

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Reproductive Biology of
***Eretmocerus warrae* Naumann and Schmidt**
(Hymenoptera: Aphelinidae)

**A thesis presented in partial fulfilment of the
requirements for the degree of**

Doctor of Philosophy in Plant Sciences
(Entomology)

at

Massey University



Palmerston North

New Zealand

Abdul Hanan

2012

Abstract

Eretmocerus warrae Naumann and Schmidt is a thelytokous and specialist parasitoid of the Greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). Prior to this research, little information was available on its reproductive biology. Emergence of *E. warrae* occurs exclusively during the photophase and peaks during the first few hours of the photophase and then decreases rapidly afterwards. However, *E. warrae* adults remain active throughout 24 hours with host feeding peaking between 4 and 6 h and oviposition peaking between 10 and 14 h after lights on. With the increase of temperature from 15 to 30°C the average daily host feeding rates increase while the pre-oviposition period, longevity of adults and developmental period of immatures decrease. The maximum fecundity and host feeding of adults, and survival and emergence of offspring occur at 20 and 25°C. *E. warrae* successfully parasitises and feeds on all four nymphal instars of the greenhouse whitefly but eggs laid under the third to fifth stages of the fourth instar nymphs fail to complete development. *E. warrae* prefers the second and third instar nymphs for feeding and oviposition with higher survival rate of offspring. However, with the increase in host stage the parasitoid offspring gain more fitness with larger body size, higher egg load and longer longevity. Experienced females can discriminate between the parasitised and unparasitised hosts and avoid to superparasitise them when host density is high. However, the naïve females frequently lay eggs under the parasitised hosts. When initially deprived of food and hosts for 5 hours, *E. warrae* increases host feeding, fecundity and longevity. Females carry some mature eggs in their ovaries at emergence, and hence this is a pro-synovigenic species. Food and host deprived females can maintain eggs for up to two days while honey-fed females can keep eggs for up to 5-7 days. With the increase of host density the fecundity, parasitism, host feeding and longevity in *E. warrae* increase while the proportion of hosts fed on, parasitised and superparasitised decreases. Superparasitism increases with the increase in parasitoid density. Parasitoids emerging from singly parasitised hosts are larger, carry higher egg load and live longer than those from superparasitised hosts. Honey-fed parasitoids live 4-5 folds longer than those not provided with food or hosts after emergence. Honey-and host-fed parasitoids also live longer than those provided with hosts only. The findings from this study in relation to biological control of *T. vaporariorum* using *E. warrae*, e.g., mass-rearing or field release, are discussed.

Acknowledgements

I am highly obliged to my supervisor, Professor Qiao Wang, for his precious time, effort and support provided throughout this research. Qiao always fed me with more scientific questions, challenged my explanations, encouraged me to publish and present my results, and had an open-door policy with kind smile on his face.

I am highly obliged to my co-supervisor, Dr. Xiong Zhao He for his time in statistical assistance and advice throughout the research. He also discussed the results and making valuable comments on the thesis and manuscript papers.

I am also thankful to BioForce, Ltd., Auckland, New Zealand, for providing me with insects for this study, the staff of the Institute of Natural Resources and Plant Growth Unit, Massey University, for providing me with materials and their technical assistance and to the staff of Massey University Library and the IT Service for their help with information access.

I am grateful to the Higher Education Commission of Pakistan for providing me with an Overseas Scholarship for study at Massey University, New Zealand. I am equally thankful to the New Zealand Plant Protection Society and Institute of Natural Resources, Massey University, for providing me with their support through travel grants, which allowed me to present my study to conferences.

I owe special thanks to my departmental and university colleagues include Muhammad Shakeel, Diwas Khatri, Anand Yadav, Jin Xu, Amir Sultan, Casey Flay, Mohammad Sajjad Hussain, Santhy Priya, and Jana Mullar, for their valuable time, ideas and comments throughout my study. In addition, I am also thankful for the support from a number of other people, including Dr. Zulfiqar Hayder Butt, Julia Rayner, Kay Kitchen, James Slater, Chris Rawlingson, Denise Stewart, Glenys Gilligan and Sandra Dunkinson.

