

Table of contents

List of tables

List of figures

List of appendixes

Part 1: Introduction	1
Chapter I: General introduction	1
General background	2
Environmental stress	4
Thermal stress: ecology of thermal adaptation	5
Thermal stress effects on life-history traits and fitness	9
Different types of responses to thermal stress	10
<i>Local adaptation to temperatures</i>	11
<i>Plasticity as a response to environment variability</i>	12
Fitness trade-offs for thermal stress	15
Seasonal thermal stress	17
Environmental gradients	18
Strategies employed by ectotherms to escape winter unfavorable conditions	21
<i>Migration</i>	21
<i>Diapause in insects</i>	22
<i>Summer diapause</i>	25
<i>Diapause Cost</i>	26
Remain active as a strategy	27
Overwintering strategies: the case of aphid parasitoids.....	28
<i>Diapause in aphid parasitoids</i>	29
<i>Remain active as strategy in aphid parasitoids</i>	31
Biological control context	32
How climate change affects biological control?.....	32
Biological model	34
Aphids.....	34
Aphid parasitoids	35
<i>Chile as study case:</i>	37
Research questions and hypotheses	40
Thesis organization	41
References	45
Part 2: Aphid-parasitoids food webs composition	58
Chapter II: Winter aphid–parasitoid food webs	58
Composition and structure of winter aphid–parasitoid food webs along a latitudinal gradient in Chile	59

Abstract	59
Introduction	60
Material and methods	63
Study site.....	63
Meteorological data.....	64
Insect sampling.....	64
Parasitoid diapause identification.....	65
Quantitative food-web metrics.....	66
Statistical analysis.....	66
Results	68
Environmental gradient determination.....	68
Aphid and parasitoid food web composition and rate of diapausing individuals.....	69
Diapausing parasitoid and hyperparasitoid species in mild and cold localities.....	71
Effects of the temperature and host on the community composition.....	75
Quantitative food web metrics across the determined winter conditions.....	75
Discussion	79
Conclusion	84
References	86
Part 3: Seasonality strategies in aphid parasitoids	89
Chapter III: Overwintering strategies of <i>Aphidius platensis</i>	89
Overwintering strategies and life-history traits of different populations of <i>Aphidius platensis</i> along a latitudinal gradient in Chile	90
Abstract	90
Introduction	96
Material and methods	99
Meteorological data and gradient determination.....	99
Insect sampling and rearing.....	99
Experimental conditions.....	100
Measure of the diapause level.....	101
Measures of physiological and life-history traits.....	102
<i>Fresh body mass and hind tibia length measurements</i>	102
<i>Critical thermal minima (CTMin)</i>	102
<i>Water and fat content</i>	103
<i>Measure of egg load and volume of the eggs</i>	104
Statistical analyses.....	104
<i>Gradient determination</i>	104
<i>Parameters measured</i>	105
Results	106
<i>Meteorological data and gradient determination</i>	106
<i>Emergence rate, diapause expression, and developmental time</i>	107
<i>Fresh body mass (FBM) and size</i>	111
<i>Critical thermal limits</i>	113
<i>Water and fat content</i>	113

<i>Egg load at emergence and volume of the eggs</i>	116
Discussion	118
References	124
Chapter IV: Diapause in <i>Aphidius ervi</i>	137
Abstract	137
Introduction	138
Material and methods	141
Biological material.....	141
<i>Aphids</i>	141
<i>Parasitoids</i>	142
Winter diapause incidence in a climatic gradient: effect of photoperiod and temperature.....	143
Host species effect on winter diapause incidence.....	144
Effect of host density on winter and summer diapause incidence.....	145
Females' competition on winter and summer diapause incidence.....	145
Statistical analyses.....	146
Results	147
Winter diapause incidence in a climatic gradient: effect of photoperiod and temperature.....	147
Host species effect on winter diapause incidence.....	148
Effect of host density on diapause incidence.....	149
Incidence of Females' competition on diapause.....	151
Discussion	153
Latitudinal gradient and proportion of winter diapause.....	153
Effect of the host species on diapause induction.....	154
Effect of host density on diapause incidence.....	156
Effect of maternal competition on diapause incidence.....	157
Conclusion	158
References	160
Part 4: General discussion	165
Chapter V: General discussion	165
Overview.....	166
Overwintering strategies in response to geographic variation.....	169
Diapause expression: effects on parasitoid community and food-web structure in a climatic gradient.....	172
How does host quality affect diapause expression in aphid parasitoids?.....	174
Implications for biological control.....	176
Perspectives and future directions	179
General conclusion	183
References	184
Resumen en extenso	185
Publications and meetings	185

