. Biological Journal of Linnean Society, 113, 439-454.

Trotta V., Pertoldi C., Rudoy A., Manenti T., Cavicchi S., Guerra D., 2010. Thermal plasticity of wing size and shape in Drosophila melanogaster, Drosophila simulans and their hybrids. Climate Research, 43: 71–79.

Truman J.W., Riddiford L.M., 2002. Endocrine insights into the evolution of metamorphosis in insects. Annual Review of Entomology, 47: 467–500.

Van Lenteren J.C., Hale A, Klapwijk JN, Van Schelt J, Steinberg S. 2003. Guidelines for quality control of commercially produced natural enemies. In: van Lenteren JC, ed. Quality control and production of biological control agents. Theory and testing procedures. Wallingford: CAB International, 265–303.

Vinson S.B., 1990. How parasitoids deal with the immune system of their host: An overview. Insect Biochemistry and Physiology, 13 (1-2): 3-27.

Visser M.F., 1994. The importance of being large: the relationship between size and fitness in females of the parasitoid Aphaereta minuta (Hymenoptera: Braconidae). Journal of Animal Ecology, 6:963–978.

Wake D., Hall B., 1999.The origin and evolution of larval forms. New York: Academic Press.

Winterhalter W.E., Mosseau T.A., 2008. The strength of temperature-mediated selection on body size in a wild insect population. Journal of Orthoptera Research, 17(2):347-351.

Wyckhuys, K.A.G., Strange-Georgeb J.E., Kulhanekb C.A., Wäckersc F.K., Heimpel G.E., 2008. Sugar feeding by the aphid parasitoid *Binodoxys communis*: How does honeydew compare with other sugar sources? Journal of Insect Physiology, 54:481–491.

CHAPTER V

Effect of the parasitism and cold hardening on aphid resistance to a sub-lethal low temperature

Introduction

Low temperature is one of the greatest challenges facing insects, both in temperate and polar regions, and it plays a predominant role in establishing insect distribution limits in the different climatic regions of the world (Chown, 2001; Addo-Bediako *et al*., 2020). Moreover, low temperature is considered one of the main abiotic factors influencing insect population dynamics. Since insects are ectotherms with small body sizes, they rapidly disperse the heat, and, to ensure survival, they must be able to tolerate exposure to sub-optimal temperatures (Teets and Denlinger, 2013). To cope with daily and seasonally cold stresses, insects exhibit a lot of cold-induced protective mechanisms (Salt, 1961; Denlinger, 1991; Chen and Walker, 1994; MacMillan and Sinclair, 2011; Teets and Denlinger, 2013). Some of these mechanisms may be activated on a short time scale, and constitute the so called "rapid cold-hardening" (RCH) process (Lee *et al*., 1987).

Powell and Bale (2005) defined the RCH in insects as "an increase in survival at a 'discriminating temperature' following a brief period of 1-3 h at a low temperature, typically at 0°C, compared with samples transferred directly from the rearing temperature to the discriminating temperature". RCH is important to ensure survival to rapid thermal changes, above all for chill susceptible species with short generation times, such as aphids (Coulson and Bale, 1990). The term "thermal acclimation" instead refers to a process that lead to an increase in survival but requires a longer period of time (at least some days; Lee *et al*., 1987). The thermal acclimation is generally induced in insects when daily temperature varies in function of seasons. Although the seasonal cold-hardening mechanisms have been studied, the physiological basis of RCH are not completely understood. Since seasonal cold acclimation and RCH lead to similar results (increased survival), it is reasonable to suppose that they share, at least in part, the same mechanisms (Chen *et al*., 1987). Loeschcke and Sørensen (2005) argue that the distinction between acclimation and cold hardening is confused. For example, changes in membrane properties or composition are two typical common responses of both acclimation and hardening (Hazel, 1995). Recent metabolomic studies have provided evidence that metabolic adjustments are fundamental in the RCH process in insects. In *Sarcophaga crassipalpis* (Diptera), the accumulation of pyruvate,

glycerol, sorbitol, glucose, alanine and glutamine have been observed during RCH (Chown and Nicolson, 2004; Michaud and Denlinger, 2007; Overgaard *et al*., 2007; Vesala *et al*., 2012). As an additional consequence of RCH the metabolic perturbations during recovery from a subsequent cold shock are minimized (Overgaard *et al*., 2007; Teets *et al*., 2012). In the fly *Sarcophaga bullata*, individuals pre-exposed to a low temperature had significantly lower levels of sugars and polyols during the recovery period after the cold shock, indicating that RCH can minimize the impact of cold shock on metabolic homeostasis (Teets *et al*., 2012). In particular, it is known that cholesterol augmentation increases the capacity to undergo RCH (Shreve *et al*., 2007).

Moreover, in insects, cell membrane adjustment is a target of RCH during seasonal acclimation. Michaud and Denlinger (2006) and Overgaard *et al*. (2005) found that a not lethal cold exposure increases cellular membrane fluidity. At molecular level, many genes have been implicated in the diapause and in the cold acclimation, such as those of the heat shock proteins (Feder and Hofmann, 1999). Functional experiments have demonstrated that these genes are overexpressed during cold-hardening (Tachibana *et al*., 2005; Rinehart *et al*., 2007), but also they are essential in conferring cold tolerance, as in the case of the hsp70 or hsp23 (Rinehart *et al*., 2007).

To trigger the physiological mechanisms that increase cold tolerance, many studies on cold-hardening have been conducted by gradually decreasing the temperature (Denlinger, 1991; Vesala *et al*., 2012). It is interesting to understand how insects react to a rapid temperature decrement. Since thermal variations will occur more rapidly in future, it is supposable that the capacity of ectotherms to promptly and sufficiently display thermal hardening mechanisms could be compromised.

Under laboratory conditions, it is possible to improve the resistance to cold stress with a brief pre-exposure to low temperatures (Czajka and Lee, 1990; Powell and Bale, 2004). Powell and Bale (2005) investigated the ability of the grain aphid *Sitobion avenae* to rapidly cold harden, that is, to inure to unfavorable cold environmental conditions. The survival rate of *S. avenae* nymphs exposed at 0°C for 2h and successively exposed at -8°C for 3h, was greater than the survival of aphids directly transferred to -8°C. Since aphids and parasitoids are seasonally exposed to suboptimal temperatures (Powell and Bale, 2004; MacMilland and Sinclair, 2011; Teets and Denlinger, 2013), this have led to the co-evolution of protective biochemical and physiological mechanisms. To ensure its survival and a correct development, immature koinobiotic parasitoids of aphids, such as the wasp *Aphidius ervi*, establish strict physiological relationship with the host (Digilio *et al*., 2000; Pennacchio and

Strand, 2005). This relationship may consist in manipulate some host traits, so to reduce the risk to die for lethal thermal stress (Lagos *et al*., 2001).

In the Ch. I of this thesis, I demonstrated that the pea aphid *Acyrthosiphon pisum*, when parasitized by the endoparasitoid *A. ervi*, shows a greater survival after an exposure to a very high temperature (39°C for 30 min) if compared with the unparasitized one.

The aims of this study were to understand if (i) an exposure of the pea aphid to a non-lethal cold temperature increases the pea aphid resistance to a subsequent stronger cold stress, and if (ii) parasitization of the pea aphid by the parasitoid *A. ervi* may increase the pea aphid resistance to a subsequent stronger cold stress, as demonstrated in Ch. I in the case of heat stress. Since any interaction between parasitism and cold stress responses may be coevolved in parasitized aphid, these two kinds of stress were jointly tested by exposing parasitized aphids to a cold-hardening treatment before a sub-lethal cold stress. In particular, it was investigated if in parasitized aphids the beneficial of a cold-hardening treatment further enhances the aphid cold resistance.

Materials and Methods

Insect rearing

In this study, aphid cohorts at the 3rd nymphal stage, unparasitized and parasitized by adult females of *A. ervi,* were exposed at two different low temperatures. Aphid and parasitoid rearing and parasitization procedure, were performed as previously described in Ch. II. In this study, aphids were parasitized 2 days after the birth, that is, at the 2nd nymphal stage.

