Trophic and Competitive Interactions among Egg Parasitoids of Stink Bugs

Joanna K. Konopka
The University of Western Ontario

Supervisor
McNeil, Jeremy N
The University of Western Ontario Co-Supervisor
Gariepy, Tara D
The University of Western Ontario

Graduate Program in Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy
© Joanna K. Konopka 2018

Follow this and additional works at: https://ir.lib.uwo.ca/etd

Recommended Citation
https://ir.lib.uwo.ca/etd/5598

This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact wlsadmin@uwo.ca.
Abstract

The intra- and inter-trophic interactions in ecosystems can be disrupted by invasive species, with lasting effects on population dynamics of native organisms. An invasive species may be attractive as a prey or host to native species, but if unsuitable for consumption or for development of the natural enemy’s progeny, it constitutes an ‘evolutionary trap’. A possibility of such a trap for native egg parasitoids (Hymenoptera: Scelionidae) exists with the introduction of the exotic brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). The objective of this thesis is to enhance the understanding of Pentatomidae-Scelionidae host-parasitoid interactions from a behavioural ecology perspective, focusing on factors influencing successful host use by egg parasitoids associated with stink bugs. Behavioural, molecular, and imaging methodologies were employed to elucidate trophic and competitive interactions of native egg parasitoids with invasive host, *H. halys*, and with an interspecific competitor, the exotic *Trissolcus japonicus* (Hymenoptera: Scelionidae) egg parasitoid. First, sentinel egg masses were exposed under natural conditions, followed by parasitism quantification, using both traditional (parasitoid emergence) and molecular analysis. Second, the outcomes of competitive interactions between exotic and native egg parasitoids using *H. halys* as host were characterized and the possibility of their coexistence assessed. Third, an L9 orthogonal array (OA) design was used to rank the influence of host egg mass parameters on parasitoid behaviour and development. Finally, the temporal pattern of parasitoid development in *H. halys* was examined using a DNA-based approach (PCR) and visualized by X-ray micro-computed tomography (micro-CT).
The key finding of this thesis is that the lack of success in *H. halys* eggs is linked to native parasitoids’ inability to develop in this host (not the rejection of the host). This failure occurs early in the parasitoid development, confirming the evolutionary trap potential of *H. halys*. The maladaptive decision to oviposit in an unsuitable host is caused by the mismatch between cues females use for host recognition and acceptance, and the expected normal development of the progeny. For some native parasitoids, the impact of the evolutionary trap can be reduced via facultative hyperparasitism of exotic parasitoids; for others, the co-existence is possible due to counterbalance competition.

Keywords

host-parasitoid interactions, trophic interactions, competition, host acceptance behaviour, brown marmorated stink bug, egg parasitoids
Co-Authorship Statement

Dr. Jeremy N. McNeil and Dr. Tara D. Gariepy will be co-authors of all manuscripts published from the contents of this thesis as they co-designed the experiments and edited all the manuscripts.

Dr. Tim Haye is a co-author on the manuscripts published from the data presented in Chapters 2, 3 and 4. Part of the data collection for the work described in Chapter 2, and all experiments described in Chapters 3 and 4 were completed in collaboration with Dr. Haye in his laboratory in CABI Switzerland. Dr. Haye assisted in experimental designs and edited the manuscripts presented in those chapters.

Dr. Jinping Zhang will be a co-author on the manuscript published from Chapter 2 as she supervised and carried out sentinel egg mass exposures and parasitoid identification in China. Dr. Ben Rubin will also be a co-author on the manuscript published from Chapter 2, as he assisted in data analysis using GLMM and provided feedback on the write-up.

Dr. Peter Mason and Dr. David Gillespie are co-authors on the manuscript published from the data presented in Chapters 4, as they provided feedback and recommendations for the manuscript.

Dr. Danny Poinapen will be a co-author on manuscripts published from the data presented in Chapters 5 and 6, as he assisted with experimental design and edited both chapters. He also assisted in orthogonal array design and analysis (Chapter 5), and completed the micro-computed tomography scanning (data acquisition) and reconstruction (Chapter 6).
Dr. David Holdsworth will be a co-author on a manuscript published from the data presented in Chapter 6, as he assisted in experimental design, and will contribute in the write up.
Acknowledgments

Among all people involved in various aspects of the work presented in this thesis, I would like to start by expressing my deepest gratitude to Dr. Jeremy McNeil for his supervision and guidance over the course of my doctoral degree. His immense knowledge in all aspects of entomology and ecology, which he is always willing to share, has always been of great value to me. Jeremy has always encouraged me to follow my research interests and develop my research ideas, and he never dictated the direction of my research or made decisions about methodologies that should be used. I had the total freedom to initiate and adopt the methods most appropriate for my experiments. By allowing me total independence in the type of work that I was doing and the way this work was done, Jeremy has ensured my progression in my academic and professional development as a confident scientist, passionate about the research I am doing. At the same time, he was always available for discussion of ideas, providing feedback and suggestions in a way that respected me and my work as his equal, not only as his student. In addition to conversations about my research, Jeremy has shared with me his advice on navigating academia while maintaining high standard of scientific integrity, especially in publishing and reviewing practices. I also really appreciate his encouragement and support in activities not directly related to my thesis (including peer reviewing, involvement in professional societies, graduate business courses that I did not need for my degree, and carrying out collaborative research not part of my PhD). While those activities sometimes meant less time spent on my thesis, they undoubtedly allowed me to contribute significantly to the discipline, and to improve my leadership and innovation skills. Although Jeremy holds his students to a very
high standard, he never fails to give credit and high praise to those that reach it, and I was humbled to discover the high regard and confidence he has in me. Jeremy’s approach to research and life has taught me about the importance of quality over quantity, and about the joy of following leads towards exciting scientific and personal discoveries. While I am ready to follow my own research and career goals, Jeremy will always be a person from whom I know I can seek advice and honest opinion, and with whom I will be happy to share my progress and future successes.

Next, I would like to thank my co-supervisor, Dr. Tara Gariepy, in whose laboratory I have completed most of the work presented in this thesis. Tara’s knowledge of molecular diagnostic tools and biological control has provided me with new understanding of host-parasitoid interactions. While in her laboratory, I was able to improve existing and gain new molecular skills, which will undoubtedly be an asset for me going forward. She has also entrusted me with supervising summer students, allowing me to improve my teaching and communication skills, while being mindful of interpersonal differences in skills and opinions. Seeing the importance of good organization and workflow in Tara’s molecular laboratory has motivated to me stay organized and pay attention to details in my work. At the same time, I have observed what is involved in running a laboratory, and how being a government research scientist differs from working in a university setting. I now have a better understanding of what is involved in managing a large international research program, and the importance of developing fruitful collaborations. I am very grateful to Tara for allowing me an opportunity to meet and work with her collaborators, especially with Dr. Tim Haye in Switzerland. Tara’s support and encouragement in traveling and completing part of my work in Switzerland meant a lot to me, and allowed me not only to
excel in my research, but also to understand how personal relationships are shaped by our professional choices. I hope that going forward I will be able to use what I have learned from Tara, and apply it effectively in my own career.

The person that I want to thank next is Dr. Tim Haye. He made my stay in Switzerland productive and memorable. From the first day I arrived in Switzerland for my 6 month stay, Tim was open to my research ideas, and provided me with all the support I needed by engaging in insightful conversations and giving helpful advice. Tim also spent time helping me run experiments, both in the laboratory (including the ‘solitary confinement’ of the CABI insect quarantine) and the field, to ensure I could complete my ambitious plans. I also want to thank Tim for providing me with opportunities to serve as a reviewer, and attend an international conference in Spain, both of which were valuable experiences. Working with Tim, I’ve observed and was inspired by his genuine excitement and passion about insects, and hobbies that he still found time for. All this with an incredible sense of humor.

Although I have not had an opportunity to meet either of them in person yet, I am thankful to Dr. Jinping Zhang and her students, especially Ting Cao, for collecting and preserving samples for me in China. Having those samples was very important and allowed me to complete a more comprehensive analysis. My confidence in the data analysis used in most of the chapters of this thesis was possible through the advice and assistance from Dr. Ben Rubin, and I want to thank him for his patience and expertise in statistical analysis of ecological data. I also want to thank Elijah Talamas (Smithsonian Institution, Washington, DC, USA), Lucian Fusu (University of Iasi, Romania), and Marie-Claude Bon (USDA-ARS European Biological Control Laboratory, Montferrier sur Lez, France)
for taxonomic and molecular identification of parasitoids collected to start laboratory colonies used in some of the experiments presented in this thesis. Furthermore, I want to thank Dr. David Holdsworth for allowing me access to his laboratory and equipment. His interest and encouragement in novel methods of insect imaging has led to a fruitful collaboration that extends beyond the contents of this thesis. Thanks to his support we were able to publish insect live imaging method that has generated worldwide interest and coverage.

I would like to thank my advisory committee Dr. Cam Donly, Dr. Nusha Keyghobadi, and Dr. Danielle Way, who provided advice and feedback in all aspects of the experiments that are part of this thesis. Their suggestions allowed me to improve my work and the way it is presented. Also, I extend my gratitude to my comprehensive examination committee for pushing me into learning about aspects of trophic dynamics (Dr. Hugh Henry), molecular ecology (Dr. Nusha Keyghodabi), and phylogenetics (Dr. M. Andre Lachance), which undoubtedly expanded my knowledge and made me more aware of the interconnectedness of all research.

A special thanks to Allison Bruin, who ensured that all the insect colonies at AAFC were always fed and well taken care of, and for always answering my questions and helping me find solutions to all kinds of problems. She is truly the kindest and most helpful laboratory technician I have ever had the pleasure of working with. I also want to thank Felix Longpre for bringing a positive attitude with a good sense of humour, and for allowing me to maintain my own parasitoid rearing after he took over as a Biologist at the centre.
I am grateful to my field assistants in Canada for their help with set up and data collection: Wesley Burbridge, Dominic Drapeau, with special thanks to Holly Deacon and Cole Drake. I also want to thank my field and lab assistants in Switzerland for being great company in quarantine, and not complaining for having to watch parasitoid behavior most of the day: Rebeca Hagedorn, Anne Hayle, and Sophie Deluca. I of course would not forget to thank the “CABI family” of summer 2015, who nicked-named me ‘quarantine queen’, with special thanks to Judith Stahl, Pierre Girod and Tessa Ramburn for their friendship and serious, as well as not so serious conversations.

I also want to acknowledge and thank administrative staff of the Biology Department at Western University, especially Arzie Chant, Carol Curtis, and Diane Gauley for always being helpful, kind, and understanding of graduate student needs and the challenges they face. I would further like to acknowledge Biotron staff, especially Karen Nygard and Dr. Richard Gardiner for providing assistance, advice, and training in sample preparation for imaging, and critical point dryer use. I am also grateful to the engineering staff at AAFC (with special thanks to John Vantyghem) for keeping the experimental room and equipment running, and making adjustments as per my request.

I would like to acknowledge that I was able to focus on my project fully thanks to scholarships from Natural Sciences and Engineering Council of Canada (NSERC), Ontario Graduate Scholarship (OGS), and financial support from University of Western Ontario for the duration of my degree. I am also grateful to the Entomological Society of Canada for awarding me a Graduate Research Travel Scholarship that enabled me to travel to Switzerland, and to the Entomological Society of America for a Student and Early Professional Travel Scholarship that allowed me to travel and present some of my findings.
at the International Congress of Entomology in 2016 in Orlando, USA. I would also like to acknowledge the Western Libraries for naming me a recipient of the Western Open Access Fund that allowed me to publish one of my chapters as ‘Open Access’, making my research more accessible.