I am very thankful to my whole family, especially my wife for her unconditional support during my studies. She always does her best to take care of our boys so that I can concentrate on my studies. I am also grateful to my brother and sisters for taking care of my mother during my PhD studies.

*This thesis is dedicated to my whole loving family,
especially my deceased elder sister for her unconditional
support when she was alive*

Table of contents

Chapter 1	General Introduction	1
1.1	Introduction.....	1
1.2	Importance of whiteflies.....	2
1.2.1	Host plants.....	3
1.2.2	Pesticide resistance in <i>T. vaporariorum</i>	5
1.3	Importance and relevance of this research.....	5
1.4	Aims and objectives.....	6
Chapter 2	Literature Review	7
2.1	Introduction.....	7
2.2	Classification of <i>E. warrae</i>	7
2.3	General Biology	7
2.3.1	Egg.....	7
2.3.2	Larva.....	8
2.3.3	Pupa.....	8
2.3.4	Adult.....	8
2.4	History of <i>T. vaporariorum</i> and <i>E. warrae</i>	11
2.4.1	History of <i>T. vaporariorum</i> in New Zealand.....	11
2.4.2	Biological control history of whiteflies.....	11
2.4.3	Parasitoid species of <i>T. vaporariorum</i>	12
2.4.3	The genus <i>Eretmocerus</i>	13

2.5	Basic Biology	13
2.5.1	Effect of temperature.....	13
2.5.2	Diel activity patterns of insects.....	14
2.5.3	Developmental strategies of parasitoids.....	16
2.6	Host Feeding and Oviposition Behaviour	17
2.6.1	Host stage preference for feeding and oviposition.....	17
2.6.2	Host discrimination behaviour and superparasitism of <i>E. warrae</i> ...	19
2.7	Factors Affecting Reproductive Fitness of <i>E. warrae</i>	20
2.7.1	Egg load.....	20
2.7.2	Host stage.....	20
2.7.3	Host density.....	21
2.7.4	Parasitoid density.....	21
2.7.5	Effect of food supply.....	22
Chapter 3	General Biology	24
3.1	General introduction.....	24
3.2	General methodology.....	24
3.2.1	Materials.....	24
3.2.2	Environmental conditions.....	26
3.2.3	Procedures.....	27
3.2.4	Definitions.....	27
3.2.5	Statistical analyses and reported values.....	28

3.3	Diurnal Rhythms of Emergence, Host Feeding and Oviposition of <i>E. warrae</i>	29
3.3.1	Introduction.....	29
3.3.2	Materials and methods.....	29
3.3.2.1	Emergence.....	29
3.3.2.2	Oviposition and feeding.....	30
3.3.2.3	Statistical analyses.....	30
3.3.3	Results.....	31
3.3.3.1	Emergence.....	31
3.3.3.2	Oviposition and feeding.....	31
3.3.4	Discussion.....	32
 3.4	 Effect of Temperature on Reproductive Fitness of <i>E. warrae</i>	 34
3.4.1	Introduction.....	34
3.4.2	Materials and methods.....	34
3.4.2.1	Experimental conditions and insects.....	34
3.4.2.2	Host feeding, fecundity, parasitism and longevity of adults.....	35
3.4.2.3	Survivorship, emergence and developmental period of immatures...	35
3.4.2.4	Pre-oviposition period.....	35
3.4.2.5	Statistical analyses.....	36
3.4.3	Results.....	36
3.4.3.1	Host feeding, fecundity, parasitism, and longevity of adults.....	36
3.4.3.2	Survivorship, emergence and developmental period.....	38
3.4.3.3	Pre-oviposition period.....	40
3.4.4	Discussion.....	40

3.5	Oviposition and Developmental Strategy of <i>E. warrae</i> in Greenhouse Whitefly	43
3.5.1	Introduction.....	43
3.5.2	Materials and methods.....	44
3.5.2.1	Egg mortality (external mortality).....	44
3.5.2.2	Mortality of the first instar larvae (external mortality).....	44
3.5.2.3	Mortality of the second and third instar larvae, and pupae (internal mortality).....	45
3.5.2.4	Period between oviposition under and penetration into the hosts.....	46
3.5.2.5	Host developmental arrest.....	46
3.5.2.6	Host stage acceptance for oviposition (choice test).....	46
3.5.2.7	Host stage acceptance for oviposition (non-choice test).....	47
3.5.2.8	Statistical analyses.....	47
3.5.3	Results.....	47
3.5.3.1	External and internal mortality of parasitoids.....	47
3.5.3.2	Period between oviposition under and penetration into the host.....	49
3.5.3.3	Host developmental arrest.....	49
3.5.3.4	Host stage acceptance for oviposition.....	50
3.5.4	Discussion.....	51
Chapter 4	Host Feeding and Oviposition Behaviour	54
4.1	General Introduction.....	54
4.2	Host Stage Preference for Oviposition and Feeding	56
4.2.1	Introduction.....	56
4.2.2	Materials and methods.....	57