Experimental treatments

All the experimental treatments were carried out by exposing groups of 10 aphids, within Eppendorf tubes, at the experimental thermal conditions. The main cold treatment was performed on parasitized (Para) and unparasitized (NoPara) aphids by exposing them at -20°C for 18 minutes. Previous tests demonstrated that this exposure causes a mortality of about 90% in the group composed of 3rd nymph stage aphids and could be considered sub-lethal. This thermal treatment was also performed on aphids previously exposed to a non-lethal cold treatment.

The non-lethal cold treatment was carried out by immersing in water containing ice the Eppendorf tubes containing 10 three days-old aphids. Previous experiments showed that this exposure is not lethal for both parasitized and unparasitized aphids since mortality recorded 24 hours after the exposure was about 2%. This mortality could be mostly attributed to aphid manipulation and not to cold exposure.

Four experimental groups were generated and exposed to the sub-lethal shock at -20°C: NoPara, NoPara+Mild, Para and Para+Mild (that is, parasitized and unpararasitized aphid previously exposed or not exposed to the mild cold stress). Each experimental group consisted of three replicates (independent plants) and each replicate was composed of five groups of ten aphids, for a total of 150 aphids per treatment.

Mortality check

After the thermal treatments at -20°C were performed, aphids were transferred on a cut broad bean leaf, with the stalk inside an Eppendorf® tube filled with water and sealed with parafilm® to prevent desiccation, and placed in a plastic cylinder (150 ml) with a mesh covered ventilation hole in the screw-top. The plastic cylinders were placed in an environmental chamber at 22 ± 1 °C (mean values ± accuracy) and the survival was recorded after 24 h. Survival was considered to occur if the aphids were able to walk or if they moved their antennae or legs when gently touched by a brush.

Statistical analysis

Data on aphid survival after the cold shock were analyzed using a mixed linear model ANOVA in which "treatment" (four levels: NoPara, Para, NoPara+Mild, and Para+Mild) was the main fixed effect and "replicate" (three levels) was nested within treatment. A Tukey post-hoc test for multiple comparisons of means was also performed. All the analyses in this study were carried out using R.3.2.4 revisited software (R Core Team, 2016).

Results

Figure 5.1. shows the aphids survival recorded 24 hours after the sub-lethal cold stress (-20°C per 18 min). Significant differences were found among the four experimental groups ($P < 0.001$), but not among replicates within group (tab. 5). A higher survival was found in the aphid group experiencing both the parasitization and the non-lethal thermal stress (Para+Mild-CS) when compared with the other groups; the three remaining groups showed the same level of survival.

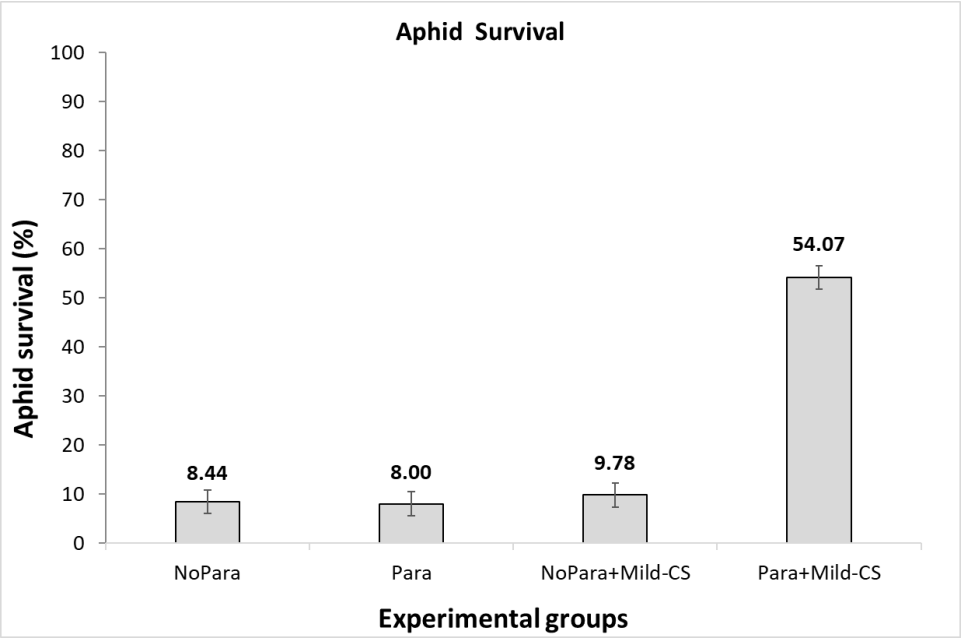

Fig. 5.1. - Mean values (± standard errors) of survival after a cold shock at -20°C for 18 min of control and parasitized aphids exposed and not exposed to a sub lethal cold shock.

Tab. 5 - Results of the mixed model ANOVA on the survival of aphids after sub-lethal cold shock.

Source of variation	*df*	MS	*F*
Treatment	3	7715.2	80.8 ***
Replicate within treatment	8	50.3	0.53 n.s.
Residuals	48	95.5	

n.s., not significant; df, degrees of freedom; MS, mean square; F, variance ratio.*** P < 0.001.

Discussion and Conclusions

In this study parasitized and unparasitized pea aphids were exposed and not exposed to a non-lethal cold stress and then exposed to a sub-lethal cold stress. Even if -20°C per 18 minutes is a strong thermal stress that rarely occurs in nature, my results suggest that the combined effects of parasitism plus cold hardening increase aphid survival after a severe cold stress. When parasitism or cold hardening were applied alone, no increase in aphid survival to a sub-lethal cold stress was observed. Even if the mechanisms implicated in the cold resistance were not investigated, in literature there are many information on these mechanisms (Zachariassen, 1985; Clark and Worland, 2008; Teets *et al*., 2012; Teets and

Denlinger, 2013). By consulting the current literature on the mechanisms implicated in both cold resistance and parasitization stress in insects, I found that only the up-regulation of some heat shock proteins is triggered by both these two kinds of stresses (Zhao and Jones, 2012).

In the Ch. II, I reported that the parasitization of the pea aphid by the wasp *A. ervi* induces a major resistance to heat stress in aphid in the following hours and days after parasitization. Among the several mechanisms determining the increment of resistance to heat stress in the parasitized aphids, it was supposed that the parasitism could up-regulate the expression of heat shock proteins in the body of parasitized aphids. Heat shock proteins are expressed in response to different kind of stresses, both abiotic and biotic, and it is well documented that they are also induced in cold stressed insects (reviewed by Zhao and Jones, 2012).

The hypothesis that the increased survival of parasitized aphids after a thermal stress (cold or heat) is linked to the upregulation of HSPs after the parasitization event was supported by two valid studies (Shim *et al*., 2008; Rinehart *et al*., 2000). Shim *et al*. (2008) reported that, when the ectoparasitic wasp *Bracon hebetor* envenomizes its host *Plodia interpunctella*, small heat shock proteins are upregulated 2-4 days after the envenomation. Rinehart *et al.* (2000) showed that some heat shock genes are upregulated in the flesh fly *S. crassipalpis* 13 hours after envenomation by the ectoparasitic wasp *Nasonia vitripenni*s.

The cold resistance could be someway linked to the up-regulation of some *hsp* genes in the pea aphid as a consequence of the parasitization stress. This up-regulation, jointly to upregulation due to the mild cold treatment, could lead to the observed survival increase after the cold stress at -20°C for 18 minutes. These results contribute to clarify the complex aphid-endoparasitoid relationships.

References

Addo-Bediako A., Chown S.L., Gaston K.J., 2000. Thermal tolerance, climatic variability and latitude. Proceedings of the Royal Society B, 267: 739-745.

Chen C.P., Denlinger D.L., Lee, R.E., 1987. Cold-shock injury and rapid cold hardening in the flesh fly *Sarcophaga crassipalpis*. Physiological Zoology, 60: 297–304.