Thank you to my family for learning the difference between bugs and insects to end my frustration of having to correct them, which is ironic since during my PhD research, I actually did work on bugs. My mom’s best intentions to understand what I actually do and why, were often sidetracked, which always made me laugh and try to think of better ways to explain my work. I also want to thank my Mauritian family, for the love and affection they bestowed upon me, and the kindness with which they have welcomed me to their home and their lives.

Finally, I want to express my deepest gratitude and appreciation to Dr. Danny Poinapen—without him this thesis would not be possible. Danny is my biggest supporter and my harshest critic, with his dual role as my research collaborator and life partner. Danny has always encouraged and pushed me to aim for competence and excellence, and to go beyond what is required of me, and offered constant encouragement, support, and guidance. Talking with Danny about my ideas, I was challenged to reflect on thought-provoking questions, and to consider many aspects of my research from a different perspective. I admire Danny’s professional yet relaxed research approach, evident in the commitment and care with which he conducts experiments, while at the same time always being open to helping others with their research. Danny holds me accountable to a high standard of scientific work and writing, so thanks to his insightful comments and constructive criticism, I was able to improve my study designs and learn how to tell a
scientific story in an exciting way. He has been an incredible source of information about scientific etiquette, practices, and communication, always offering advice and guidance. Danny has taught me many strategies for effective writing and preparing presentations, allowing me to excel in both. His help with orthogonal array and micro-CT imaging has been invaluable, with countless hours spent making sure that the data I was getting were of highest possible quality. Danny is also the person who knows my thesis as well as I do, thanks to his help in editing and proofreading, even if it meant spending his free time doing it. Simultaneously, Danny showed care and forethought in encouraging me to take courses and participate in workshops that could assist me in professional development, and is unrivaled in his excitement for my future. I am extremely grateful to him for his understanding in me spending time in Switzerland, no matter how hard it was for both of us. While I was away, Danny was the person whom I knew I could count on for encouragement and comfort when I needed it the most. Danny has always been there for me, and I am so fortunate to have him in my life, both professionally and personally. Together, we engage in insightful discussions and follow our scientific curiosities as far as they take us. Danny is an amazing scientist and partner and I look forward to continue exploring the unexplored with him.
# Table of Contents

Abstract.............................................................................................................................................. i

Co-Authorship Statement.................................................................................................................. iii

Acknowledgments............................................................................................................................... v

Table of Contents ............................................................................................................................ xii

List of Tables ..................................................................................................................................... xvii

List of Figures .................................................................................................................................... xix

List of Appendices ............................................................................................................................ xxiii

List of Abbreviations ......................................................................................................................... xxiv

**Chapter 1** ...................................................................................................................................... 1

1. General introduction ...................................................................................................................... 1

1.1 Animal trophic strategies ......................................................................................................... 2

1.2 Parasitism in insects .................................................................................................................. 4

1.3 Host- parasitoid interactions in Hymenoptera ........................................................................ 5

1.4 Scelionidae: egg parasitoids ................................................................................................... 11

1.5 Host-parasitoid interactions and ecological roles of parasitoids ........................................ 13

1.6 Invasive species introductions ................................................................................................. 15

1.7 Brown marmorated stink bug: an evolutionary trap for Scelionidae .................................. 16

1.8 Competitive interactions between egg parasitoids .................................................................. 19

1.9 Objectives .................................................................................................................................. 20

1.10 Thesis outline ........................................................................................................................... 22

1.11 References ................................................................................................................................ 24

**Chapter 2** .................................................................................................................................... 35

Exploitation of native and exotic pentatomids by native egg parasitoids in the introduced range of *Halyomorpha halys*: a molecular approach using sentinel egg masses

2.1 Introduction.................................................................................................................. 35

2.2 Materials and Methods............................................................................................... 39

2.2.1 Field sites.................................................................................................................. 39

2.2.2 Insect colonies.......................................................................................................... 39

2.2.3 Sentinel egg mass preparation, field exposure and collection ......................... 41

2.2.4 DNA extraction and amplification........................................................................ 43

2.2.5 DNA sequencing...................................................................................................... 45

2.2.6 Statistical analysis.................................................................................................. 45

2.3 Results ........................................................................................................................ 48

2.3.1 Parasitism in reared sentinel egg masses ......................................................... 48

2.3.2 Parasitism based on molecular analysis.............................................................. 51

2.3.3 Parasitoid identification........................................................................................ 54

2.4 Discussion.................................................................................................................... 59

2.5 References.................................................................................................................. 65

Chapter 3......................................................................................................................... 71

Possible co-existence of native and exotic parasitoids and their impact on control of *Halyomorpha halys*

3.1 Introduction................................................................................................................... 71

3.2 Materials and Methods............................................................................................... 74

3.2.1 Insect Rearing......................................................................................................... 74

3.2.2 Larval Competition................................................................................................. 74

3.2.3 Adult competition.................................................................................................. 75

3.2.4 Statistical analysis................................................................................................. 76
3.3 Results .................................................................................................................. 77
   3.3.1 Host acceptance and larval competition ......................................................... 77
   3.3.2 Adult competition ......................................................................................... 80
3.4 Discussion .......................................................................................................... 81
3.5 References ......................................................................................................... 85
Chapter 4 ................................................................................................................. 89
   An exotic parasitoid provides an invasional lifeline for native parasitoids ......... 89
   4.1 Introduction .................................................................................................... 90
   4.2 Materials and Methods ................................................................................ 92
      4.2.1 Stink bug rearing .................................................................................. 92
      4.2.2 Parasitoid rearing ............................................................................... 92
      4.2.3 Time-course developmental study ...................................................... 93
      4.2.4 Egg mass (patch) guarding behavior ............................................... 94
      4.2.5 Statistical analysis ............................................................................ 94
   4.3 Results .......................................................................................................... 95
      4.3.1 Host acceptance behavior ................................................................. 95
      4.3.2 Developmental success and interspecific larval competition .......... 95
      4.3.3 Egg mass guarding behavior ............................................................... 98
   4.4 Discussion .................................................................................................... 99
   4.5 References .................................................................................................. 104
Chapter 5 ............................................................................................................ 108
   Understanding the mismatch between behaviour and development in a novel host-parasitoid association ............................................................. 108
   5.1 Introduction .................................................................................................. 109
   5.2 Materials and Methods .............................................................................. 112
5.2.1 Insect colonies ................................................................. 112
5.2.2 Orthogonal array .............................................................. 113
5.2.3 Experimental set up ......................................................... 117
5.2.4 Statistical analysis ............................................................. 118
5.3 Results .................................................................................. 119
5.3.1 Behaviour ......................................................................... 119
5.3.2 Development ..................................................................... 123
5.4 Discussion ............................................................................ 126
5.5 References ........................................................................... 134

Chapter 6 .................................................................................. 141

Timing of failed parasitoid development in *Halyomorpha halys* eggs ....... 141

6.1 Introduction .......................................................................... 142
6.2 Materials and Methods .......................................................... 144
  6.2.1 Egg mass parasitization and processing .................................. 144
  6.2.2 DNA extraction and amplification ........................................ 145
  6.2.3 Sample preparation for micro-CT data acquisition ..................... 146
  6.2.4 Image reconstruction and 3D rendering .................................... 147
  6.2.5 Statistical analysis .................................................................. 147
6.3 Results .................................................................................. 147
  6.3.1 DNA analysis ....................................................................... 147
  6.3.2 Micro-CT 3D reconstruction of development ............................ 148
6.4 Discussion ............................................................................ 155
6.5 References ........................................................................... 160

Chapter 7 .................................................................................. 166

General discussion ....................................................................... 166
7.1 Ecological interactions

7.2 Towards enhanced understanding of Pentatomidae-Scelionidae host parasitoid interactions: summary of results

7.3 General discussion

7.4 Limitations and future work

7.5 Conclusion

7.6 References

Appendices

Curriculum Vitae
List of Tables

Table 1.1 Selected hymenopteran parasitoid families (and members) representing several common parasitoid life history strategies. .................................................................10

Table 2.1 Geographic coordinates and characteristics of natural, agricultural, and urban field sites in China, Canada, and Switzerland used for exposure of native and exotic (*H. halys*) stink bugs (Pentatomidae: Hemiptera) sentinel egg masses in 2014 and 2015 field seasons. ...........................................................................................................40

Table 2.2 Odds ratio and 95% confidence intervals of the fixed effects in the generalized linear mixed effects model (GLMM) characterizing the effect of date, country, type of host, type of site, and interaction between date and country on the presence of parasitism in sentinel stink bug egg masses. ........................................................................................................52

Table 2.3 Odds ratio and 95% confidence intervals of the fixed effects in the generalized linear mixed effects model (GLMM) characterizing the effect of date, country, type of host, type of site, and interaction between date and country on the proportion of parasitized eggs in sentinel stink bug egg masses. ........................................................................................................53

Table 5.1 L9 standard orthogonal array indicating combination of factors (4 factors each with 3 levels) and experiments (9 independent ones) to be performed. .........................114

Table 5.2 Factors (and their respective levels) for the no-choice tests of *Trissolcus euschisti* (Hymenoptera: Scelionidae) host acceptance and development on stink bug (Hemiptera: Pentatomidae) egg masses. ........................................................................................................114

Table 5.3 An L9 orthogonal design with factor combination for the no-choice tests of *Trissolcus euschisti* (Hymenoptera: Scelionidae) host acceptance and development on stink bug (Hemiptera: Pentatomidae) egg masses. ........................................................................................................115
**Table 5.4** Ranking of factor based on influence on *T. euschisti* egg parasitoid host acceptance, patch residence, patch exploitation, and development from stink bug host egg masses. ...........................................................................................................................................121

**Table 5.5** Summary of decision making of foraging egg parasitoid (*Trissolcus euschisti*) on stink bug host egg masses based on the ranking of critical factors. ........................................128
List of Figures

Figure 1.1 Schematic diagram of dichotomies used to determine which of the major three trophic strategies an animal belongs to, highlighting the parasitoid strategy.................................3

Figure 1.2 The approximate relative percentage of parasitoids belonging to different insect orders..................................................................................................................................................5

Figure 1.3 Cladogram depicting the evolution of parasitism (indicated by a grey circle) in Hymenoptera..................................................................................................................................................6

Figure 1.4 Schematic of a general host searching and acceptance behaviours of a foraging parasitoid female, with selected factors that may influence these behaviours. ....................8

Figure 1.5 A general schematic diagram of a simple host-parasitoid interaction between two parasitoids and two herbivorous host species. .................................................................................................14

Figure 1.6 Brown marmorated stink bug, Halyomorpha halys a) adult b) egg mass and c) current distribution in Canada and USA (as of September 2017). .................................................................17

Figure 1.7 A Trissolcus cultratus female on Halyomorpha halys egg mass.. ..................19

Figure 1.8 Possible interspecific interactions between exotic Trissolcus japonicus and European native egg parasitoids (here Anastatus bifasciatus is used as an example) on egg masses of exotic stink bug, Halyomorpha halys.......................................................................................................................21

Figure 2.1 Mean (±SE) egg mass parasitism detected using rearing and molecular methods in sentinel egg masses of a) stink bugs native to each area and b) introduced H. halys exposed in native (China) and introduced (Canada and Switzerland) ranges of H. halys over the 2014 and 2015 field seasons. ..................................................................................................................49

Figure 2.2 Mean (±SE) egg parasitism detected using rearing and molecular methods in sentinel egg masses of a) stink bugs native to each area and b) introduced H. halys exposed in native (China) and introduced (Canada and Switzerland) ranges of H. halys over the 2014 and 2015 field seasons. ..................................................................................................................50
Figure 2.3 Parasitoid adults recovered from reared sentinel egg masses of stink bugs native to each area and of *H. halys* exposed in a) Canada b) Switzerland and c) China over the 2014 and 2015 field seasons. Sample size (n) refers to the number of emerged parasitoids across all sites within each country .......................................................... 55