List of tables

Part 2

Chapter II

- Table 2.1.** Total number of fields sampled and frequency of cereal aphids, mummies, emerged parasitoids, hyperparasitoids, non-emerged parasitoids (dead larva/adult individuals), and diapausing individuals during winter on cereal crops in the central-south valley of Chile for each locality and year. Localities are presented from North (top of the table) to South (bottom of the table). The total percentage of diapausing individuals in each location and year is shown in parentheses. 73
- Table 2.2.** Parasitoid (*Aphidius ervi*, *Aphidius uzbekistanicus* and *Praon volucre*) and hyperparasitoid (*Phaenoglyphis villosa*) species recorded in diapause during winter on different aphid species in cereal crops in the central-south valley of Chile for each locality and year sampled. The numbers of emerged non-diapausing and diapausing individuals and their respective percentages are shown in parentheses. The remaining percentage corresponds to the non-emerged (dead) individuals. 74
- Table 2.3.** Results of model selection for the main aphid and parasitoid species present in winter. The influence of temperature (T_{min} and $h < 0^{\circ}C$) on the abundance of the dominant aphid species, *Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum*, and the dominant parasitoid species, *Aphidius platensis*, *Aphidius ervi* and the complex *Aphidius uzbekistanicus/rhopalosiphii*, was tested. The influence of the exploited aphid species on the abundance of each parasitoid species was tested. Columns correspond to the tested dependent variables, the AIC value, and the estimate values determined in the analysis. Statistically significant p-values (≤ 0.05) are presented in bold. 76

Part 3

Chapter III

- Table 3.1.** Results of exposure of aphids parasitized by five populations of *A. platensis* in three conditions of temperatures and photoperiod: Emergence rate, N° of mummies formed, N° of non-emerged mummies dissected 15 days after the last emergence and Proportion of dissected non-emerged mummies containing a dead adult the remaining proportion corresponds to dead larvae. No mummies in diapause were found. Mean \pm SE are shown. The white part shows the mild winter localities and grey parts the cold winter ones. 110
- Table 3. 2.** Influence of different conditions on fresh body mass (mg) and hind tibia length (mm) at emergence (N = 50 ♂ and N = 75 ♀) of five populations of *A. platensis* from central south valley of Chile. Mean \pm SE are shown. The shaded part shows the cold winter localities and the white part the mild winter localities. 112
- Table 3.3.** Values of water content (WC) and fat content (FC) of *A. platensis* from different populations of the central south valley of Chile tested in three different conditions. Mean \pm SE are shown. The shaded part shows the cold winter localities and the white part the mild winter localities. 115

Chapter IV

- Table 4. 1.** Total number of mummies formed and emerging pattern of *A. ervi* parasitizing *A. pisum* at different levels of host density and competition (- C = without competition and + C = with competition) between parasitoid females at 10°C and 8:16 LD and 24°C and 16:8 LD temperature and photoperiod regimen. Values in brackets are proportions. Different letters indicate a significant difference between treatments (Tukey HSD post-hoc test ($P < 0.05$)). 152