Chen C.P., Walker V.K., 1994. Cold-shock and chilling tolerance in *Drosophila*. Journal of Insect Physiology, 40: 661-669.

Chown S.L., 2001. Physiological variation in insects: hierarchical levels and implications. Journal of Insect Physiology, 47(7): 649-660.

Chown, S.L., Nicolson, S.W., 2004. Insect physiological ecology. mechanisms and patterns. Oxford University Press, Oxford.

Clark M.S., Worland M.R., 2008. How insects survive the cold: molecular mechanism-a review. Journal of Comparative Physiology, 178: 917-933.

Coulson S.C., Bale J.S., 1990. Effect of rapid cold hardening on reproduction and survival of offspring in the housefly *Musca domestica*. Journal of Insect Physiology, 38(6): 421-424.

Czajka, M.C., Lee Jr., R.E., 1990. A rapid cold-hardening response protecting against cold shock injury in Drosophila melanogaster. Journal of Experimental Biology 148, 245–254.

Denlinger D.L., 1991. Relationship between cold hardiness and diapause. Insects at Low Temperature (ed. by R. E. Lee and D. L. Denlinger), pp. 174–198. Chapman & Hall, New York, New York.

Digilio, M.C., Isidoro, N., Tremblay, E., Pennacchio, F., 2000. Host castration by Aphidius ervi venom proteins. Journal of Insect Physiology, 46, 1041–1050.

Feder M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual Review of Physiology, 61: 243-282.

Hazel J.R., 1995. Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? Annual Reviews of Physiology, 57, 19–42.

Lagos, N.A., Fuentes-Contreras, E., Bozinovic, F., Niemeyer, H.M., 2001. Behavioural thermoregulation in Acyrthosiphon pisum (Homoptera: Aphididae): The effect of parasitism by Aphidius ervi (Hymenoptera: Braconidae). Journal of Thermal Biology, 26, 133–137.

Leather S. R., Walters K. F. A., Bale, J. S., 1993. The Ecology of Insect Overwintering. Cambridge University Press

Lee R.E., Chen C.P., Denlinger D.L., 1987. A rapid cold-hardening process in insects. Science, 238, 1415 –1417

Loeschcke V., Sørensen J.G., 2005. Acclimation, heat shock and hardening-a response from evolutionary biology. Journal of Thermal Biology, 30:255–257.

MacMillan H.A., B.J. Sinclair, 2011. The role of the gut in insect chilling injury: cold-induced disruption of osmoregulation in the fall field cricket, Gryllus pennsylvanicus. Journal of Experimental Biology, 2011 214: 726-734.

Michaud M.R., Denlinger D.L., 2006. Oleic acid is elevated in cell membranes during rapid cold-hardening and pupal diapause in the flesh fly, Sarcophaga crassipalpis. Journal of Insect Physiology, 52, 1073–1082.

Michaud M.R., Denlinger D.L., 2007. Shifts in the carbohydrate, polyol, and amino acid pools during rapid cold-hardening and diapause-associated cold-hardening in flesh flies (Sarcophaga crassipalpis): a metabolomic comparison. Journal of Comparative Physiology B, 177, 753–763.

Overgaard J., Malmendal A., Sørensen J.G., Bundy J.G., Loeschcke V., Nielson N.C., Holmstrup M., 2007. Metabolomic profiling of rapid cold hardening and cold shock in Drosophila melanogaster. Journal of Insect Physiology, 53, 1218–1232.

Overgaard J., Sørensen, J.G., Petersen S.O., Loeschcke V., Holmstrup M., 2005. Changes in membrane lipid composition following rapid cold hardening in Drosophila melanogaster. Journal of Insect Physiology, 51, 1173–1182.

Pennacchio F., Strand M.R., 2005. Evolution of developmental strategies in parasitic Hymenoptera. Annual Reviews of Entomology. 51, 233–258

Powell S.J., Bale J.S., 2004. Cold shock injuries and ecological costs of rapid cold hardening in the grain aphid Sitobion avenae (Hemiptera:Aphididae). Journal of Insect Physiology, 50(4):277-284.

Powell S.J., Bale J.S., 2005. Low temperature acclimated populations of the grain aphid Sitobion avenae ability to rapidly cold harden enhanced fitness. Journal of Experimental Biology, 208: 2615-2620.

R Core Team, 2016. R: A language and environment for statistical computing.

Rinehart J.P., Li A., Yocum G.D., Robich R.M., Hayward S.A.L., Denlinger D.L., 2007. Up-regulation of heat shock proteins is essential for cold survival during insect diapause. Proceedings of the National Academy of Sciences of the United States of America, 104, 11130–11137.

Rinehart J.P., Yocum G.D., Denlinger D.L. 2000. Developmental upregulation of inducible hsp70 transcripts, but not the cognate form, during pupal diapause in the flesh fly, Sarcophaga crassipalpis. Insect Biochemistry and Molecular Biology, 30, 515–521.

Salt R.W., 1961. Principles of insect cold-hardiness. Annual Review of Entomology, 6, 55–74.

Shim J.K., Ha D.M., Nho S.K., Song K.S., Lee K.Y., 2008. Upregulation of heat shock protein genes by envenomation of ectoparasitoidBraconhebetor in larval host of Indian meal moth Plodiainterpunctella. J. Invertebr. Pathol. 97: 306-309.

Shreve, S.M., Yi, S.X., Lee, R.E., 2007. Increased dietary cholesterol enhances cold tolerance in Drosophila melanogaster. CryoLetters, 28, 33–37.

Tachibana S.I., Numata H., and Goto S.G. 2005. Gene expression of heat-shock proteins (Hsp23, Hsp70 and Hsp90) during and after larval diapause in the blow fly Lucilia sericata. Journal of Insect Physiology, 51, 641–647.

Teets M.T., D.L. Denlinger, 2013. Physiological mechanisms of seasonal and rapid cold-hardening in insects. Physiological Entomology, 105-116.

Teets, N.M., Peyton, J.T., Ragland, G.J., Colinet H., Renault D., Hahn D.A., Denliger D.L., 2012. Combined transcriptomic and metabolomic approach uncovers molecular mechanisms of cold tolerance in a temperate flesh fly. Physiological Genomics, 44, 764–777.

Vesala L., Salminen T.S., Kankare M., A. Hoikkala, 2012. Photoperiodic regulation of cold tolerance and expression levels of regucalcin gene in Drosophila Montana. Journal of Insect Phisiology, (58:5): 704-709

Zachariassen K.E., 1985. Physiology of cold tolerance in insects. Physiological Reviews, 65(4):799-832.

Zhao L., Jones W.A., 2012. Expression of heat shock protein genes in insect stress responses. ISJ, 9: 93-101.

CHAPTER VI

Effects of arena and rearing temperature on the predation of *Hippodamia variegata* (Goeze) at different temperatures

Ladybirds (Coleoptera: Coccinellidae) are mainly generalist predators feeding on a wide prey range, due to their voracity, they play a fundamental role in reducing pest populations on crops (Obrycki, 1998; Dixon, 2000; Lanzoni *et al*., 2004). Small arthropods are the principal food for aphidophagous ladybirds, while nectar and mildew are secondary food that positively affect ladybird fitness (Bianchi and Van DerWerf, 2004). Since the early 20th century, ladybirds are used as a component of integrated or biological control against aleyrodids, aphids, coccids and diaspids (Obrycki and Kring, 1998), both in open field and in greenhouses and tunnels (Hodek, 1970; Oerke, 1994; Hodek and Michaud 2008; Cabral *et al.* 2009). Moreover, in last decades, the role of ladybirds in biological control has been recognized through conservation and enhancement techniques (Symondson *et al*., 2002; Obrycki *et al*. 2009). *Hippodamia variegata* (Goeze), the species tested in this study, is an aphidophagous ladybird of Palearctic origin, currently present in different world regions (Franzmann, 2002). It has been reported in many countries as an efficient control agent of at least 12 different aphid species, including the pea aphid *A. pisum* (Franzmann, 2002; Kavallieratos *et al*., 2004; Kontodimas and Stathas, 2005; Mandour *et al*., 2012). Moreover, *H. variegata* is massive reared and commercialized at an industrial scale.