4.2.2.1	Choice experiment.....	57
4.2.2.2	Non-choice experiment.....	58
4.2.2.3	Statistical analyses.....	58
4.2.3	Results.....	59
4.2.3.1	Host stage preference behaviour observation.....	59
4.2.3.2	Choice test.....	59
4.2.3.3	Non-choice test.....	62
4.2.4	Discussion.....	66
4.3	Superparasitism and Host Discrimination Behaviour in <i>E. warrae</i>	68
4.3.1	Introduction.....	68
4.3.2	Materials and methods.....	69
4.3.2.1	Statistical analyses.....	70
4.3.3	Results.....	70
4.3.4	Discussion.....	74
4.4	Host Feeding and Oviposition Behaviour of <i>E. warrae</i> After a Certain Period of Host and Food Deprivation	76
4.4.1	Introduction.....	76
4.4.2	Materials and methods.....	77
4.4.2.1	Effect of food and host deprivation on host feeding and oviposition behaviour and longevity.....	77
4.4.2.2	Effect of food and host deprivation on egg resorption.....	77
4.4.2.3	Statistical analyses.....	78
4.4.3	Results.....	78

4.4.3.1	Effect of food and host deprivation on host feeding and oviposition behaviour and longevity.....	78
4.4.3.2	Effect of food and host deprivation on egg resorption.....	80
4.4.4	Discussion.....	82
 Chapter 5 Factors Affecting Reproductive Fitness of <i>Eretmocerus warrae</i>		84
5.1	General Introduction.....	84
5.2	Functional Response of <i>E. warrae</i> under Different Host Densities	86
5.2.1	Introduction.....	86
5.2.2	Materials and methods.....	87
5.2.2.1	Experiments.....	87
5.2.2.2	Statistical analyses.....	87
5.2.3	Results.....	88
5.2.4	Discussion.....	90
5.3	Effect of Parasitoid and Host Density on Reproductive Success of Adult and Progeny Fitness in <i>E. warrae</i>	92
5.3.1	Introduction.....	92
5.3.2	Materials and methods.....	92
5.3.2.1	Effect of parasitoid and host density on host feeding, fecundity, parasitism and superparasitism in <i>E. warrae</i>	92
5.3.2.2	Effect of parasitoid and host density on offspring fitness in <i>E. warrae</i>	93
5.3.2.3	Statistical analyses.....	93

5.3.3	Results.....	94
5.3.3.1	Effect of parasitoid and host density on host feeding, fecundity, parasitism and superparasitism in <i>E. warrae</i>	94
5.3.3.2	Effect of parasitoid and host density on offspring fitness in <i>E. warrae</i>	96
5.3.4	Discussion.....	97
5.4	Effect of Food Supply on Reproductive Potential of <i>E. warrae</i>	100
5.4.1	Introduction.....	100
5.4.2	Materials and methods.....	101
5.4.2.1	Experiments.....	101
5.4.2.2	Statistical analyses.....	101
5.4.3	Results.....	102
5.4.4	Discussion.....	104
5.5	Effect of Host Stage on Reproductive Potential in <i>E. warrae</i>	106
5.5.1	Introduction.....	106
5.5.2	Materials and methods.....	107
5.5.2.1	Effect of host stage on adult fitness.....	107
5.5.2.2	Effect of host stage on offspring fitness.....	107
5.5.2.3	Statistical analyses.....	108
5.5.3	Results.....	108
5.5.3.1	Effect of host stage on adult fitness in <i>E. warrae</i>	108
5.5.3.2	Effect of host stage on offspring fitness in <i>E. warrae</i>	110
5.5.4	Discussion.....	111