List of figures

Part 1

Chapter I

- Figure 1.1.** Thermal performance curves of ectotherms in temperate environments. T_{\min} and T_{\max} represent the minimum and maximum temperature at which organisms can perform, and T_{opt} is the optimal temperature for performance. The green curve under the thermal performance curve is the temperature that the organism is exposed to during an average time and the average is represented as T_{hab} . ΔT is the distance between T_{hab} and T_{opt} (Johansson et al. 2020). 6
- Figure 1.2.** Diagram of behavioral and physiological thresholds measured. Onset of critical thermal minima is highlighted in light blue, beginning at the temperature at which locomotor efficiency is first compromised (locomotor trouble CT_{Min1}). The threshold temperatures at which spontaneous movements and coordination are lost (entry to chill coma. Chill coma (CT_{Min2}) the physiological state is highlighted in dark blue (Modified from Hazell and Bale, 2011). 8
- Figure 1.3.** Ecological tolerance curve. The green shaded area represents the population frequency distribution of one species along the environmental gradient (e.g., cool to warm temperature) (Faith and Lyman 2019)..... 11
- Figure 1.4.** Phenotypic plasticity in terms of reaction norms: A) A group of genotypes adapted to their environment of origin is exposed to a novel environment, which results in the expression of phenotypes that were not observed in the original environment. B) Proxy of fitness differences between phenotypes results in the retention of a subset of the genotypes, which increases the mean fit between the phenotype and the new environment, and C) Fine-fit response of reaction norms of genotypes compared to the origin population. The blue lines represent the reaction norms of one genotype which show an increased in plasticity (Modified from Uller et al. 2020)..... 14
- Figure 1.5.** Effects of environmental variation on individuals, populations and communities..... 19
- Figure 1.6.** Description of phases of insects' diapause during the different ontogeny stages, from embryo to adult according to Košťál (2006). Changes in diapause intensity are presented: dotted branches (a and b) apply to the constant conditions, while solid branch (c) applies to the change of environmental conditions. 24
- Figure 1. 7.** Golden-yellow prepupae diapausing of *Aphidius ervi*, 36 days following mummification..... 30
- Figure 1.8.** (A) Parthenogenetic grain aphid, *Sitobion avenae*, a cosmopolitan species on grasses and cereals. (B) Typical holocyclic life cycle of aphids. The species alternates between the primary winter host, and secondary summer hosts. Anholocyclic life cycle of aphids. Continuous asexual reproduction occurs with the winged forms moving from different host plants. Modified of Finlay & Luck (2011)..... 35
- Figure 1.9.** (A) *Aphidius platensis* ovipositing on *Rhopalosiphum padi* (B) Biological cycle of an aphid parasitoid (<https://twitter.com/KoppertCa>) 36
- Figure 1.10.** (A) Map of Chile showing the regions corresponding to the central- south valley (Modified from [https://es.wikipedia.org/wiki/Archivo:Mapa_de_Chile_\(regiones\).svg](https://es.wikipedia.org/wiki/Archivo:Mapa_de_Chile_(regiones).svg)). (B) Mean annual temperature near surface (2 m) highlight the pronounced climate gradients along latitudinal gradient in Chile (Mutz et al 2021)..... 39

Part 2

Chapter II

- Figure 2. 1.** (A) Map showing the nine localities where aphids and parasitoids were sampled during three consecutive years (2016–2018) along a latitudinal gradient in the central-south valley of Chile and (B) Principal components analysis (PCA) biplot based on climatic variables (mean minimal temperature 'Tmin' and number of hours below zero 69

Figure 2. 2. Quantitative aphid–parasitoid food webs in the central-south valley of Chile. Horizontal bars represent the relative abundance of each aphid species (lower) and their parasitoids, including hyperparasitoids (upper points). Arrows represent the interaction strength (% of relative abundances) between host–parasitoid pairs. 72

Figure 2. 3. Generalized linear model results showing the relationship between the abundance per field for the main active aphid species (*Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum*) and the abundance of the main active parasitoid aphid species: A) *Aphidius platensis* B) *Aphidius ervi* and C) *Aphidius uzbrho*. The trend line shows the significant links between aphid species and parasitoids based on the negative binomial regression..... 77

Figure 2. 4. Box plot of the climatic gradient (warm, mild and cold winters areas) showing (A) Shannon’s diversity of interaction; (B) Connectance, the overall complexity of the food web (realized proportion of potential links); (C) Web-asymmetry, the balance between the numbers of parasitoid and aphid species (negative values indicate more species in higher than in lower trophic-level); (D) Vulnerability, the weighted mean number of parasitoid species attacking a given aphid species; (E) Generality, the weighted mean number of aphid species exploited by each parasitoid species; and (F) ‘H2’, the level of specialization within a network, from 0 (no specialization) to 1 (perfect specialization). There were no differences among climatic areas for the evaluated metrics. 78

Part 3

Chapter III

Figure 3. 1. Geographic and environmental gradient along the central south valley of Chile in winter 2018: (A) Study sites, (B) Biplot of the principal component analysis (PCA) for environmental variable in the meteorological stations near to the sampling localities. The PC1 and PC2 shows clear partitioning according to minimal temperatures ‘Tmin’ and numbers of hours below 0°C; (C) Total hours below 0°C (grey bars) and mean ± SE of minimum temperature (dots) for each sampled latitudinal locality (RN: Rancagua, TL: Talca, CH: Chillan, PN: Pinto and TM: Temuco)..... 107

Figure 3. 2. Probability of emergence in days since oviposition to emergence of *A. platensis* from five populations exposed to three conditions of temperatures and photoperiod (spring, fall and winter conditions). Different letters indicate a significant difference between populations and conditions. Tukey HSD post hoc test ($P < 0.05$). The white part shows the mild winter localities and grey parts the cold winter ones..... 109