In order to predict how the predator will control the target pest population in a biological control plan, it is essential to know its predatory efficiency under environmental conditions different from those at which it was reared (Dixon, 2000). To ensures a rapid and efficient industrial production level, entomophagous species are generally reared in thermal conditions that maximize their reproduction and reduce the developmental time (Bigler, 1994). For the ladybirds the optimal thermal rearing temperature is generally around the 25°C (Fisher, 1963; Babu and Azam, 1987; Schüder *et al*., 2004). In most cases, rearing temperature highly differ from those in the field and predators, after their releasing in the field, can be stressed due to a drastic and rapid thermal variation. As consequence, ladybird efficiency as biological control agents may be reduced (Bigler, 1994; Sørensen *et al*., 2013). To improve their performances, biological control agents may be thermal acclimated to the conditions that they will experience in the field (Hoffmann, 1995; Prasad *et al*., 1999; Chidawanyika and Terblanche, 2011; Sørensen *et al*., 2013; Terblanche, 2014).

Acclimation is definable as "a phenotypic alteration in physiology that occurs in response to the environmental conditions experienced by an animal and is often thought to enhance performance, thereby improving fitness" (Angilletta, 2009). The "beneficial acclimation theory" has been frequently debated in last decades (Angilletta, 2009). Some studies in *Drosophila* spp. support this theory (Nunney and Cheung, 1997; Thomson *et al*., 2001; Sørensen *et al*., 2013), while others, in *Drosophila* (Huey *et al*., 1999; Gibert *et al*., 2001) and in dragonflies (Smolinský and Gvoždík, 2013), do not. Sørensen *et al*. (2013) studied the effects of acclimation on the adult of the two-spotted ladybirds *Adalia bipunctata* L. They tested the effects of three rearing temperatures (15, 20 and 25°C) on the aphid predation at three different temperatures (15, 20 and 25°C). Ladybirds tested at the same thermal environment at which they were reared, and then acclimated, consumed more aphids compared to individuals acclimated to a different temperature.

Apart from temperature, another factor affecting predation activity in the field is the physical space in which predation occur. To obtain more reliable predictions of the predatory activity, many laboratory studies considered the effects of the experimental arena on the number of consumed preys (Noppe *et al*., 2012; Uiterwaal *et al*., 2017; Uiterwaal and De Long, 2018). Generally, it is more difficult to find the prey in a more complex arena than in a simpler one; as a consequence, predation should be lower in complex arenas (Uiterwaal and DeLong, 2018; Zarei *et al*., 2019). This aspect is however controversial and it has been widely discussed in literature. Some studies suggest that arena size may play a role in determining space clearance rate (Uiterwaal *et al*., 2017; Uiterwaal and DeLong, 2018), while other studies reports that this parameter is not important (van Rijn *et al*., 2005).

In this preliminary study, I tested if and how (i) the predation of *H. variegata* on *A. pisum* is affected by the experimental arena complexity at two temperatures, and if (ii) the acclimation influences the predation rate. For the second question, I specifically tested if the rearing thermal conditions affect prey consumption in two different thermal environments.

Material and Methods

Insect rearing

The pea aphid population and the broad bean cultures have been previously described in this thesis. For this study, aphids at the 3rd nymphal stage (3-days-old) were used. *H. variegata* culture started from few specimens found in an alfalfa field on July 2020, in Lleida (Catalogna, Spain). The massive culture was stocked at 25±1°C, 75±5% RH, with a photoperiod consisting of 18 hours of light and six hours of dark. The massive culture consisted in several glass pots (5 litres), each of them containing 13-15 adult females and at

4-5 adult males. Each pot was closed with a mesh covered ventilation hole in the screw-top. To reduce the ladybird cannibalism, internal surface of the pot was augmented by inserting crumpled paper napkins. Paper napkins were also used by the ladybirds as ovipositon place, allowing an easier egg collection by cutting with scissors the paper napkins around the eggs. Adults were daily fed *ad libitum* with eggs of *Ephestia kueniella* Zeller and *Artemia* spp, pollen, and pea aphids (3rd and 4th nymphal instars). Eggs were daily collected and placed on a wet cotton disc within a Petri dish. In accordance with the treatments of this study, the Petri dishes were then placed at the different thermal conditions (see the description of the experiments below). After hatching, larvae were isolated within glass vials (6 cm long, 1 cm diameter), plugged with cotton, and daily fed *ad libitum* as done for the adult ladybird. Since larvae prefer to fix themselves on soft surface before the moulting, a paper strip (5 cm long and 0.5 cm wide) was inserted within each vial. In order to standardize the effect of the age, coetaneous individuals were used: moults were checked twice a day, and, after the moult, the exuvia was removed from the vial. In this study, 3rd larval instar of *H. variegata* were used because the larval stages are often less tolerant than adults to thermal variations (Krebs and Loeschcke, 1995; Pumhan *et al*., 2020).

Effects of arena on predation

Two different kinds of experimental arenas were tested: a small plastic cylinder (7 cm high; 5 cm diameter) containing a cut broad bean leaf, and a large plastic cylinder (20 cm high; 8 cm diameter) containing a broad bean plant. Both cylinders were transparent and had a mesh covered ventilation hole in the screw-top. The stalk of the cut broad bean leaf was inserted inside an Eppendorf® tube, containing water to prevent desiccation, and sealed with parafilm®. The bigger plastic cylinder was partially interred (about 2 cm deep) within a plastic pot (10 cm diameter, 15 cm tall) hosting a small broad bean plant (6-8 cm tall), with a well-developed pair of leaves.

Twenty pea aphids at the 3rd nymphal stage (3-days-old) were gently placed inside the experimental arenas paying attention to put them on the cut leaf or on the broad bean plant, in accordance with the arena type. To allow aphids to dispose on the leaf and on the broad bean plant without disturbance, *H. variegata* larva was introduced with a soft paint brush in the arenas one hour after the aphids. After the introduction of the ladybird, experimental arenas were placed at the same thermal conditions at which larvae developed, that are 20°C and 25 °C. Predation was stopped after 24 hours by removing the larva from the arena, and the number of preyed aphids were recorded. Twelve-thirteen replicates were done for each experimental treatment.

Effect of the rearing temperature on predation

H. variegata eggs were obtained by the culture stocked at 25°C and were then reared at two different temperatures: 20±1 °C and 25±1°C. All larvae used in this study were at the 3rd instar at least from 8 hours. To test the effect of acclimatization on predation, four experimental groups were originated: ladybirds reared at 20°C and predation test carried out at 20°C and at 25°C; ladybirds reared at 25°C and predation test carried out at 20°C and at 25°C. This study was carried out in the small cylinder (5 cm diameter and 7 cm tall) containing the cut broad bean leaf. Twelve-thirteen replicates were done for each experimental treatment.

Statistical analysis

To test for the effect of the arena and of the temperature on the predation of *H. variegata*, prey consumption was analyzed using a binomial generalized linear model (GLM) with a logit link function in which "experimental temperature" (two levels: 20°C and 25°C) and "treatment" were the main fixed effects. The levels of "treatment" were two for the "arena experiments" (small and large ones) and two for "temperature experiment" (rearing temperature of 20°C and 25°C). Each experimental treatment was composed of about twelve-thirteen replicates.

Results

Effect of experimental arena.

Mean values (± standard errors) of predation rate of *H. variegata* larvae measured in the two arena types and at the two different temperatures are shown in Fig. 6.1. The predation rate was slightly lower (even if not significant, $\chi^2_{(1)}$= 1.15, P = 0.28) in the large cylinders than in the smaller ones, both at 20°C (the mean numbers of consumed aphids were 6.73 and 7.7, respectively) and at 25°C (the mean numbers of consumed aphids were 11.9 and 12.9, respectively). The predation rate was instead higher at 25°C than at 20°C, independently from the experimental arena ($\chi^2_{(1)}$= 58.3, P < 0.001). No interaction between temperature and arena was found ($\chi^2_{(1)}$= 0.004, P = 0.95).