Chapter 6	General Discussion and Conclusion	113
6.1	Introduction.....	113
6.2	General biology.....	113
6.3	Host feeding and oviposition behaviour.....	115
6.4	Reproductive fitness in <i>E. warrae</i> in relation to different factors.....	116
6.5	Conclusion.....	117
References		118

APPENDIX: Published Papers from PhD Study

- | | | |
|----------|--|-----|
| 1 | Hanan A, He XZ, Shakeel M, Q Wang 2009. Diurnal Rhythms of Emergence, Host Feeding and Oviposition of <i>Eretmocer</i> | 141 |
| | warrae (Hymenoptera: Aphelinidae). New Zealand Plant Protection 62: 156-160. | |
| 2 | Hanan A, He XZ, Shakeel M, Q Wang 2010. Effect of food supply on reproductive potential of <i>Eretmocer</i> | 146 |
| | warrae (Hymenoptera: Aphelinidae). New Zealand Plant Protection 63: 113-117. | |
| 3 | Hanan A, He XZ, Q Wang 2012. Host feeding and oviposition strategy of <i>Eretmocer</i> | 151 |
| | warrae (Hymenoptera: Aphelinidae) under different host densities. New Zealand Plant Protection 65: 80-85. | |

List of Tables

Table 1.1	The host plants of <i>T. vaporariorum</i>	3
Table 2.1	Parasitoid species of <i>T. vaporariorum</i>	12
Table 3.1	Parasitoid mortality (%) with respect to host stage parasitised	48
Table 4.1	Oviposition and host feeding behaviour of <i>E. warrae</i> in response to different stages of <i>T. vaporariorum</i> in choice test	60
Table 4.2	Oviposition and host feeding behaviour of <i>E. warrae</i> on different stages of <i>T. vaporariorum</i> in the non-choice test	62
Table 4.3	Time allocation (%) of <i>E. warrae</i> on different behaviour on different stages of <i>T. vaporariorum</i> in the non-choice test	63
Table 4.4	Six treatments used in the experiment	70
Table 4.5	Superparasitism (%) of <i>E. warrae</i> at different host densities	71
Table 5.1	Effect of host and/or honey solution on lifetime host feeding and reproduction in <i>E. warrae</i>	104
Table 5.2	Effect of host stage on parasitoid progeny fitness	111

List of Figures

Figure 2.1	Life cycle of <i>E. warrae</i> : (a) egg, (b) first instar larva, (c) second instar larva, (d) third instar larva, (e) Pupa, and (f) adult. All pictures were taken in the present study	10
Figure 3.1	Breeding cage for <i>T. vaparariorum</i>	25
Figure 3.2	Breeding cages for <i>E. warrae</i>	25
Figure 3.3	System used for behaviour observation and recording	26
Figure 3.4	Emergence of <i>E. warrae</i> in the photophase. Columns with the same letters are not significantly different ($P > 0.05$)	31
Figure 3.5	The number of eggs laid and hosts fed on by <i>E. warrae</i> throughout the photophase and scotophase. Means (\pm SE) followed by the same English letters within the oviposition line and the same Greek letters within the host feeding line are not significantly different ($P > 0.05$). Data from the photophase and scotophase were analysed separately	32
Figure 3.6	Effect of temperature on the total number of eggs laid (A), hosts parasitised (B), hosts fed on (C), average daily parasitism rate (D), host feeding rate (E), and longevity (F) in <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$)	37
Figure 3.7	Effect of temperature on survival rate (A), emergence rate (B), and developmental time (C) in <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$)	39
Figure 3.8	Effect of temperature on pre-oviposition period in <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$)	40
Figure 3.9	Survival of the parasitoid first instar larvae after hatching. Columns with the same letters are not significantly different ($P > 0.05$)	48