Figure 2.4 Parasitoid adults or DNA recovered from sentinel egg masses exposed in a natural field site in Canada (LORDC-forest) using a) rearing (identified via DNA extraction and analysis) and b) molecular methods ............................................................. 57

Figure 2.5 Parasitoid adults or DNA recovered from a natural field site in Switzerland (Délemont) using a) rearing (identified via DNA extraction and analysis) and b) molecular methods ........................................................................................................ 58

Figure 3.1 Mean proportion (± SE) of previously parasitized *H. halys* eggs attacked by a) *A. bifasciatus* and b) *T. japonicus*. .......................................................................................................................... 78

Figure 3.2 Mean proportion of *H. halys* eggs giving rise to *T. japonicus*, *A. bifasciatus*, *H. halys* nymphs, or nothing, following multiparasitism by *T. japonicus* and *A. bifasciatus* at different time intervals (ages) between attacks: (a) parasitized by *T. japonicus* first (b) parasitized by *A. bifasciatus* first .................................................................................................................. 79

Figure 3.3 General schematic showing development outcomes from *H. halys* eggs parasitized by a) *T. japonicus* b) *A. bifasciatus* c) *T. japonicus* followed by *A. bifasciatus* and d) *A. bifasciatus* followed by *T. japonicus* ........................................................................................................ 84

Figure 4.1 Mean proportion (±SE) of (unparasitized) fresh or frozen *H. halys* egg masses, as well as fresh egg masses previously parasitized by *T. japonicus* which were a) drilled and b) marked by *T. cultratus* as a function of age/time since parasitized by *T. japonicus* .................................................................................................................. 96

Figure 4.2 Mean proportion of *T. japonicus*, *T. cultratus*, *H. halys* nymph or nothing emerging from different aged *H. halys* eggs that were a) fresh, b) frozen or c) multiparasitized by *T. japonicus* and *T. cultratus* at different time intervals (ages). ........ 97
**Figure 4.3** General schematic showing the temporal pattern of suitability of parasitized and unparasitized *H. halys* eggs for *T. cultratus* as a function of their age. ..........................101

**Figure 5.1** Proportion of host egg masses accepted by *T. euschisti* females in nine experiments based on L9 (3⁴) orthogonal array (OA) design.................................................120

**Figure 5.2** Mean (± SE) a) time spent on egg mass b) total time spent drilling, and c) time spent drilling per egg (for successful parasitization attempts) by *T. euschisti* females in nine experiments based on L9 (3⁴) orthogonal array (OA) design, summarizing behaviour of the parasitoid on host egg masses..............................................................................................................................122

**Figure 5.3** Mean (± SE) proportion of eggs drilled, marked, and superparasitized by *T. euschisti* females in nine experiments based on L9 (3⁴) orthogonal array (OA) design, summarizing behaviour of the parasitoid on host egg masses..............................................................................................................................124

**Figure 5.4** Mean proportion of *Trissolcus euschisti*, *Telenomus podisi*, host nymphs (*Podisus maculiventris*, *Halyomorpha halys*, or *Euschistus variolarious*) or nothing emerging from egg masses parasitoid by *T. euschisti* females in nine experiments based on L9 (3⁴) orthogonal array (OA) design, summarizing development of the parasitoid on host egg masses..............................................................................................................................125

**Figure 6.1** Temporal pattern of COI parasitoid DNA (ng/µL; mean ±SE) following parasitization by *Trissolcus euschisti* in fresh parasitized, frozen parasitized, and non-parasitized stink bug eggs of a) *Halyomorpha halys* and b) *Podisus maculiventris*......149

**Figure 6.2** Temporal pattern of COI host DNA (ng/µL; mean ±SE) following parasitization by *Trissolcus euschisti* in fresh, frozen, and non-parasitized stink bug eggs of a) *Halyomorpha halys* and b) *Podisus maculiventris*. .................................................................150

**Figure 6.3** 2D slices and 3D-CT volume reconstruction at 6 µm isotropic voxel spacing of non-parasitized eggs of *Podisus maculiventris* (PN) (a and b) and *Halyomorpha halys* (BN) (c and d) over 120 h of development. ..............................................................................................................................152
Figure 6.4 2D slices and 3D-CT volume reconstruction at 6 µm isotropic voxel spacing of parasitized frozen eggs of *Podisus maculiventris* (PZ) (a and b) and *Halyomorpha halys* (BZ) (c and d) over 120 h of development (from the time of parasitization). .................153

Figure 6.5 2D slices and 3D-CT volume reconstruction at 6 µm isotropic voxel spacing of parasitized fresh eggs of *Podisus maculiventris* (PF) (a and b) and *Halyomorpha halys* (BF) (c and d) over 120 h of development (from the time of parasitization). .........................154
List of Appendices

Appendix A  Field site locations and sentinel egg mass preparation and exposure ......180

Appendix B  Seasonal parasitism based on rearing and molecular analysis of sentinel egg masses from Canada, Switzerland, and China.................................................................186

Appendix C  Parasitoid development in fresh and frozen *P. maculiventris* eggs (7-11 days) and in fresh *H. halys* eggs at 120 h (5 days)..........................................................203
List of Abbreviations

2D- two dimensional
3D- three dimensional
ANOVA- analysis of variance
BIN- Barcode Index Number
BMSB- brown marmorated stink bug
BF- fresh parasitized eggs of *H. halys*
BN- normal (non-parasitized) eggs of *H. halys*
BZ- frozen parasitized eggs of *H. halys*
BOLD- Bar code of Life Data System
CI- confidence interval
COI- cytochrome oxidase I
CPD- critical point drying
DNA- deoxyribonucleic acid
GLMM- generalized linear mixed model
HU- Hounsfield units
IPM- integrated pest management
Micro-CT- micro-computed tomography
NA- North America
OA- orthogonal array
ON- Ontario
OR- odds ratio
PCR- polymerase chain reaction
PF- fresh parasitized eggs of *P. maculiventris*
PN- normal (non-parasitized) eggs of *P. maculiventris*
PZ- frozen parasitized eggs of *P. maculiventris*
RH- relative humidity
RFU- relative fluorescence unit
SE- standard error
S/N - signal to noise ratio
USA - United States of America
Chapter 1

General introduction
1.1 Animal trophic strategies

Within any given ecosystem, individuals of different species co-exist and the intra- and interspecific interactions (positive, negative, or neutral) ensure ecosystem stability (Smith and Smith 2009). The acquisition of nutritional resources is one of the most ubiquitous interactions and constitute the food webs within the ecosystem (Molles and Cahill 2008). The strategy organisms use to obtain energy for survival and reproduction will depend on their trophic position within the food chain (Chapin et al. 2002). Unlike plants, most animals are consumers as they cannot synthesize their own food and therefore, must feed directly or indirectly on other living organisms.

An organism is considered a natural enemy when it exploits another species to fulfill dietary and/or reproductive needs, and in doing so either reduces (i.e. kills) or reduces its fitness (Lafferty and Kurtis 2002). There are three general categories of natural enemies using different strategies: predators, parasites, and parasitoids (Figure 1.1), although they may be subdivided into as many as 10 categories (Lafferty and Kuris 2002; Poulin 2011). The classification is based on the number of victims the natural enemies attack and exploit (at a particular stage of their life history), whether that attack reduces or eliminates the fitness of the prey or host, and if this effect on host or prey is intensity-dependent (Lafferty and Kuris 2002).

The parasitic life history, including different types of parasites and parasitoids (Poulin and Randhawa 2015), has evolved independently multiple times across numerous phyla. This life history represents one of the most striking cases of convergent evolution (i.e. phenotypic similarity in phylogenetically unrelated organisms due to similar selection
pressures) (Poulin and Morand 2000; de Meeûs and Renaud 2002). Convergence of morphology, function, transmission modes, and body size in many distinct taxa have been achieved by following one of the few trajectories towards highest probability of survival and reproduction, equivalent to one of few adaptive peaks on the fitness landscape (Wright 1984; Poulin 2011).

Figure 1.1 Schematic diagram of dichotomies used to determine which of the major three trophic strategies an animal belongs to, highlighting the parasitoid strategy. Reading from left to right: predators (e.g. lion) and micropredators (e.g. mosquito) attack multiple prey, either killing and consuming the prey, or taking multiple small meals that do not significantly impact the prey’s fitness, respectively. Parasites (e.g. tapeworm) exploit a single host without killing it, but they may significantly reduce their host’s fitness; while parasitoids (e.g. parasitic nematode) kill a single host during the process of offspring development on or in that particular host (modified from Lafferty and Kuris 2002).
Parasitoids represent one of the stable adaptive peaks. This strategy is distinct from other parasitic ones because it always leads to host death (i.e. host fitness is zero), as opposed to reducing the fitness and keeping the host alive, as seen in typical parasites (Figure 1.1). Parasitoids are generally smaller than their hosts, as their growth is constrained by the hosts’ body size. Although organisms such as hairworms, nematodes and polychaetes are parasitoid animals, insects are by far the most numerous and well known group of parasitoids (Godfray 1994).

1.2 Parasitism in insects

Almost all insects with a parasitoid life history belong to the orders Coleoptera (e.g. ground, rove, and blister beetles), Diptera (e.g. tachinid flies), or Hymenoptera (e.g. wasps) (Fig. 1.2). Beetles and flies represent less than 20% of all insect parasitoids, but this life history strategy evolved multiple times (more than 100 times, in ~14 and 21 families, respectively) (Eggleton and Belshaw 1992; Quicke 1997). In contrast, the order Hymenoptera contains the majority (~80%) of insect parasitoid species, but with a single evolutionary origin of the parasitic life history (Eggleton and Belshaw 1992; Quicke 1997).

Sawflies and wood wasps, the basal lineages in Hymenoptera, are phytophagous (i.e. feed on plant material), and xylophagous/mycophagous (i.e. feed on wood and fungi), respectively (Grimaldi and Engel 2005). The parasitic life history in these basal lineages first appeared in Orrusidae wood wasps, with subsequent changes leading to the evolution of Aculeata (stinging wasps, bees, ants) and Parasitica (parasitoid wasps) (Whitfield 1998; Ronquist 1999; Grimaldi and Engel 2005; Sharkey 2007) (Fig. 1.3).
Although some groupings have been questioned, revised, or acknowledged to be faulty (Brothers 1975; Schulmeister et al. 2002; Sharanowski et al. 2010), the single origin of parasitism in Hymenoptera from xylophagy/mycophagy is supported by all morphological and molecular analyses.

### 1.3 Host-parasitoid interactions in Hymenoptera

Hymenoptera underwent considerable radiation following the evolution of parasitism, giving rise to a multitude of life history strategies. Many of those strategies
Figure 1.3 Cladogram depicting the evolution of parasitism (indicated by a grey circle) in Hymenoptera. A cladogram depicts tree topology only, and it does not contain information on evolutionary time, age of nodes, or rates of evolution. Outgroup (reference group used when determining the evolutionary relationships) indicates the direction of evolutionary change (i.e. from left to right). Group names (along the top) in bold font are considered monophyletic (consisting of all descendants of a common ancestor). Black and grey circles indicate some important character changes (i.e. modifications from the basal state) over evolutionary time (read from left to right along the bottom diagonal line). Not all groups, and not all character changes are included. Once a character change appears along the bottom line, it is present in all the groups to the right of that character change, unless otherwise indicated by character reversals. For example, parasitism is present in Orussidae, Aculeata, and Parasitica, but not in Phyllophaga, Xylophaga, or the outgroup. Haplodiploidy (sex-determination system where males develop from unfertilized egg and hence carry one set of genes making them haploid, and females develop from fertilized eggs, carrying two sets of genes, making them diploid) is a characteristic present in all Hymenoptera. Line colour indicates feeding strategy of each group: green = phytophagous (consumes plants); brown = xylophagous/mycophagous (consumes wood/fungi); yellow = predatory; and red = parasitic. All feeding strategies except xylophagy/mycophagy are represented in Aculeata (i.e. reversal to phytophagy and evolution of predation). Aculeata includes stinging wasps, bees, and ants. Modified from Grimaldi and Engel 2005; Sharkey 2007.
were dictated by the interaction with hosts. In parasitic Hymenoptera, the adults are free-living, while the larvae are parasitic and consume their host to complete development (Eggleton and Belshaw 1992).