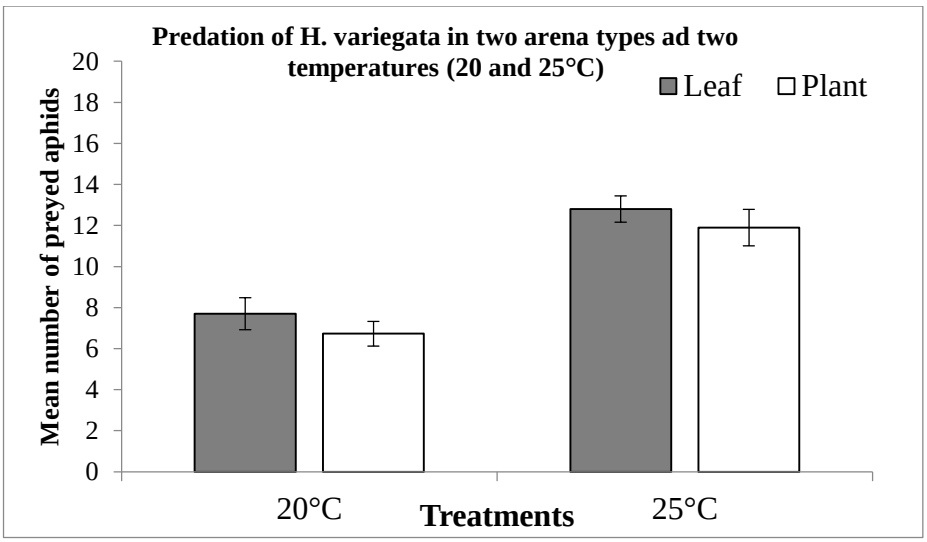

Fig. 6.1 - Mean values (± standard errors) of predation rate of *Hippodamia variegata* larvae measured in two arena types and at two temperatures.

Effect of rearing temperature on predation

Predation rate of the ladybirds developed at 25°C was significantly higher when the predation occurs at 25°C than at 20°C (the mean numbers of consumed aphids were 12.1 and 6.45, respectively). In the case of ladybirds reared at 20°C, no differences in terms of predation rate were found when they preyed at 20°C and 25°C (the mean numbers of consumed aphids were 6.7 and 7.7, respectively; Fig. 6.2). This pattern is confirmed since the interaction between rearing temperature and predation was highly significant ($\chi^2_{(1)}$= 11.96, $P < 0.001$).

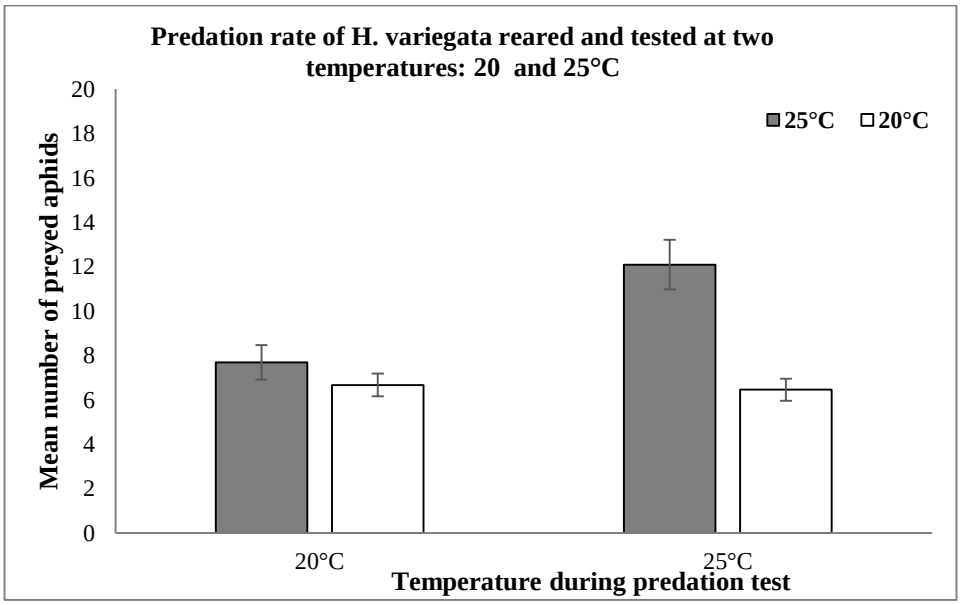

Fig. 6.2 - Mean values (± standard errors) of predation rate of Hippodamia variegata larvae that developed and preyed at 20°C and 25°C.

Discussions and conclusions

Many studies on the effects of the experimental arenas on insect predation activity reported a negative relationship between the structural complexity degree of the arena and the predation rate (Noppe *et al.*, 2012; Reynolds and Cuddington 2012; Uiterwaal and DeLong, 2018; Zarei *et al*., 2019). This is basically due to higher prey research times and prey handling times, which are due to the difficulty to find the prey in a more complex microcosm (Solomon, 1949). In this study, I tested two experimental arenas: a cylinder containing a cut broad bean leaf and a cylinder containing a potted broad bean plant. Regardless of temperature, the mean number of preyed aphids was slightly higher in the small than in the large cylinder. However, the statistical analysis showed no significant difference in the number of preyed aphids in these two arena types. On the contrary, some authors found that aphidophagous predators consume significantly more aphids in a homogeneous environment than in a heterogeneous one like a plant (Reynolds and Cuddington, 2012; Zarei *et al*., 2019). Zarei *et al*. (2019) confirmed the negative relationship between arena complexity and the number of consumed preys by testing the predation of the ladybird *H. variegata* and the lacewing *Crysoperla carnea* Stephens in plastic containers containing a simple cut broad bean leaf and or a whole broad bean plant. Similarly, Reynolds and Cuddington (2012) compared the aphid predation rate of Asian lady beetle, *Harmonia axyridis* Pallas, and the larvae of *C. carnea* in Petri dishes and on the whole plant. They found that predators consumed significantly more aphids on leaf tissue in petri dishes than on whole plants with the same surface area. Although my findings are in contrast with other studies conducted with other aphidophagous predator species, in fact I did not find any significant differences in terms of predation rate between the two arena type, it is not excluded that the predation rate of *H. variegata* may vary by testing more experimental arenas, composed by bigger plants and or by more voluminous containers.

Quantitative information on the impact of natural enemies is essential to compose models predicting prey consumption in field. In particular, as well as knowing the effective number of consumed preys during a certain period of time, it is necessary to quantify the rate of a successful predator attack and the handling time exhibited by the predator. These two factors strongly determine the rate at which a natural enemy kills its prey (Solomon, 1949). Moreover, to obtain realistic models, the effects of the temperature and of the arena type on prey behaviour should be considered. In aphids, escape behaviour from aphidophagous species is affected by temperature (Brodsky and Barlow, 1986; Ma and Ma, 2012). In particular, Ma and Ma (2012) reported that the pea aphids dropping from the host plant increase at high temperatures. Lagos *et al*., (2001) reported that parasitized pea aphids could

escape from the heat by moving toward different microhabitats of the same host plant. All these aphid behaviors could determine variation in the aphid-predator meeting rate and also could reduce the success of a predator attack.

I found that *H. variegata* consumes more aphids when it preys at 25°C than at 20°C, but only when it was reared at 25°C. When the *H. variegata* larvae developed at 20°C and preyed at 25°C, the aphid consumption was lower than those of the larvae that were both reared and preyed at 25°C. Interestingly, when the larvae reared at 20°C preyed at 25°C, the aphid consumption rate did not increase if compared with larvae both reared and tested at 20°C. Then, as found by Sørensen *et al*., (2013) in the case of two-spotted ladybird *A. bipunctata*, aphid predation rate of *H. variegata* is maximum at the temperature at which it developed than at other that it did not experience before the predation tests. Considering this finding, it is possible to affirm that the predation of *H. variegata* is a trait subjected to thermal plasticity. This finding is not obvious as in literature different studies debate on the thermal plasticity of the predation activity (Jensen *et al*., 2017 for the predatory mite *Gaeolaelaps aculeifer* Canestrini, and Bar-Ziv and Scharf, 2018 for the wormlion *Vermilio* sp.).