Figure 3.10	Mean period between oviposition and penetration in <i>E. warrae</i> under different stages of hosts. Columns with the same letters are not significantly different ($P > 0.05$)	49
Figure 3.11	Host arrest caused by <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$)	50
Figure 3.12	Oviposition of <i>E. warrae</i> under the different stages of the fourth instar nymphs in choice (A) and non-choice test (B). Columns with the same letters are not significantly different ($P > 0.05$)	51
Figure 4.1	Mean duration of antennation and circling (A), probing for oviposition (B), probing for feeding (C), and total time spent on each instar of whiteflies (D) in the choice test of <i>E. warrae</i> behaviour. Columns with the same letters are not significantly different ($P > 0.05$)	61
Figure 4.2	Mean duration of antennation/circling (A), probing for oviposition (B), probing for host feeding (C) and total time spent on each instar (D) by <i>E. warrae</i> in the non-choice test. Columns with the same letters are not significantly different ($P > 0.05$)	65
Figure 4.3	Rejection rate (A), number of encounters (B) and total time spent (C) by <i>E. warrae</i> in the first and second oviposition at the density of 10 hosts after 1 and 24 hours of oviposition. SEP = same experienced parasitoid; NP = naïve parasitoid; DEP = different experienced parasitoid. Columns with the same letters in each category are not significantly different ($P > 0.05$)	73
Figure 4.4	Effect of host and food deprivation on the total number of eggs laid (A), hosts parasitised (B), hosts fed (C), and longevity (D) in <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$). H* = 10% honey feeding for 24 hours	79

Figure 4.5	Effect of host and food deprivation on the average daily rates of host feeding (A) and parasitism (B) in <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$). $H^* = 10\%$ honey feeding for 24 hours	80
Figure 4.6	Effect of host and food deprivation and 24 hours honey feeding on egg load in <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$). $H^* = 10\%$ honey feeding for 24 hours	81
Figure 4.7	Effect of honey feeding on egg resorption in <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$)	81
Figure 5.1	Effect of host density on the number of eggs laid (A), number of hosts parasitised (B), number of hosts fed (C), and longevity (D) in <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$)	89
Figure 5.2	Effect of host density on host feeding (A), superparasitism (B) and parasitism rates (C) in <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$)	90
Figure 5.3	Effect of host and parasitoid density on (A) host feeding = $\exp(0.3238.9817 + 0.0109 \text{ HD} + 0.6342 \text{ PD} - 0.0623 \text{ PD}^2)$; (B) parasitism = $\exp(0.8948 + 0.0166 \text{ HD} + 0.3744 \text{ HD} - 0.0339 \text{ PD}^2)$; (C) fecundity = $\exp(0.9474 + 0.0140 \text{ HD} + 0.5410 \text{ PD} - 0.05403 \text{ PD}^2)$; and (D) number of emerged offspring = $\exp(-1.4487 + 0.0795 \text{ HD} - 0.0005 \text{ HD}^2 + 0.5036 \text{ PD} - 0.0576 \text{ PD}^2)$. HD = host density, PD = parasitoid density	95
Figure 5.4	Effect of host and parasitoid density on (A) host feeding rate = $\exp(2.6937 - 0.0032 \text{ HD} + 0.3361 \text{ PD} - 0.0329 \text{ PD}^2)$ and (B) superparasitism rate = $\exp(-3.1949 - 0.0068 \text{ HD} + 3.7262 \text{ PD} - 0.4557 \text{ PD}^2)$	96

Figure 5.5	Effect of host and parasitoid density on (A) development time = $\exp (3.2116 - 0.0005 \text{ HD} + 0.0132 \text{ PD})$; (B) body size = $\exp (-0.4072 + 0.0012 \text{ HD} - 0.0348 \text{ PD})$; (C) egg load = $\exp (3.2162 + 0.0052 \text{ HD} - 0.1128 \text{ PD})$; and (D) longevity of emerged offspring = $\exp (2.0439 + 0.0055 \text{ HD} - 0.3036 \text{ PD} + 0.0335 \text{ PD}^2)$	97
Figure 5.6	Effect of food supply on the longevity of <i>E. warrae</i>	102
Figure 5.7	Daily number of hosts fed (a) and eggs laid (b) by <i>E. warrae</i> when provided with hosts only and honey + hosts. All data were pooled for regression but means (\pm SE) were presented	103
Figure 5.8	Effect of host stage on number of hosts fed (A), number of eggs laid (B), and longevity (C) in <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$)	109
Figure 5.9	Effect of host stage on host feeding rate (A) and parasitism rate (B) in <i>E. warrae</i> . Columns with the same letters are not significantly different ($P > 0.05$)	110