The success of a parasitoid is largely dependent on the host-searching abilities of the adult female. She must first find a suitable habitat (habitat location), and then locate a suitable host (host location), through host recognition and host acceptance (Vinson 1998; Steidle and van Loon 2002). The cues used in these steps differ in their relative detectability (the ease of cue recognition) and reliability (actual indication of host presence), and are not constant in the environment. Therefore, females must be able to cope with high variability and complexity of odours while foraging (Vet and Dicke 1992; Hilker and McNeil 2008). Host searching and acceptance by parasitoid females are influenced by both intrinsic and extrinsic factors, such as the physiological state of the female (e.g. age, egg load, mating status, host search appetite), and environmental cues (Vinson 1976; Lewis et al. 1990; Vinson 1998; Fatouros et al. 2008).

Foraging parasitoid females locate a habitat and a host by using olfactory cues from the host (e.g. pheromones, excrement) or food plants of the host. Once in the vicinity of a potential host, visual, mechano-sensory, and gustatory cues provide additional information on its identity and suitability. The final decision whether to deposit eggs or not can depend on the information obtained via antennation of the host and internal cues obtained by ovipositor probing. These behaviours allow the female to determine important factors such as host age, or presence of competitors (Fig. 1.4).

Once a suitable host has been located, the females’ oviposition behaviour determines if their progeny will feed on the outside (ectoparasitoid), or the inside of the
Figure 1.4 Schematic of a general host searching and acceptance behaviours of a foraging parasitoid female, with selected factors that may influence these behaviours. The stages of host searching and selection are: habitat location, host location, host recognition, and host acceptance. The steps (and the sequence) involved in each stage are indicated by rectangles and the arrows that connect them. Blue ovals indicate factors that influence decision of foraging parasitoid females. Recognition and detection of cues indicative of suitable habitat and host are listed as a series of questions and specific decisions that females must make. Following eclosion (emergence) from the host, female might develop a preference for a host from which she developed (host imprinting). She will then make a decision to leave or remain in the habitat, and mate. Following host-associated cues, the female’s search appetite will be influenced by intrinsic (e.g. physiological state) and extrinsic (e.g. environment) factors. If the host is located, novel (not previously encountered) and known cues associated with the host will be used to determine if oviposition in or on that host should be attempted. Lastly, the final host acceptance and oviposition will be made by assessment of suitability and likelihood of successful larval development, which may include determination of the host age, host quality, and presence of adult or larval competitors. Modified after Vinson 1976; Vinson 1998; Fatouros et al. 2008.
host (endoparasitoid) (Askew and Shaw 1986; Quicke 1997). Furthermore, most parasitoid taxa have become specialized to exploit only a specific life stage of the host (i.e. egg, nymph, larva, pupa, or adult), either killing or paralyzing it during parasitization (idiobiont), or allowing further host development post-parasitization (koinobiont) (Quicke 1997; Harvey 2005) (Table 1.1).

The vulnerability of a host to parasitoid attack will in turn depend on how easily it can be located, the behavioural and morphological defences it employs, and its ability to eliminate the parasitoid via physiological reaction (e.g. encapsulation) once attacked (Gross 1993; Reed et al. 2007). To overcome such host defences, many parasitoid females inject venoms or other substances (e.g. polydnaviruses, Dufour’s gland secretions) during parasitization. These secretions can alter host behaviour, physiology, and metabolism, which increases the likelihood of successful parasitism, and create a more suitable environment for the larval parasitoid. For example, some of these substances can induce permanent paralysis, cause toxicity, up- or down-regulate metabolism, or arrest development (Vinson and Iwantsch 1980; Stoltz 1986; Fleming 1992; Doury et al. 1997; Nakamatsu and Tanaka 2003; Pennacchio and Strand 2006; Mitra 2013).

Once an egg/eggs has been deposited by the parasitoid female, the larva/larvae must utilize a finite and limited amount of resources to successfully complete development (Slansky 1986). The larvae are not, however, solely reliant on the factors injected by the female during oviposition to overcome the host defences. They can overcome the defensive reaction of the host by the production of protective coatings (Salt 1965), attrition of the host through continuous feeding (Salt 1968), larval salivary secretions (Doury et al. 1997), and release of teratocytes (Strand et al. 1988; Dahlman 1990; Dahlman 1991; Volkoff and
Table 1.1 Selected hymenopteran parasitoid families (and members) representing several common parasitoid life history strategies.

<table>
<thead>
<tr>
<th>Life history strategy</th>
<th>Hymenoptera families</th>
<th>Example species</th>
<th>Stage and host attacked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectoparasitoid</td>
<td>idioibont</td>
<td>Pteromalidae</td>
<td><em>Nasonia vitripennis</em>¹</td>
</tr>
<tr>
<td></td>
<td>Megaspilidae</td>
<td><em>Dendrocerus carpenteri</em>²</td>
<td>parasitoid prepupae/pupae inside aphids (e.g. <em>Aphidius ervi</em> parasitoid in <em>Acythosiphon pisum</em> primary host)</td>
</tr>
<tr>
<td></td>
<td>Ichneumonidae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ectoparasitoid</td>
<td>koinobiont</td>
<td>Eulopidae</td>
<td><em>Euplectrus comstockii</em>³</td>
</tr>
<tr>
<td></td>
<td>Ichneumonidae</td>
<td><em>Zatypota albicosa</em>⁴</td>
<td>adult spiders (e.g. <em>Achaearanea tepidariorum</em>)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endoparasitoid</td>
<td>idioibont</td>
<td>Trichogrammatidae</td>
<td><em>Trichogramma evanescens</em>⁵</td>
</tr>
<tr>
<td></td>
<td>Braconidae</td>
<td><em>Rhysipolis decorator</em>⁶</td>
<td>moths larvae (e.g. <em>Caloptilia sp.</em>)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endoparasitoid</td>
<td>koinobiont</td>
<td>Aphelinidae</td>
<td><em>Aphelinus mali</em>⁷</td>
</tr>
<tr>
<td></td>
<td>Eucolilidae</td>
<td><em>Leptopilina boulardi</em>⁸</td>
<td>fly larvae (e.g. <em>Drosophila simulans</em>)</td>
</tr>
</tbody>
</table>

Furthermore, parasitoids can be challenged by intra- or inter-specific competitors, both as an adult and during the larval stages (Cusumano et al. 2016). Adult females can compete directly for resources when they locate a host simultaneously, or when one has remained on a host following parasitization to defend it from potential competitors. The actual outcome of the adult competition will largely depend on fighting abilities and aggressiveness of the females, but it can be mediated by reproductive capacity, or parasitism efficiency (Hardy et al. 2013; Wang et al. 2015; Cusumano et al. 2016). When more than one female of the same species (superparasitism) or of different species (multiparasitism) oviposits onto/into the same host, the developing larvae must compete for the same limited resources. The winner of such competition will be determined by several factors including rate of development, host quality, as well as the order of and time interval between parasitization events (De Moraes et al. 1999; De Moraes and Mescher 2005; Irvin et al. 2006). In cases when several females oviposit into the same host, facultative hyperparasitism can occur. This process is the opportunistic utilization of a primary parasitoid as a host by another primary parasitoid (van Baaren et al. 1995; Boivin and Brodeur 2006).

1.4 Scelionidae: egg parasitoids

Among the parasitic Hymenoptera, wasps in the family Scelionidae are solitary idiobiont endoparasitoids (i.e. they develop inside the host while preventing its further development) of insect and spider eggs (Krombein et al. 1979; Jones 1988; Ehler 2002). Host searching and acceptance of scelionid egg parasitoids are well described (Bin et al.
1993; Quicke 1997; Vinson 1998; Fatouros et al. 2008). Since eggs are small, immobile, and inconspicuous in the environment, with no exploitable intense long-range odors (Hilker and Meiners 2002; Fatouros et al. 2008), foraging females use chemical cues from host plants (e.g. induced by herbivore feeding and oviposition), and cues left by the adult stage of their host (e.g. kairomones such as sex pheromones, aggregation pheromones, walking traces) (Colazza et al. 1999; Borges et al. 2003; Laumann et al. 2009; Mumm and Dicke 2010; Fatouros et al. 2012; Hilker and Fatouros 2015; Michereff et al. 2016; Ponzio et al. 2016). Once an egg or egg mass has been located and its suitability assessed by antennation and ovipositor probing (Bin et al. 1993; Mattiacci et al. 1993; Borges et al. 2003; Silva et al. 2006; Laumann et al. 2009; Michereff et al. 2016), the female lays an egg inside each individual host egg, using a hypodermic needle-like ovipositor (Austin 1983; Bin et al. 1993; Field and Austin 1994). Following oviposition, the female deposits a marking pheromone and she may remain on the egg mass to deter further oviposition by other con- or heterospecifics (Roitberg and Mangel 1988; Hofsvang 1990; Bin et al. 1993; Mattiacci et al. 1993; Colazza et al. 1996; Field and Calbert 1999).

Insect eggs are thought to lack immune response (at least at the early stages of development before hemocyte formation in the host) (Salt 1968; Godfray 1994; Strand and Pech 1995). Consequently, egg parasitoids do not need to inject venom to paralyze the host, and many Scelionidae lack venom glands (Strand et al. 1983; Rosi et al. 2001). It has however been suggested that female common oviduct secretions arrest development of the host (Strand et al. 1983; Strand et al. 1986; Rosi et al. 2001). The host is further weakened by the teratocytes produced by the immature parasitoid larva (Salt 1968; Dahlman 1990; Dahlman 1991; Volkoff and Colazza 1992; Cônsoli et al. 2001). Therefore, given the
relatively well described biology of scelionid egg parasitoids, and the ability to effectively control population dynamics of their hosts, they are ideal for studying host-parasitoid interactions.

1.5 Host-parasitoid interactions and ecological roles of parasitoids

Host-parasitoid interactions can be influenced by spatial patchiness, intra- and interspecific interactions, and metapopulation dynamics (Hassell 2000). Spatial and temporal variation in host attack by parasitoids and the variation in the outcome of the attack influence host population stability and spatial distribution of their mortality (Hassell 2000). Direct or indirect competition for resources between host or parasitoid species regulates population growth rates, which shapes community structure (Holt 1977; Godfray 1994). Finally, the dispersal ability and frequency of the host or their natural enemies can lead to a spatially separated local populations connected by movement of individuals (i.e. metapopulations) affecting population persistence. All of these interactions determine the existence and stability of diverse natural host-parasitoid communities (Fig. 1.5).