This preliminary study focalized only on the first day after predator releasing in new thermal environments. It is supposable that the ladybird *H. variegata* could acclimate to the new temperature, and no differences in terms of mean number of consumed aphids could be found in the following days, as reported by Zarei *et al*. (2019). It is well known that acclimation to temperatures different from those at which the predator was reared could have relevant metabolic costs, affecting other fitness-related life history traits, such as longevity and reproduction (Jensen *et al*., 2017). The effect of this kind of thermal stress on the daily number of eggs production and on the mortality for the whole ladybird life could be observed, as in the case of the pea aphid (see Ch. I).

This experiment is therefore to be considered as a preliminary study that will allow me to plan subsequent studies on the long-term effects of a thermal stress on the ladybird life history traits. For example, it will be very interesting to investigate if (and eventually how) the different prey consumption, at different temperatures, influences the developmental times of the ladybird larval and pupal stages, their body size and, eventually, the fitness.

Another future goal of my research on ladybirds will be to understand if the temperature influences the intraguild predations (IGP). The IGP consists in the behaviour of killing and eating potential competitors that exploit the same, often limiting, resources (Polis *et al.*,1989). Since the predation rate of an organism is influenced by developmental and/or acclimational temperatures, even the interaction among aphidophagous species that occupy the same trophic level foraging on the same prey could be affected by temperatures (Müller

and Brouder, 2002). One of the issues of biological control is the opportunity to release predators and parasitoids together (Cardinale *et al.*, 2003). Experimental studies and field observations seem to indicate that, at some release ratios, predators and parasitoids may have a complementary action in the control of the pest (Cardinale *et al.*, 2003, Bilu and Coll, 2007; Gontijo *et al.*, 2015). However, if predators have a strong preference for parasitized versus unparasitized prey, IGP may have a disruptive effect on parasitoid populations (Snyder and Ives, 2003). The behaviour change mediated by temperature in the subjects involved in IGP (ladybirds and parasitoids) will be a matter of my future researches.

References

Angilletta M.J., 2009. Thermal adaptation. A theoretical and empirical synthesis. Oxford University press, Oxford.

Babu T.R., Azam K.M., 1987. Biology of *Cryptolaemus montrouzieri* Mulsant (Coccinellidae, Coleoptera) in relation with temperature. Entomophaga, 32: 381–386.

Bar-Ziv M.A., Scharf I., 2018. Thermal acclimation is not induced by habitat-of-origin, maintenance temperature, or acute exposure to low or high temperature in a pit-building wormlion (*Vermileo* sp). Journal of Thermal Biology, 74: 181-186.

Bianchi, F.J.J.A., Van DerWerf W., 2004. Model evaluation of the function of prey in non-crop habitats for biological control by ladybeetles in agricultural landscapes. Ecological Modelling, 171(1–2): 177-193.

Bigler, F., 1994. Quality control in *Trichogramma* production. In: Wajneberg, E., Hassans, S.A. (Eds.), Biological control with egg parasitoids. CAB International Oxon, U.K., pp. 93–112.

Bilu E., Coll M., 2007. The importance of intraguild interactions to the combined effect of a parasitoid and a predator on aphid population suppression. BioControl, 52: 753-763.

Brodsky L.M., Barlow C.A., 1986. Escape responses of the pea aphid, Acyrthosiphon pisum (Harris): influence of predator type and temperature. Canadian Journal of Zoology, 64, 937–939.

Cabral S, Soares AO, Garcia P (2009) Predation by Coccinella undecimpunctata L. (Coleoptera: Coccinellidae) on Myzus persicae Sulzer (Homoptera: Aphididae): effect of prey density. Biological Control, 50:25–29.

Cardinale B. J., Harvey C. T., Gross K., Ives A. R., 2003. Biodiversity and biocontrol: emergent impacts of a multienemy assemblage on pest suppression and crop yield in an agroecosystem. Ecology Letters, 6: 857-865.

Chidawanyika, F., Terblanche, J.S., 2011. Costs and benefits of thermal acclimation of codling moth, Cydiapomonella (Lepidoptera: Tortricidae): implications for pest control and the sterile insect release programme. Evolutionary Applications 4, 534–544.

Dixon A. F. G., 2000. Insect predator-prey dynamics. Ladybird beetles and biological control. Cambridge University Press, Cambridge, UK.

Fisher T.W., 1963. Mass culture of Cryptolaemus and Leptomastix: natural enemies of the citrus mealybug. Bulletin California Agricultural Experiment Station No. 797.

Franzmann B.A., 2002. Hippodamia variegata (Goeze) (Coleoptera: Coccinellidae), a predacious ladybird new in Australia. Australian Journal of Entomology, 41:375–377.

Gibert, P., Huey, R.B., Gilchrist, G.W., 2001. Locomotor performance of Drosophila melanogaster: interactions among developmental and adult temperatures, age, and geography. Evolution 55, 205–209.

Gontijo L. M., Beers E. H., Snyder W. E., 2015. Complementary suppression of aphids by predators and parasitoids. Biological Control, 90: 83-91.

Hodek I, Michaud J.P., 2008. Why is Coccinella septempunctata so successful? (a point-of-view). European Journal of Entomology, 105:1–12.

Hodek I., 1970. Coccinellids and the modern pest management. BioScience 20, 543–552.

Hoffmann A., 1995. Acclimation: increasing survival at a cost. Trends in Ecology and Evolution, 10, 1–2.

Huey, R.B., Berrigan, D., Gilchrist, G.W., Herron, J.C., 1999. Testing of adaptive significance of acclimation: a strong inference approach. American Zoologist, 39, 323–336.

Jensen K., Kristensen T., Overgaard J., S. Toft, Sorensen J.G., M. Holmstrup, 2017. Cold acclimation reduces predation rate and reproduction but increase cold- and starvation tolerance in the predatory Gaeolaelaps aculeifer Canestrini. Biological Control, 114: 150-157.

Kavallieratos N. G., AthanassiouC. G., TomanovicŽ., Papadopoulos G. D., VayiasB. J., 2004. Seasonal abundance and effect of predators (Coleoptera, Coccinellidae) and parasitoids (Hymenoptera: Braconidae, Aphidiinae) on Myzus persicae (Hemiptera, Aphidoidea) densities on tobacco: a two-year study from Central Greece. Biologia -Section Zoology, 59 (5): 613-619.

Kontodimas D.C., Stathas, G.J., 2005. Phenology, fecundity and life table parameters of the predator Hippodamia variegata reared on Dysaphiscrataegi. Biocontrol 50, 223–233.

Krebs R.A., Loeschcke V., 1995. Resistance to thermal stress in preadult Drosophila buxxatii variation among populations and changes in relative resistance across life stages. Biological Journal of Linnean Society, 56, 517–531

Lagos N.A., Fuentes-Contreras E., Bozinovic F., Niemeyer H.M., 2001. Behavioural thermoregulation in Acyrthosiphon pisum (Homoptera: aphididae): The effect of parasitism by Aphidius ervi (Hymenoptera: Braconidae). Journal of Thermal Biology, 26, 133–137.

Lanzoni A., Accineli G., Bazzocchia, Burgio G., 2004. Biological traits and life table of the exotic Harmonia axyridis compared with Hippodamia variegata, and Adalia bipunctata (Col, Coccinellidae), Journal of Applied Entomology, 128: 298 – 306.

Ma G., C-S Ma, 2012. Climate warming may increase aphid's gropping probabilities in response to high temperatures. Journal of Insect Physiology, 58(11): 1456-1462.