Figure 3. 3. Critical minimal temperatures (CTMin) of males and females of five populations (RN: Rancagua, CH: Chillan, TM: Temuco, TL: Talca and PI: Pinto) of *A. platensis* exposed to three different conditions: (A) CT of loss of muscular function (CTMin1); (B) Fresh body mass of all tested individuals, correlated with the CTMin1, (C) CT of chill coma (CTMin 2), and (D) Fresh body mass of all tested individuals correlated with CTMin2. Plots show the mean ± SE. Different letters indicate a significant difference between populations of *A. platensis*, conditions and sexes. Tukey HSD post hoc tests ($P < 0.05$). The white part shows the mild winter localities and grey parts the cold winter ones..... 114

Figure 3. 4. Effect of the three conditions on the five populations of *A. platensis* for the following parameters: (A) Mean number of mature eggs, (B) Correlation between the number of eggs and the tibia length, (C) Mean volume of eggs in mm³ and (D) Correlation between the volume of the eggs and the tibia length. Twenty-five females at their emergence were used to measure these life-history traits. Box plots showed the median; lower and upper quartiles; whiskers: smallest and largest non-outlier observations; dots: outliers. 117

Chapter IV

Figure 4. 1. Map of sampling locations in central-south of Chile and proportions of emerged adults, dissected non emerged mummies containing a dead larvae/adult and dissected non emerged mummies containing a prepupal diapausing individual produced by *Aphidius ervi* females sampled from cereal fields on *Sitobion avenae* and tested at two different

conditions of temperature and photoperiod (winter and non-diapausing conditions). A total of 1282 mummies were formed in this laboratory experiment for both conditions tested. 148

Figure 4. 2. Proportion of diapause incidence in *Aphidius ervi* parasitizing *Acyrtosiphum pisum* and *Sitobion avenae* at two conditions of temperature and photoperiod (winter and laboratory-controlled conditions). The number of mummies is provided on the figure for each aphid host species and condition. A total of 1116 mummies were formed. Significant differences are shown (Post hoc Tukey test; $P < 0.05$): < 0.001 ‘***’, < 0.01 ‘**’, < 0.05 ‘*’, and ns ‘no significant’ 149

Figure 4. 3. Proportion of diapause incidence in *Aphidius ervi* parasitizing *Acyrtosiphum pisum* at different conditions (winter and summer) and two levels of host density. The number of mummies formed is provided on the figure for each aphid host and condition. A total of 1235 of mummies were formed. Significant differences are shown (Post hoc Tukey test; $P < 0.05$): < 0.001 ‘***’, < 0.01 ‘**’, < 0.05 ‘*’, and ns ‘no significant’ 150

Figure 4. 4. Proportion of diapause incidence in *Aphidius ervi* parasitizing *A. pisum* at two conditions (winter and summer) in relation to female parasitoid competition (- C=without competition and + C=with competition). The number of mummies formed is provided on the figure for each aphid host and condition. A total of 1838 of mummies were formed. Significant differences are shown (Post hoc Tukey test; $P < 0.05$): < 0.001 ‘***’, < 0.01 ‘**’, < 0.05 ‘*’, and ns ‘no significant’ 152

List of appendixes

Part 2

Chapter II

Appendix 2.1. Figure S2.1. Climatic data in nine sampled localities and three consecutive years. T mean: Mean temperature (°C), T min: minimal temperatures over winter season and Mean hours $< 0^{\circ}\text{C}$: Occurrence of temperatures below 0°C over winter season. 93

Appendix 2.2. Figure S2.2. Representation of the relationship between the abundance per field of the main aphid species (*Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum*) and the main parasitoid species (*Aphidius platensis*, *Aphidius ervi* and *Aphidius uzb/rho*) with the temperature variables: (A) Aphids vs Tmin, (B) Parasitoids vs Tmin, (C) Aphids vs hours below 0°C and (D) Parasitoids vs hours below 0°C 94

Part 3

Chapter III

Appendix 3. 1. Table S3.1. General results of the different models tested for populations of *A. platensis* along the latitudinal gradient of Chile, when faced to three different conditions of temperature and photoperiod. For each level, the number of freedom degrees (df), the statistic test and the p value are represented. 132

Appendix 3. 2. Figure. S3.2. Global Spearman correlations between changes in physiological parameters (**p < 0.05) of parasitoids (males and females). Coefficients of correlations (R) and probabilities of R Pearson tests are shown. 134

Appendix 3. 3. Figure S3.3 Global Spearman correlations between fresh body mass and tibia length (**p < 0.05) of parasitoids (males and females). Coefficients of correlations (R) and probabilities of R Pearson tests are shown. 135