Scelionid egg parasitoids (including members of the genus *Telenomus* and *Trissolcus*) have high searching and dispersal capacities, the ability to cause high host mortality, and exhibit positive host density responsiveness (Orr 1988). These characteristics allow them to significantly impact the population dynamics of their hosts, including stink bugs (Hemiptera: Pentatomidae) (Jones 1988; Hoffmann and Davidson 1991; Ehler 2002). However, clearly identifying and characterizing the multitrophic interactions (i.e. across trophic levels including plants, herbivores, and their natural enemies) is difficult (Vet and Godfrey 2008). mortality, and exhibit positive host density
responsiveness (Orr 1988). These characteristics allow them to significantly impact the population dynamics of their hosts, including stink bugs (Hemiptera: Pentatomidae) (Jones 1988; Hoffmann and Davidson 1991; Ehler 2002). However, clearly identifying and characterizing the multитrophic interactions (i.e. across trophic levels including plants, herbivores, and their natural enemies) is difficult (Vet and Godfrey 2008).

Our understanding of the interspecific interactions in the Pentatomidae-Scelionidae host-parasitoid system is limited, at least in part due to the poor characterization of scelionid communities. Naturally occurring stink bug egg masses are hard to locate, and

**Figure 1.5** A general schematic diagram of a simple host-parasitoid interaction between two parasitoids and two herbivorous host species. Parasitoid species #1 preferentially attacks host species #1, and parasitoid species #2 preferentially attacks host species #2. Solid and dashed lines represent direct and indirect interactions, respectively. Line colours indicate type of interaction: solid black = direct competition; dashed black = indirect or apparent competition; solid blue = direct effect on host mortality through parasitism; dashed blue = indirect effect of host on parasitoid, or parasitoid on host (e.g. facilitation); and solid green = direct effect of host on parasitoid (e.g. defensive reaction causing parasitoid mortality).
conventional methods for estimating parasitism in naturally-occurring or sentinel (i.e. laboratory reared, field exposed) egg masses may underestimate the actual levels of parasitism (Jones et al. 2014; Cornelius, Dieckhoff, Hoelmer, et al. 2016). Furthermore, morphological identification of emerged parasitoid adults (obtained from reared host eggs) and the immature larval stages (obtained from dissected host eggs) can be challenging. Additionally, rearing and dissection data from field-exposed egg masses do not allow the detection of multi- or hyper-parasitism, or the identification of parasitoid species involved in such interactions under natural conditions. Understanding such interspecific interactions at the same (e.g. between parasitoid species) or different (e.g. between stinkbug hosts and their parasitoids) trophic levels is further complicated when invasive species are involved. Those introduced species (either accidental or intentional) can disrupt the stability of existing interactions, and hence the stability of the system.

1.6 Invasive species introductions

Invasive species can have positive or negative effects on native species at the same or different trophic levels. Depending on whether these interactions are positive or negative, the invasive species may increase or decrease the biodiversity of the native community, respectively (Simberloff 1981; Schmitz and Simberloff 1997; Elton 2000; Pimentel et al. 2000; Rodriguez 2006). Negative effects on native communities usually result from competition or predation, while positive ones occur in the form of facilitation or introduction of novel resources for the native species to exploit (Mooney and Cleland 2001; Rodriguez 2006; Martinson et al. 2013; Berthon 2015; Sugiura 2016).

The presence of an exotic species can benefit an indigenous natural enemy if it is a new exploitable resource. This introduced species serves as a ‘trophic subsidy’ that
increases the survival and reproduction of the native natural enemy (Rodriguez 2006). However, success (fitness) of the native species can be reduced if the introduced species produces cues normally indicative of an adaptive outcome (e.g. high reproductive success), which are no longer reliable. For example, if a native parasitoid female accepts an invasive species as a host, but her progeny fails to develop, then this host serves as an ‘evolutionary trap’ for the parasitoid (Schlaepfer et al. 2002; Schlaepfer et al. 2005; Berthon 2015). Potential of such an evolutionary trap for native scelionid parasitoids exists with the introduction of the invasive exotic brown marmorated stink bug (Abram et al. 2014; Haye et al. 2015).

1.7 Brown marmorated stink bug: an evolutionary trap for Scelionidae

The brown marmorated stink bug (BMSB) (*Halyomorpha halys* Stål) is native to East Asia, and has become established in North America, Europe, and more recently in Eurasia, and South America (Hoebeke and Carter 2003; Gariepy et al. 2013; Xu et al. 2013; Gapon 2016; Faúndez and Rider 2017; Valentin et al. 2017) (Fig. 1.6). This insect causes severe agricultural and nuisance problems as it is highly polyphagous and tends to aggregate in large numbers in man-made structures (Leskey et al. 2012; Rice et al. 2014). While life history, ecology, and management of *H. halys* have been well studied (Lee et al. 2013; Haye et al. 2014; Rice et al. 2014; Lee 2015), less attention has been given to behavioural and population ecology of native natural enemies (predators and parasitoids) attacking *H. halys* in North America and Europe. In fact, the main focus of research on parasitoids and predators attacking *H. halys* has been to determine their potential as biological control agent for this insect (i.e. characterizing and assessing *H. halys* mortality

While *H. halys* poses agricultural and nuisance problems in the introduced areas and it is important to determine effective control measures against this insect, its presence also provides an excellent opportunity to learn about interspecific interactions involving

**Figure 1.6** Brown marmorated stink bug, *Halyomorpha halys* a) adult b) egg mass and c) current distribution in Canada and USA (as of September 2017). *Halyomorpha halys* is native to East Asia (China, Japan, Korea, and Taiwan), and it was first detected in USA in Allentown, Pennsylvania in 1996. The occurrence of *H. halys* has been regularly reported since 2003 and 2012 in USA and Canada, respectively. Adult and egg mass images are not to scale (adult *H. halys* ~ 17 mm long; individual *H. halys* eggs ~ 1 mm in diameter. Photo credits: Tim Haye; map source: www.stopbmsb.org.
exotic and native species. Species-specific host-parasitoid associations between the Pentatomidae and the Scelionidae are poorly documented, and the interaction between exotic *H. halys* and native parasitoids in recently invaded areas is largely unknown, despite the increased attention it has received. Parasitism of *H. halys* egg masses under field conditions is reported to be very low in the USA (Cornelius, Dieckhoff, Vinyard, et al. 2016; Cornelius, Dieckhoff, Hoelmer, et al. 2016; Ogburn et al. 2016; Dieckhoff et al. 2017), but it is unclear if the lack of parasitism is due to the rejection of *H. halys* as a host by foraging female egg parasitoids, or due to the inability of larvae to develop in this host (i.e. evolutionary trap for the parasitoids). The possibility of an evolutionary trap is quite likely because, under laboratory conditions, females of native North American *Telenomus podisi* (Ashmead) and European *Trissolcus cultratus* (Mayr) species (Fig. 1.7) readily attack freshly laid BMSB egg masses, but their progeny seldom successfully complete development (Abram et al. 2014; Haye et al. 2015). Conversely, adaptations that permit these species to exploit the novel host, will allow them an escape from this mismatch.

This apparent mismatch between the behavioural acceptance and lack of development by scelionid egg parasitoids in the invasive *H. halys* currently lacks a mechanistic explanation. According to the preference-performance hypothesis, females should oviposit in hosts most suitable for their offspring’s development (Gripenberg et al. 2010). To understand why scelionid egg parasitoids, at least under laboratory conditions, deviate from predictions of this hypothesis, it is necessary to assess which cues foraging females use, and their relative importance in the host selection process. Furthermore, after the decision to oviposit in a host is made, it is unclear why the development of the parasitoid larva fails. Most importantly, it is unclear at which point following oviposition parasitoid
Figure 1.7 A *Trissolcus cultratus* female on *Halyomorpha halys* egg mass. Photo credit: Tim Haye.

larval development fails within the *H. halys* egg. Establishing this timeframe is crucial to understand the reason(s) or mechanisms behind failed development in the exotic *H. halys* host.

### 1.8 Competitive interactions between egg parasitoids

The establishment of one exotic species can facilitate the establishment and spread of additional non-indigenous species (Simberloff and Von Holle 1999; Simberloff 2006). After the introduction of *H. halys*, the presence of *Trissolcus japonicus* (Ashmead) (Yang et al. 2009; Talamas et al. 2013) was reported in the USA (Talamas et al. 2015; Herlihy et al. 2016; Milnes et al. 2016). *Trissolcus japonicus* is a dominant parasitoid of *H. halys* in Asia, but it is not host-specific (Yang et al. 2009; Zhang et al. 2017). The lack of host specificity means that *T. japonicus* could attack and develop in eggs of native Pentatomidae in North America and Europe, and could thus affect stink bug population dynamics.
In addition to affecting native pentatomids, \textit{T. japonicus} will encounter and interact with native parasitoids. The outcomes of potential competitive interactions between the Asian \textit{T. japonicus} and native parasitoid species in North America and Europe are largely unknown. Such interactions can be both extrinsic (between adult females competing for access to the same egg mass) or intrinsic (between immature stages of developing larvae inside multiparasitized eggs) (Fig. 1.8). The host-parasitoid model system involving \textit{H. halys}, thus presents an excellent opportunity to assess the possibility of different interspecific interactions (e.g. competition, facilitation), between a native and exotic parasitoid on a novel invasive host (\textit{H. halys}), and to evaluate the impacts of these interactions.

\subsection*{1.9 Objectives}

To elucidate the interaction between \textit{H. halys} and scelionid egg parasitoids, a combination of behavioural, molecular, and imaging techniques was employed to:

1) determine which North American and European native species of parasitoids are associated with \textit{H. halys} in Canada and Switzerland (part of the introduced range of \textit{H. halys});

2) study potential competitive interactions between the exotic \textit{T. japonicus} parasitoid and native parasitoids;

3) determine the ability of North American native egg parasitoids to exploit \textit{H. halys} as a host (in terms of host acceptance behaviour and progeny development); and

4) determine the timing of failed development by native parasitoids in fresh \textit{H. halys} eggs.
Together, the data generated from these experiments will serve to address the main goal of this thesis: to enhance the understanding of Pentatomidae-Scelionidae host-parasitoid interactions for successful or unsuccessful host use by egg parasitoids associated with native and exotic stink bugs.
1.10 Thesis outline

This thesis is organized in the following way:

Chapter 1 introduces parasitism as a life history in animals including insects, and details host-parasitoid interactions of hymenopteran parasitoids with their native and exotic hosts. In this chapter, the current knowledge gaps associated with interspecific interactions involving exotic species are identified. The occurrence of an evolutionary trap for native egg parasitoids associated with the introduction of exotic *Halyomorpha halys*, establishes the foundation for the work presented in this thesis.

Chapter 2 deals with Objective 1, using DNA-based molecular methods to detect and identify the association of native parasitoids with potential hosts, using sentinel egg masses of *H. halys* and native stink bugs in different habitats (natural, agricultural, and urban) in Canada, Switzerland, and China. The data identify important host-parasitoid interactions and demonstrates that egg masses of both native and exotic host species are equally likely to be attacked under field conditions, supporting the evolutionary trap potential of *H. halys* for native egg parasitoids.

Chapter 3 is based on Objective 2, and examines the outcome of intrinsic and extrinsic competitive interaction between the exotic *T. japonicus* and the European native eulopelmid egg parasitoids (*Anastatus bifasciatus*), both of which are capable of development in fresh *H. halys* egg masses. The data show coexistence of native and exotic parasitoids under natural conditions is possible, as a result of counterbalance competition.

Chapter 4, also based on Objective 2, investigates the outcome of competitive interactions between the exotic *T. japonicus* (a parasitoid that is developmentally compatible with fresh *H. halys* eggs) and the European native *T. cultratus* scelionid egg
parasitoids (generally not developmentally compatible with fresh \( H. \) \( halys \) eggs). The data from this chapter demonstrate that the native parasitoid has a narrow window of opportunity to escape the evolutionary trap posed by \( H. \) \( halys \), by acting as a facultative hyperparasitoid of the exotic \( T. \) \( japonicus \).