Mandour N.S., Sarhan A.A., El-Basha N.A., Abdel-Motaal D.S., 2012. Effect of different temperature regimes on the biology, reproduction and predation of Hippodamia variegata (Goeze) (Coleoptera: Coccinellidae). Egyptian Journal of Biological Pest Control, 21(1): 305-312.

Müller C. B., Brouder J., 2002. Intraguild predation in biological control and conservation biology. Biological Control, 25 (3): 216-223.

Noppe C., Michaud J.P., De Clercq P., 2012. Intraguild predation between lady beetles and lacewings: outcomes and consequences vary with focal prey and arena of interaction. Annals of the Entomological Society of America, 105(4): 562-571.

Nunney, L., Cheung, W., 1997. The effect of temperature on body size and fecundity in female Drosophila melanogaster: evidence for adaptive plasticity. Evolution 51, 1529–1535.

Obrycki J., Kring T., 1998. Predaceous coccinellidae in biological control. Annual Review of Entomology, 43: 295-321.

Obrycki J.J., Harwood J.D., Kring T.L., O'Neil R.J., 2009. Aphidophagy by Coccinellidae: Application of biological control in agroecosystem. Biological Control, 51(29): 244-254.

Obrycki, J. 1998. Predaceous Coccinellidae in biological control. Annual Review of Entomology, 43:295-321.

Oerke, E.C., 1994. Crop Production and Crop Protection: Estimated Losses in Major Food and Cash Crops. Elsevier, Amsterdam.

Polis G. A., Myers C. A., Holt R. D., 1989.- The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics, 20: 297-330.

Prasad R.P., Roitberg B.D., Henderson D., 1999. The effect of rearing temperature on flight initiation of Trichogramma sibericumsorkina at ambient temperatures. Biological Control 16, 291–298.

Pumhan N., Tian M., Xu L.L., Jiang J., Liu T., Zhang S. 2020. Effects of heat stress and exposure duration on survival characteristics of different developmental stages of Propylaea japonica, a dominant aphidophagous ladybeetle in China. Crop Protection 130: 105054.

Reynolds P.G., Cuddington K., 2012. Effects of plant gross morphology on predator consumption rates. Environmental Entomology, 41: 508-515.

Schüder I., Hommes M., Larink O., 2004. The influence of temperature and food supply on the development of Adalia bipunctata (Coleoptera: Coccinellidae). European Journal of Entomology 101, 379–384.

Smolinský R., Gvoždík L., 2013. Does developmental acclimatization reduce the susceptibility to predation in newt larvae? Biological Journal of Linnean Society, 108, 109–115.

Snyder W. E., Ives A. R., 2003. Interactions between specialist and generalist natural enemies: parasitoids, predators, and pea aphid biocontrol. Ecology, 84: 91-107.

Solomon, M.E., 1949. The natural control of animal populations. Journal of Animal Ecology, 18: 1-35.

Sørensen C. H., Søren T., Kristensen T.N., 2013. Cold-acclimation increases the predatory efficiency of the aphidophagous coccinellid Adalia bipunctata. Biological Control, 65: 87-94.

Symondson W.O.C., Sunderland K.D., Greenstone M.H., 2002. Can generalist predators be effective biocontrol agents? Annual Review of Entomology, 47:561-594.

Terblanche J.S., 2014. Physiological performance of field-released insects. Current Opinion in Insect Science, 4, 60–66.

Thomson, L., Robinson, J.M., Hoffmann, A.A., 2001. Field and laboratory evidence for acclimation without costs in an egg parasitoid. Functional Ecology 15, 217–221.

Uiterwaal S., DeLong J.O., 2018. Multiple factors, including arena size, shape the functional responses of ladybird beetles. Journal of Applied Entomology, 55(5): 2429-2438.

Uiterwaal S., Mares C., DeLong J.O., 2017. Body size, body size ratio, and prey type influence the functional response of damselfly nymphs, Oecologia, 185(3): 1-8.

van Rijn, P. C. J., Bakker, F. M., van der Hoeven, W.A.D., Sabelis, M.W., 2005. Is arthropod predation exclusively satiation-driven? Oikos, 109, 101– 116.

Zarei M., Madai H., Zamani A.A., Nedved O., 2019. Predation rate of competing Chrysoperla carnea and Hippodamia variegata on Aphis fabae at various prey densities and arena complexities. Bullettin of Insectology, 72(2): 273-289.

GENERAL CONCLUSIONS AND FUTURE RESEARCHES

The experiments conducted in this thesis are related with the thermal biology of the pea aphid *Acyrthosiphon pisum*, the parasitic wasp *Aphidius ervi* and the aphidophagous ladybird *Hippodamia variegata*.

In most studies, the effects of climate change have been considered with regard to an increasing of mean temperature (Asin and Pons, 2001; Mehrparvar and Hatami, 2007). On the contrary, the studies in this thesis are all based on rapid and high thermal variations, which aims to produce useful knowledge to understand how insects could respond in nature when similar conditions occur. Rapid thermal variations often occur in nature, and represent a common challenge for these insect species. Moreover, these scenarios will be more frequent in future due to global warming.

In the Ch. I, I found that a sub-lethal heat exposure (39°C for 30 min), determining about 80% of mortality in the experimental group, decreased both the longevity and nymph production of specimens survived to the stress. This result highlights the negative effects of a rapid thermal variation on aphid abundance, by determining a direct mortality during and soon after the stress, and by a subsequent long-term scale effect on other life history traits affecting dynamic and structure of aphid population. This result is useful to explain why in nature aphid populations usually reduce in summer, when diurnal temperatures increase.

In the Ch. II, I exposed the pea aphid, parasitized and unparasitized by the braconid wasp *A. ervi*, to a sub-lethal temperature (39 °C for 30 min) and I recorded the effects on aphid survival. I found a greater survival rate of parasitized aphids compared with unparasitized ones after exposure to the sub-lethal temperature. After the heat shock, the survival of unparasitized aphids decreases according to their age at the heat shock treatment, suggesting a different adaptation of the aphid life stage to the different microclimatic conditions they experience. Survival of parasitized aphids does not change according to parasitoid stage at time of the heat shock treatment, but it is always significantly higher compared with the unparasitized aphids. Parasitized aphids are very quickly subjected to a wide range of physiological modifications which may be the cause of the increased survival to the heat shock treatment. Lagos *et al.* (2001) observed a different thermal sensitivity in *A. pisum* parasitized by *A. ervi* mediated by a different behavioural response (walking activity). In the present chapter, I show evidence that parasitism, in the same aphid-parasitoid system, can also affect the host thermal sensitivity through a physiological mechanism whose exact

nature needs to be further investigated. High temperatures and drought are known to induce many changes in hosts and parasitoids and can generate a disjunction between their population growth rates, affecting the role of parasitoids in aphid population control (Romo and Tylianakis, 2013). Taking this into account, it could be interesting to investigate if the phenomenon I observed could affect the host-parasitoid population dynamics in nature. This study is particularly important because it reports for the first time a greater survival after a heat shock due to a metazoan parasite.

In the Ch. III, I tested the effect of a mild heat treatment and parasitization on the aphid survival to a subsequent sub-lethal stress. In literature the beneficial effects of non-lethal heat exposures on the insect heat tolerance to a subsequent heat stress is well documented (Angilletta, 2009). I suppose that the stress induced by parasitization could increase aphid heat resistance through the activation of a hardening-like mechanism. In this chapter, I then investigate if parasitism and a thermal hardening may or may not have any cumulative effects on aphid thermal resistance and if (and to what extent) these effects influence parasitoids survival. I found that the mild heat exposure increases the thermal tolerance of unparasitized aphid to a subsequent heat stress, and that, although this mild exposure did not determine significant mortality in the aphid group during the first 24 hours after the stress, it has a relevant negative long-term effect on aphid vitality. Since the aphids experiencing both the mild stress and the parasitization before the sub-lethal stress did not have greater survival than those experiencing just the parasitization, no additive effect in terms of increment heat resistance were found among parasitization and the mild exposure. Moreover, I found that both the mild and sub-lethal exposure, and their combination, reduce mummification rate and adult eclosion rate. Interestingly, no differences in mummification rate and adult eclosion rate were found among all the groups experiencing the heat.

In the Ch. IV, I investigated if the parasitoid *A. ervi*, at various stages of development, shows differences in adult body size and body allometries after the exposure at two stressful temperatures (35°C and 39°C). Body size was estimated considering leg and wing length. Parasitized aphids were subjected to a thermal treatment at two different developmental stages (egg and 3rd larval stage) for an exposure time of 30 minutes. I found that this temperature exposure reduces the wing and tibia length of the parasitoids developed in heat stressed aphid, if compared with those emerged from not heat stressed aphids. I found that just the exposure at 39°C determines a significant wing and tibia length reduction in the adult parasitoids, both when they experienced the stress at the egg and at the 3rd larval stage.

Moreover, I found that the heat exposure determined an allometry change in the body of the adult parasitoids. This allometric change had different intensity, and varied both in function of the temperature and the developmental stage at which the parasitoid experienced the stress.
Body size reduction and allometric change in adult parasitoids, due to the heat stress, could be associated with fitness reduction and might impact on their population structure and dynamic.

In the Ch. V, I investigated if an exposure of the pea aphid to a non-lethal cold temperature increases the pea aphid resistance to a subsequent stronger cold stress. I also investigated if parasitization of the pea aphid by the parasitoid *A. ervi* may increase the pea aphid resistance to a subsequent stronger cold stress, as demonstrated in Ch. II in the case of heat stress. Since any interaction between parasitism and cold stress responses may be coevolved in parasitized aphids, these two kind of stress were jointly tested by exposing parasitized aphids to a cold-hardening treatment before a sub-lethal cold stress. In particular, it was investigated if in parasitized aphids the beneficial of a cold-hardening treatment further enhances the aphid cold resistance. I found that the mild cold exposure increased survival just when aphids were parasitized by the parasitoid *A. ervi*. This result suggests a cumulative and synergic effect of the parasitism and of the mild cold exposure on the increment of aphid cold resistance. This result confirms the capability of *A. ervi* to increase thermal tolerance to stressful temperature when thermal variations rapidly occur.

In the Ch. VI, I tested if and how the predation of *H. variegata* on *A. pisum* is affected by the experimental arena complexity at two temperatures (20 and 25°C), and if the acclimation influences the predation rate when this ladybird preys at temperatures different from those at which it was reared. For the second question, I specifically tested if the rearing thermal conditions affect prey consumption in two different thermal environments. I found that the experimental arenas tested in this study did not affect the aphid consumption rate during a 24h predation test, and I found that the aphid consumption is maximum at the temperature at which the predator developed. Then, I found that *H. variegata* plastically responded to the tested temperatures. This last result provides information on the thermal plasticity of this ladybird species, and represents a starting point to accurately predict its efficiency as a consequence of the change in thermal conditions. Moreover, predation studies at fluctuating thermal conditions and in semi-field conditions could be carried out to obtain more detailed

information on the effect of the rearing temperature on the predatory performances of this ladybird.

Findings in the Ch. II, III and V confirm the existence of a strict physiological relationship between the pea aphid and its parasitoid *A. ervi*. The physiological mechanism at the base of the host manipulation were not identified in these studies, but the available literature and the results reported in the Ch.II allows to suppose that the up-regulation of some heat shock proteins may have a fundamental role in the aphid resistance to heat stress after parasitization, in the heat resistance increment found in the pea aphid after a heat hardening treatment (see Ch. III) and in the cold resistance increment due to parasitization (see Ch. V). To ascertain the role of the heat shock proteins in these observed phenomena, studies aiming to identify the implicated genes, and quantify their up-regulation due to heat, cold and parasitization stress, could be carried out. Moreover, taking into account the results of Ch. II, III and V, it could be interesting to investigate, from an ecological perspective, if and how these thermal stresses affect the host-parasitoid population dynamic in nature.

My results confirm that rapid and extreme thermal variations could strongly affect some of the main fitness-related life history traits of the pea aphid and two of the main natural enemies, the endoparsiotid *A. ervi* and the ladybird *H. variegata*. Moreover, these results represent a contribution to the knowledge on the aphid-endoparasitoid relationship under extreme thermal conditions and on the thermal biology of the ladybird *H. variegata*.

SCINETIFIC PRODUCTION

Scientific papers on the PhD activity

- Trotta V., Forlano P., Falabella P., Battaglia D., Fanti P., 2018. The aphid Acyrthosiphon pisum exhibits a greater survival after a heat shock when parasitized by the wasp Aphidius ervi. Journal of Thermal Biology, 72: 53-58.

- Short and long-term effects of a heat stress on the survival and reproduction in the pea aphid Acyrthosiphon pisum (Harris) (Hemiptera: Aphididae). Physiological Entomnology, DRAFTING.

- Effects of two heat stresses and their combination on the survival of the pea aphid Acyrthosiphon pisum and its parasitoid Aphidius ervi. Physiological Entomnology, DRAFTING.

Others papers during PhD period

- Trotta V., Toma I., Forlano P., Fanti P., Duran Prieto J. Battaglia D., 2020. The age of tomato plants affects the development of Macrosiphum euphorbiae (Thomas, 1878) (Hemiptera: Aphididae) colonies. ACCEPTED by Agronomia Colombiana.

- Duran Prieto J., Trotta V., Di Nardo E., Forlano P., Fanti P., Battaglia D., 2018. Intraguild predation between Macrolophus pygmaeus and Aphidius ervi. Bulletin of Insectology, 71(1): 113-120.

- Trotta V., Forlano P., Caccavo V., Fanti P., Battaglia D., 2021. A survey of potential insect vectors of the plant pathogenic bacterium Xylella fastidiosa in the Basilicata Region, Italy. ACCEPTED by Bulletin of Insectology.

Conference partecipations

- Trotta V., Caccavo V., Forlano P., Fanti P., Battaglia D., 2000. A survey of potential insect vectors of the plant photogenic bacterium Xylella fastidiosa in the Basilicata Region, Italy. National Congress of Entomology of Italy. June/2021. SUMBITTED.

- Trotta V., Caccavo V., Forlano P., Fanti P., Battaglia D., 2000. Impacts of single and repeated heat shocks applied at different developmental stages in an aphid-parasitoid system. National Congress of Entomology of Italy. June/2021. SUMBITTED.

- Fanti P., Trotta V., Forlano P., Falabella P., Battaglia D., Dealing with high temperatures: the aphid Acyrthosiphon pisum exhibits a greater survival after a heat shock when parasitized by the wasp Aphidius ervi. XI European Congress of Entomology, 2-6 July 2018. Napoli, Italy. pp 9-10.

- Forlano P, Battaglia D., Preliminary studies on the effects of high temperatures on some aphid life history traits and on aphid-parasitoid interactions. - IX Annual Meeting of European PhD Network "Insect Science", 14-16 November 2018. Firenze, Italy. p. 17.

- Forlano P., Progettare la biodiversità in città: effetto della complessificazione della componete botanica sulle comunità di insetti. VII Convegno Nazionale Apicoltura Urbana dal titolo, presso la sezione di Legambiente di Potenza, 20/10/2018.

ACKNOWLEDGEMENTS - I would like to give my profound thanks Prof. Donatella Battaglia, who give me the chance to work under their supervision as a PhD student and introduced me into the knowledge of the applied entomology and the biological control. My gratitude goes also to Dr. Vincenzo Trotta and Prof. Paolo Fanti for their support and advice and especially for experiment planning and their contribution on the statistical analysis of the data presented in this thesis and for their valuable comments to improve this work.

I also wish to make a grateful acknowledgement to Prof. Xavier Pons, Dr. Roberto Meseguer and Dr. Alex Levi for hosting me at the Entomology Laboratory of Lleida University (Catalogna, Spain). I am very grateful with them because they help me to solve any kind of question or problem with the experimental tests and for sharing with me their expertise in the entomological field.