Chapter 5 addresses Objective 3 through the use of an orthogonal array (OA) design method to assess and rank the influence of several critical factors (host species, age of eggs, status of the eggs, and surface wash) characterizing host resource on behaviour (acceptance, patch residence and patch exploitation) and development (successful emergence) of the North American \( Trissolcus \) \( euschisti \) egg parasitoid on native \( (Podisus \) \( maculiventris \) and \( Euschistus \) \( variolarius \)) and exotic \( (H. \) \( halys \)) host egg masses. The data indicate that the relative importance of individual factors changes at different steps in the sequence of behaviours, suggesting that the maladaptive decision to oviposit in an unsuitable host is a result of a mismatch between the cues used by foraging female parasitoids and the expected outcome of this choice.

Chapter 6 addresses Objective 4 by examining the temporal parasitoid development within suitable \( (Podisus \) \( maculiventris \)) and unsuitable \( (Halyomorpha \) \( halys \)) host eggs using DNA-based molecular approach targeting the cytochrome oxidase I (COI) region of the mitochondrial DNA, and \textit{in situ} 3D visualization by X-ray micro-computed tomography (micro-CT). The findings suggest there is limited or no larval development past 24 h following initial parasitization, thus providing a time window for further investigation of the mechanism behind failed development in \( H. \) \( halys \).

Chapter 7 discusses the implications of the major findings of Chapters 2-6 of this thesis, addressing the objectives for improved understanding of host-parasitoid interaction.
in Pentatomidae-Scelionidae system, set out for this thesis (Chapter 1). Limitations of the current work and future studies in the context of this host-parasitoid interactions are also discussed.

1.11 References


Gapon DA. 2016. First records of the brown marmorated stink bug *Halyomorpha halys*
(Stål, 1855) (Heteroptera, Pentatomidae) in Russia, Abkhazia, and Georgia. Entomol Rev. 96:1086–1088.


Milnes JM, Wiman NG, Talamas EJ, Brunner JF, Hoelmer KA, Buffington ML, Beers EH. 2016. Discovery of an exotic egg parasitoid of the brown marmorated stink bug,


Pimentel D, Lach L, Zuniga R, Morrison D. 2000. Environmental and economic costs of


Salt G. 1965. Experimental studies in insect parasitism XIII. The haemocytic reaction of a


Chapter 2

Exploitation of native and exotic pentatomids by native egg parasitoids in the introduced range of *Halyomorpha halyss*: a molecular approach using sentinel egg masses

A version of this chapter will be submitted for publication in *Journal of Pest Science* (https://www.springer.com/life+sciences/entomology/journal/10340)

2.1 Introduction

Natural enemies play an important role in the population dynamics of their prey and hosts and the top-down effects of natural enemies (predators and parasitoids) on herbivorous terrestrial insects are especially strong (Vidal and Murphy 2018). Parasitoids have the ability to suppress host populations (through numerical and/or functional responses), and the capacity to maintain long-term stability in ecosystems. These properties, combined with their high reproductive potential, and ease of rearing, have made parasitoids preferred model systems when testing evolutionary and ecological hypotheses (e.g. host-parasitoid interaction at individual or population levels), within both theoretical and applied contexts (Vinson 1976; Hassell and Waage 1984; Gross 1993; Mills and Getz 1996; Vinson 1998; Fatouros et al. 2008; Jervis et al. 2008; Wajnberg et al. 2016; Okuyama 2017).

The natural or artificial introduction of exotic species (as an alternative host or a competitor) may have positive (e.g. trophic subsidy) or negative (e.g. competition, predation) impacts on indigenous parasitoids and their host community composition (Schmitz and Simberloff 1997; Elton 2000; Pimentel et al. 2000; Rodriguez 2006). Newly introduced invasive species can serve as a potential alternate host for indigenous parasitoids thereby enhancing their survival and/or reproductive success. However, if the new potential host elicits proper oviposition behavioural response by an indigenous parasitoid, but does not support the development of progeny, then the parasitoid may be faced with an evolutionary trap (Schlaepfer et al. 2002; Schlaepfer et al. 2005; Berthon 2015).
The introduction and spread of the invasive brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in North America and Europe has resulted in a potential evolutionary trap for some indigenous hymenopteran egg parasitoids. Generalist egg parasitoids in the Eupelmidae and Encyrtidae families can attack and develop on fresh eggs of Pentatomidae, including *H. halys* (Cusumano et al. 2013; Choi et al. 2014; Roversi et al. 2017). Conversely, Scelionidae egg parasitoids (associated more closely with pentatomid hosts) (Yeargan 1979; Orr et al. 1986; Orr 1988) accept this new host but their offspring generally fail to develop (Abram et al. 2014; Haye et al. 2015).

The interactions involving Scelionidae egg parasitoids and *H. halys* (Jones et al. 2014; Cornelius, Dieckhoff, Vinyard, et al. 2016; Cornelius, Dieckhoff, Hoelmer, et al. 2016; Ogburn et al. 2016; Roversi et al. 2016; Dieckhoff et al. 2017) and native pentatomids (Orr et al. 1986; Koppel et al. 2009; Tillman 2011; Tillman 2016) have received a lot of attention. Experiments assessing the impact of *H. halys* on indigenous parasitoid communities, both under laboratory and field conditions, have given variable results, depending on habitat, geographical location or the type *H. halys* egg masses used (e.g. naturally laid or sentinel; fresh or frozen) (Abram et al. 2017).

The focus of many studies has been to assess the potential of native parasitoids as biological control agents against *H. halys*, while the impact of this alien invasive host on the behavioural ecology and population dynamics of native parasitoid community remains relatively unexplored. Additionally, while providing valuable information about the interaction between *H. halys* and the native parasitoids, few studies include a direct comparison of parasitism between *H. halys* and native Pentatomidae (Ogburn et al. 2016; Dieckhoff et al. 2017). Field experiments of this nature are essential to (i) assess the relative
attraction and acceptance of *H. halys* egg masses by native parasitoids and the native hosts they co-evolved with, (ii) determine the impact of *H. halys* on the native parasitoid community dynamics, and (iii) quantify the relative search effort and acceptance of the native and alien host egg masses by native parasitoids.

The conventional methods of assessing egg parasitism (e.g. direct observation of parasitoid emergence or by dissection) are time consuming, and often underestimate the actual level of parasitism (Jones et al. 2014; Cornelius, Dieckhoff, Vinyard, et al. 2016). Molecular tools are well suited to assess parasitism with a higher degree of accuracy in host-parasitoid interactions (Gariepy et al. 2014; Hrček and Godfray 2015). In particular, a DNA-based approach with Scelionidae-specific primers for the DNA barcode region of the cytochrome oxidase subunit I (COI) gene (Gariepy et al. 2014) is more accurate at detecting and quantifying egg parasitism, thus enhancing the characterization of the parasitoid community, as well as the identification of associations between native parasitoids and native and exotic pentatomid hosts.

In this study, we estimated both egg mass and individual egg parasitism of the exotic *H. halys* and native pentatomids under field conditions, using traditional rearing methods and a molecular approach (PCR with Scelionidae-specific primers). We completed this assessment by exposing paired sentinel egg masses (to allow for comparison of parasitism) in native (China) and introduced (Switzerland and Canada) ranges of *H. halys*, in three different types of habitats (natural, agricultural, and urban), across the summer season (May-September). The aims of the present study were to evaluate the relative utility of traditional rearing versus molecular approaches to assess parasitism, and
to determine whether native and exotic pentatomids are equally exploited by native parasitoids in the introduced range of *H. halys*.

### 2.2 Materials and Methods

#### 2.2.1 Field sites

Exotic *H. halys* and native pentatomid sentinel egg masses were exposed in natural, agricultural, and urban locations in the province of Ontario (ON, Canada) during June-September 2014, and the canton of Jura (Switzerland) during May-September 2015. Additionally, *H. halys* sentinel egg masses were exposed in natural and agricultural locations in Beijing and Haidian (China) during May-August 2014 (Table 2.1, Appendix A, Figs. A1-A3).

#### 2.2.2 Insect colonies

All stink bug colonies were established using field collected individuals from Hamilton and London (ON, Canada; 2012), Zurich and Basel (Switzerland; 2012), and Lengquan and Langfang (China; 2012). All stink bugs (except *Podisus maculiventris* (Say 1832), described below) were kept in BugDorm mesh cages (45×45×45 cm in Canada and Switzerland, and 60×60×60 cm in China) at 26 °C, 70% RH, 16L:8D.

In Canada, adult colonies of *H. halys* and native stink bugs (*Euschistus variolarius* (Palisot de Beauvois 1805), *Euschistus servus* (Say 1832), *Euschistus tristigmus* (Say 1832), and *Thyanta custator* (Fabricius 1803)) were fed with romaine lettuce (*Lactuca sativa* L. var. longifolia), carrots (*Daucus carota* L.), apples (*Malus* sp.), dry peanuts (*Arachis hypogaea* L.) and soybean (*Glycine max* L.), supplemented with zucchini (*Cucur-
Table 2.1 Geographic coordinates and characteristics of natural, agricultural, and urban field sites in China, Canada, and Switzerland used for exposure of native and exotic (*H. halys*) stink bugs (Pentatomidae: Hemiptera) sentinel egg masses in 2014 and 2015 field seasons.

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Type of site</th>
<th>City or region</th>
<th>Site</th>
<th>Coordinates (latitude, longitude)</th>
<th>Habitat type</th>
<th>Host plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>China</td>
<td>N</td>
<td>Beijing</td>
<td>Bai Wang Mountain</td>
<td>40.03167, 116.25472</td>
<td>forest edge</td>
<td>wild peach</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>Haidian</td>
<td>Fragrant Hills</td>
<td>40.0611, 116.05005</td>
<td>forest edge</td>
<td>smoketree</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Haidian</td>
<td>Yangtaishan village</td>
<td>40.07083, 116.08139</td>
<td>forest edge</td>
<td>wild peach</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Haidian</td>
<td>Beianhe village</td>
<td>40.06903, 116.08167</td>
<td>fruit orchard</td>
<td>wild peach</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Haidian</td>
<td>Lengquan village</td>
<td>40.03500, 116.21139</td>
<td>fruit orchard</td>
<td>wild peach</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Haidian</td>
<td>Shuijiaotuo village</td>
<td>40.06611, 116.11278</td>
<td>fruit orchard</td>
<td>wild peach</td>
</tr>
<tr>
<td></td>
<td>Canada</td>
<td>N</td>
<td>London</td>
<td>LORDC forest</td>
<td>43.02877, -81.21329</td>
<td>forest edge</td>
<td>maple, dogwood, buckthorn, walnut,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>City Orchard</td>
<td>43.02959, -81.17838</td>
<td>abandoned orchard</td>
<td>apple, dogwood, buckthorn, walnut</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>London</td>
<td>LORDC farm</td>
<td>43.03035, -81.21146</td>
<td>crop/ vegetable patch</td>
<td>sorghum, zucchini, sunflower</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ESW</td>
<td>43.07505, -81.33591</td>
<td>crop/ vegetable patch</td>
<td>corn, pumpkin, sunflower</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Blenheim</td>
<td>Blenheim</td>
<td>42.31105, -82.17838</td>
<td>peach orchard edge</td>
<td>apple, walnut, buckthorn, mulberry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hamilton</td>
<td>Princess Point/</td>
<td>43.27188, -79.90015</td>
<td>hiking path/</td>
<td>mulberry, buckthorn, mountain holly</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sam Lawrence Park</td>
<td>43.24494, -79.86573</td>
<td>city park</td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>Switzerland</td>
<td>N</td>
<td>Delémont</td>
<td>Delémont</td>
<td>47.37599, 7.34044</td>
<td>forest edge</td>
<td>dogwood, buckthorn, hazel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>Courtételle</td>
<td>Courtemelon</td>
<td>47.35440, 7.32235</td>
<td>cherry orchard edge</td>
<td>dogwood, buckthorn</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Basel</td>
<td>St. Alban Park</td>
<td>47.55262, 7.60095</td>
<td>city park</td>
<td>buckthorn, maple, catalpa</td>
</tr>
</tbody>
</table>

*Note: N=natural; A= agricultural; U=urban*
*bita pepo* var. Cylindrica), and green beans (*Phaseolus vulgaris* L.). Adults of native *P. maculiventris* were kept in plastic buckets (height = 15 cm; diameter = 15 cm) and fed *Tenebrio molitor* larvae.

In Switzerland, *H. halys* and native stink bug colonies (*Carpocoris fuscispinus* (Boheman 1851), *Eurydema ornatum* L., and *Graphosoma lineatum* L.) (collected in 2015) were fed a mix of bean (*P. vulgaris*) and corn (*Zea mays* L.), supplemented with fresh branches of cherry (*Prunus avium* L.), buckthorn (*Rhamnus* sp.) and hazelnut (*Corylus* sp.). In China, *H. halys* were fed green beans (*P. vulgaris*), and corn (*Z. mays*), supplemented with fresh branches of peach (*Prunus persica* L.).

### 2.2.3 Sentinel egg mass preparation, field exposure and collection

Stink bug egg masses were collected daily and the number of egg masses of each species and the number of eggs per mass were counted. In Canada and Switzerland, *H. halys* eggs were freeze-killed at -80 °C for five minutes prior to exposure in the field. This step avoids the possibility of introducing *H. halys* into new locations, but these eggs are still suitable for parasitoid attack and development (Kivan and Kilic 2005). Egg masses of *H. halys* and native pentatomids, with approximately the same number of eggs, were prepared and 2-9 pairs of each species (depending on availability) were exposed at each site every week. Pairs consisted of two egg masses of the same species exposed on the same host plant, one that would be reared for parasitoids following field exposure, and one that would be frozen immediately after field exposure for subsequent molecular analysis. Egg masses with the substrate they were laid (approximately 1 cm²) on were attached to Heavy Duty Duct Tape (3M™, London, ON, Canada), and the remaining sticky surface was coated with 400 µm acid washed silica beads (VWR International, Mississauga, ON,
Canada) or fine quartz sand (Sandy Vogeland, Vitakraft, Switzerland), and stapled to small (~2×4 cm) pieces of labeled filter paper (Whatman® qualitative filter paper, Oakville, ON, Canada) (Appendix A, Fig. A4).

Pairs of prepared *H. halys* and native pentatomid egg masses were stapled to the underside of leaves of stink bug host plants at each location, and individually enclosed in clip cages. The mesh of the clip cages was large enough to permit movement of parasitoids in and out, but small enough to exclude most predators (Appendix A, Fig. A5). Host plants included: dogwood (*Cornus* sp.), buckthorn (*Rhamnus* sp.), maple (*Acer* sp.), mountain holly (*Ilex* sp.), mulberry (*Morus* sp.), walnut (*Juglans* sp.), apple (*Malus* sp.), sorghum (*Sorghum* sp.), corn (*Z. mays*), zucchini (*Cucurbita* sp.), pumpkin (*Cucurbita* sp.), and sunflower (*Helianthus* sp.) in Canada; dogwood, buckthorn, maple, cigar tree (*Catalpa* sp.), and hazelnut (*Corylus* sp.) in Switzerland; and peach (*Prunus* sp.), and smoketree (*Cotinus* sp.) in China (Table 2.1). The same host plants were used for the entire field season, with the paired egg masses exposed and collected every 7(±1) days at each location in Canada and Switzerland. Egg masses of each pair were attached to leaves on different branches of a host plant, at least 0.5 m apart. A total of 526 *H. halys* and 494 native pentatomid egg masses were exposed in Canada (Appendix B, Tables B1-B4), and 430 and 154 in Switzerland (Appendix B, Tables B5-B8). In China, only fresh *H. halys* egg masses (with no other native species) were exposed. Every two weeks paired egg masses were placed on either peach or smoketree and collected after 3-8 days. A total 347 *H. halys* egg masses were exposed over the season in China (Appendix B, Tables B9-B10).

Following collection, in both Canada and Switzerland one egg mass from each pair was frozen at -20 °C for subsequent molecular analysis, while the other was kept at 26 °C,
70%RH, 16L:8D. The latter was checked daily for the emergence of pentatomid nymphs (native species) or parasitoids for up to five weeks, or until no unhatched eggs remained (unhatched eggs from reared masses were not dissected). In China, one egg mass of each pair was stored in 70% ethanol, while the other one was reared out, as described above for the Canadian and Swiss samples. All emerging parasitoids from samples in China were collected and identified to species using a taxonomic key (Talamas et al. 2017). A subsample (≤ 5 each) of the small number of parasitoids that emerged from individual samples in Canada and Switzerland were DNA barcoded and identified to species using a DNA barcode database of expertly-identified, vouchered Scelionidae.

2.2.4 DNA extraction and amplification

For those egg masses that were immediately stored at -20 °C following field exposure, DNA from each individual egg within an egg mass was extracted using a modified Chelex DNA extraction method (Walsh et al. 1991; Gariepy et al. 2014). Each egg was transferred with sterilized forceps into a well of a 96-well microplate containing 2 µL of Proteinase K (20 mg/mL; Ambion™ Life Technologies, Burlington, ON) and crushed using a sterilized micropestle. One hundred µL of 5% Chelex® 100 resin (Bio-Rad Laboratories Canada Ltd., Mississauga, ON, Canada) was added to each well. Each microplate contained a negative control comprised of Proteinase K and Chelex solutions, but no insect tissue. The entire microplate was incubated at 55 °C overnight, followed by centrifugation for 5 min to pellet the Chelex solution. The supernatant (containing extracted DNA) was collected and used for PCR (Polymerase Chain Reaction) following deactivation (denaturation) of Proteinase K via incubation at 99 °C for 10 min.
PCRs targeting the COI of scelionid mitochondrial DNA were performed in 25 µL volumes containing 0.125 µL of Taq Platinum, 2.5 µL of 10×PCR buffer, 1.25 µL of 50 mM MgCl₂, 0.125 µL of 10 mM dNTPs (Invitrogen/ Life Technologies, Burlington, ON, Canada), 0.25 µL of 10 mM HCO-2198 (5’-TAAACTTCAGGGTGACCAAAAAATCA-3’) (Folmer et al. 1994), 0.25 µL of 10 mM SCEL_F1 (5’-GCAAATAATTCGAATAGAA TTAAAGGT-3’) (Gariepy et al. 2014), 19.5 µL of ddH₂O, and 2 µL of the template DNA in skirted 96-well PCR plates (VWR International, Mississauga, ON, Canada). The thermal cycle profile was programmed for 1 min at 94 °C for initial denaturation, followed by 35 cycles of 40 s at 94 °C for denaturation, 40 s at 52 °C for annealing, 1 min at 72 °C for extension, and 5 min at 72 °C for the final extension. To increase the detection of small quantities of DNA (particularly of undeveloped native parasitoids in H. halys), samples from Canada and Switzerland were subjected to a re-PCR using the PCR product from the first round of PCR as template. This second 25 µL PCR was as described above, but using 1 µL of the template, and 20.5 µL of ddH₂O. Extracted parasitoid (Scelionidae) DNA served as positive control: 2 µL or 1 µL (for original PCR and re-PCR, respectively) of previously extracted scelionid DNA was used as a template in a single well of each PCR plate to ensure optimal PCR conditions and DNA amplification.

All PCR products were examined using a QIAxel Advanced (Qiagen Sciences, Germantown, MD, USA) multicapillary electrophoresis instrument and visualized using QIAxel ScreenGel software (v. 1.2.0). Samples of the correct size peaks (600-800 bp for Scelionidae) with ≥0.1 relative fluorescence units (RFUs) were scored as positive.
2.2.5 DNA sequencing

High quality PCR products from one natural site each in Canada (LORDC-forest) and in Switzerland (Delémont) were selected for further analysis and purified using ExoSAP-it (Affymetrix, Santa Clara, CA, USA), following the manufacturer’s instructions. PCR products were bi-directionally sequenced (using ScelF1 forward and HCO-2198 reverse primers) on an ABI 3730 DNA Analyzer at the Robarts Research Institute (London Regional Genomics Centre, London, ON, Canada). The forward and reverse sequences were assembled and edited using CodonCode Aligner (v. 4.2.7) software (CodonCode Corporation, Centerville, MA, USA). The DNA sequence files, tracefiles, and specimen information were uploaded to the project JSENT in the Barcode of Life Data System (BOLD; www.boldsystems.org), and assigned Barcode Index Numbers (BINs) and/or taxonomic identifications based on matching sequences in the BOLD identification engine (Ratnasingham and Hebert 2013).

2.2.6 Statistical analysis

The proportion of native and exotic (H. halys) parasitized eggs and egg masses based on traditional rearing and molecular approaches (using Scelionidae-specific primers) were compared using Chi square tests (contingency table analysis incorporating country) in SPSS (v. 25) (IBM 2016) statistical software.

Due to relatively low incidence of parasitism in reared egg masses, subsequent analysis was performed exclusively on the molecular dataset. The egg mass parasitism (scored as presence of at least one parasitoid per egg mass) and egg parasitism (proportion of parasitized eggs per egg mass) were both fitted with a generalized linear mixed model (GLMM) with binomial distribution and logit link function, using centered Julian date (i.e.
day of year), country (Canada, Switzerland, and China), type of host (*H. halys* or native), and type of site (natural, agricultural, or urban) as fixed effects in the model. The following equations characterize both models:

\[ Y_{ijklm} \sim \text{Bin}(1, p_{ijklm}) \text{ for egg mass parasitism} \]

and

\[ Y_{ijklm} \sim \text{Bin}(n_{ijklm}, \pi_{ijklm}) \text{ for proportion of parasitized eggs per egg mass} \]

where \( p_{ijklm} \) is a probability of egg mass \( i \) in country \( j \), of host type \( k \), in type of site \( l \), in specific site \( m \) being parasitized (\( Y_{ijklm} \) is 1 if an egg mass is parasitized, and 0 if not) (model for presence of parasitism per egg mass), and where \( n_{ijklm} \) is a number of eggs exposed, and \( \pi_{ijklm} \) is a probability in an egg mass \( i \) of an egg being parasitized (\( Y_i \) is a number of eggs in an egg mass that are parasitized represented as a proportion) (model for proportion of parasitism per egg mass).

For both models:

\[ \text{logit}(p_{ijklm}) = \beta_{\text{Country}} + \beta_{\text{Type of host}} + \beta_{\text{Type of site}} + \beta_1 \times \text{Date} + \beta_{\text{Country} \times \text{Date}} + \alpha_{\text{Country}} + \alpha_{\text{Type of host}} + \alpha_{\text{Type of site}} + \text{Random effect} \]

The random part of the model is composed of a random intercept (for a specific random factor used in the model) and residual error (\( e_{ijklm} \)). The random intercept introduces a compound symmetrical structure (i.e. allows for different intercept based on the random factor).

Random intercept\( \sim N(0, \sigma_a^2) \)

or
\[ E(\text{Y}_{ijklm}) = \pi_{ijklm} \times n_{ijklm} \quad \text{and} \quad \text{var}(\text{Y}_{ijklm}) = \pi_{ijklm} \times n_{ijklm} \times (1 - \pi_{ijklm}) \]

The model for the incidence of egg mass parasitism incorporated specific sites in each country as a random effect (as the probability of egg mass being parasitized is correlated with the probability of other egg masses in the same site being parasitized). The data on presence and absence of parasitism per egg mass were analyzed in R (v. 3.4.3) statistical software using MASS package (glmmPQL) (Vanables and Ripley 2002), and the model was validated by plotting normalized residuals against fitted values, as well as against all explanatory variables (to ensure homogeneity and independence).

The model for the proportion of parasitized egg masses incorporated a random effect for each egg mass (as the probability of an egg in an egg mass being parasitized is correlated with the probability of other eggs in the same egg mass being parasitized), with the number of eggs in each egg mass used as an offset (to control for the relative effect based on egg mass size). The data on proportion of parasitized eggs were analyzed in R (v. 3.4.3) statistical software using lme4 package (glmer) (Bates et al. 2015), and checked for overdispersion with blmeco package (Korner-Nievergelt et al. 2015). The model was validated by plotting normalized residuals against fitted values, as well as against all explanatory variables (to ensure homogeneity and independence).

The obtained coefficients of each model were converted to odds ratio (ORs), and their respective 95% confidence intervals (CI) computed. Odds ratios are a way of representing probability, and indicate a strength of the relationship as a relative measure of effect between treatment and a reference condition, where an odds ratio of 1 indicates no difference between the groups (Bland and Altman 2000; Szumilas 2010). Confidence
intervals are used as an estimate of the precision of the ORs (large CI indicates low precision; small CI indicates high precision).

2.3 Results

2.3.1 Parasitism in reared sentinel egg masses

The estimates of egg mass parasitism (defined as at least one egg in an egg mass being parasitized) of native pentatomids were at least 60% lower when calculated using reared egg masses than those obtained with molecular methods in Canada (5.8% vs. 71.0%; \( \chi^2(1, N=487) = 218.6, p < 0.001 \)) and Switzerland (8.0% vs. 69.3%; \( \chi^2(1, N=150) = 59.5, p < 0.001 \)) (Fig. 2.1). In all three countries the values for egg mass parasitism of *H. halys* were also significantly lower (by ~ 80% in Canada and Switzerland and by ~20% in China) using the rearing techniques compared to values obtained with molecular ones: Canada (0.4% vs 83.5%; \( \chi^2(1, N=525) = 373.2, p < 0.001 \)), Switzerland (1.4% vs 79.9%; \( \chi^2(1, N=418) = 266.7, p < 0.001 \)) and China (54.8% vs 73.4%; \( \chi^2(1, N=313) = 11.8, p < 0.001 \)) (Fig. 2.1).

Egg parasitism (total number of eggs parasitized per egg mass) of native pentatomids was also significantly lower (by ~30%) for reared egg masses than for those analyzed with molecular methods in Canada (6.7% vs 33.1%; \( \chi^2(1, N=7697) = 892.0, p < 0.001 \)) and Switzerland (3.8% vs. 35.8%; \( \chi^2(1, N=1711) = 293.1, p < 0.001 \)) (Fig. 2.2). A similar situation was seen for the egg parasitism of *H. halys* in Canada (0.0% vs. 23.7%; \( \chi^2(1, N=13556) = 1793.5, p < 0.001 \)), Switzerland (1.2% vs. 15.1%; \( \chi^2(1, N=11175) = 721.3, p < 0.001 \)) and China (41.9% vs. 58.7%; \( \chi^2(1, N=8096) = 229.7, p < 0.001 \)) (Fig 2.2).
Figure 2.1 Mean (±SE) egg mass parasitism (presence of at least one parasitoid per egg mass) detected using rearing and molecular methods in sentinel egg masses of a) stink bugs native to each area and b) introduced *H. halys* exposed in native (China) and introduced (Canada and Switzerland) ranges of *H. halys* over the 2014 and 2015 field seasons. Asterisks (*) indicate significant difference between reared and molecular methods ($\alpha = 0.05$) based on $\chi^2$ tests in contingency table analysis. Sample sizes for native egg masses: Canada (n= 242 and n=245 for reared and molecular, respectively); Switzerland (n= 75 and n=75 for reared and molecular, respectively). Sample sizes for *H. halys* masses: Canada (n= 260 and n=261 for reared and molecular, respectively); Switzerland (n= 209 and n=209 for reared and molecular, respectively); (China: n=155 and n=158 for reared and molecular, respectively).
Figure 2.2 Mean (±SE) egg parasitism (proportion of parasitized eggs per egg mass) detected using rearing and molecular methods in sentinel egg masses of a) stink bugs native to each area and b) introduced *H. halys* exposed in native (China) and introduced (Canada and Switzerland) ranges of *H. halys* over the 2014 and 2015 field seasons. Asterisks (*) indicate significant difference between reared and molecular methods ($\alpha = 0.05$) based on $\chi^2$ tests in contingency table analysis. Sample sizes for native eggs: Canada (n= 4401 and n=4273 for reared and molecular, respectively); Switzerland (n= 951 and n=962 for reared and molecular, respectively). Sample sizes for *H. halys* masses: Canada (n= 6687 and n=6893 for reared and molecular, respectively); Switzerland (n= 5536 and n=5648 for reared and molecular, respectively); (China: n=4017 and n=4092 for reared and molecular, respectively).
The use of molecular methods significantly improved the ability to detect parasitism at the egg mass level in native (ϕ= 0.670, p<0.001 in Canada; ϕ= 0.630, p<0.001 in Switzerland) and exotic (i.e. *H. halys*) (ϕ= 0.843, p < 0.001 in Canada; ϕ= 0.799, p<0.001 in Switzerland, and ϕ= 0.194, p<0.001 in Canada) pentatomid hosts (ϕ is equivalent to Pearson correlation, and defines the strength of the relationship between method used and ability to detect parasitism). The molecular methods also significantly improved the ability to detect parasitism at the individual egg level in native (ϕ= 0.340, p<0.001 in Canada; ϕ= 0.414, p<0.001 in Switzerland) and exotic (i.e. *H. halys*) (ϕ= 0.364, p < 0.001 in Canada; ϕ= 0.254, p<0.001 in Switzerland, and ϕ= 0.168, p<0.001 in China) pentatomid hosts.

### 2.3.2 Parasitism based on molecular analysis

Egg mass parasitization was not affected by date (i.e. parasitism was consistent throughout the season), country, type of host, type of site, and there was no interaction between the effect of date and the country (i.e. the seasonal effect was the same in all countries) (Table 2.2). The intercept in the GLMM was 0.856 and the random effect was assumed to be normally distributed with mean 0 and variance 0.527² ($\sim N(0, 0.527^2)$).

In contrast, the proportion of parasitized eggs was affected by country, type of host, and to some extent by date, with the seasonal effect being different depending on the country (Table 2.3). The type of site (i.e. natural vs. agricultural vs. urban) had no effect on level of parasitism at an egg mass level. The intercept in the GLMM was 1.08 and the random effect was assumed to be normally distributed with mean 0 and variance 4.983² ($\sim N(0, 4.983^2)$). There was no overdispersion in the model (dispersion = 1.00865).
Table 2.2 Odds ratio and 95% confidence intervals of the fixed effects in the generalized linear mixed effects model (GLMM) characterizing the effect of date, country, type of host, type of site, and interaction between date and country on the presence of parasitism in sentinel stink bug egg masses.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Fixed effect</th>
<th>Odds ratio</th>
<th>95% CI (odds ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Date</td>
<td>1.006</td>
<td>[0.993, 1.019]</td>
</tr>
<tr>
<td>Country</td>
<td>China</td>
<td>reference</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Switzerland</td>
<td>1.652</td>
<td>[0.517, 2.788]</td>
</tr>
<tr>
<td></td>
<td>Canada</td>
<td>1.616</td>
<td>[0.626, 2.607]</td>
</tr>
<tr>
<td>Type of host</td>
<td>BMSB</td>
<td>reference</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Native</td>
<td>0.771</td>
<td>[0.385, 1.158]</td>
</tr>
<tr>
<td>Type of site</td>
<td>Natural</td>
<td>reference</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Agricultural</td>
<td>1.866</td>
<td>[1.035, 2.697]</td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>1.308</td>
<td>[0.304, 2.311]</td>
</tr>
<tr>
<td>Date × Country</td>
<td>Date × China</td>
<td>reference</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Date × Switzerland</td>
<td>0.996</td>
<td>[0.980, 1.013]</td>
</tr>
<tr>
<td></td>
<td>Date × Canada</td>
<td>0.994</td>
<td>[0.977, 1.011]</td>
</tr>
</tbody>
</table>

Note: Odds ratio represents a relative measure of the effect compared to the reference condition. Odds ratio = 1 means no effect of the factor on the outcome (presence of parasitism in egg mass); odds ratio > 1 means that the factor has more effect than the reference condition; odds ratio < 1 means that the factor has less effect than the reference condition. Confidence intervals are used as proxy of significance (if CI includes 1, the effect is likely not significant). Significant CIs are indicated in bold font.
Table 2.3 Odds ratio and 95% confidence intervals of the fixed effects in the generalized linear mixed effects model (GLMM) characterizing the effect of date, country, type of host, type of site, and interaction between date and country on the proportion of parasitized eggs in sentinel stink bug egg masses. Significance assessed at $\alpha = 0.05$.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Fixed effect</th>
<th>Odds ratio</th>
<th>95% CI (odds ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Date</td>
<td>1.035</td>
<td>[1.021, 1.049]</td>
</tr>
<tr>
<td>Country</td>
<td>China</td>
<td>reference</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Switzerland</td>
<td>0.029</td>
<td>[-0.578, 0.636]</td>
</tr>
<tr>
<td></td>
<td>Canada</td>
<td>0.056</td>
<td>[-0.558, 0.669]</td>
</tr>
<tr>
<td>Type of host</td>
<td>BMSB</td>
<td>reference</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Native</td>
<td>2.443</td>
<td>[2.066, 2.820]</td>
</tr>
<tr>
<td>Type of site</td>
<td>Natural</td>
<td>reference</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Agricultural</td>
<td>1.398</td>
<td>[0.991, 1.806]</td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>1.030</td>
<td>[0.589, 1.470]</td>
</tr>
<tr>
<td>Date × Country</td>
<td>Date × China</td>
<td>reference</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Date × Switzerland</td>
<td>0.966</td>
<td>[0.949, 0.983]</td>
</tr>
<tr>
<td></td>
<td>Date × Canada</td>
<td>0.951</td>
<td>[0.934, 0.969]</td>
</tr>
</tbody>
</table>

Note: Odds ratio represents a relative measure of the effect compared to the reference condition. Odds ratio $=1$ means no effect of the factor on the outcome (presence of parasitism in egg mass); odds ratio $>1$ means that the factor has more effect than the reference condition; odds ratio $<1$ means that the factor has less effect than the reference condition. Confidence intervals are used as proxy of significance (if CI includes 1, the effect is likely not significant). Significant CIs are indicated in bold font.
For each week of sentinel egg mass exposure, the probability of more eggs in an egg mass being parasitized increased slightly, but stayed similar throughout the season (odds ratio of 1.035). The egg parasitism in Canada and Switzerland was lower (0.95 and 0.97 times, respectively) with each week of exposure, compared to parasitism in China throughout the season. Eggs in egg masses exposed in Canada and Switzerland were 0.06 and 0.03 times as likely to be parasitized compared to those in China. Eggs in egg masses of native pentatomids were 2.44 times more likely to be parasitized compared to those of *H. halys*, but the egg parasitism was similar for different types of sites tested.

### 2.3.3 Parasitoid identification

Three species of parasitoids were reared from sentinel egg masses of native pentatomids in Canada: 81.0% were identified as *Telenemus podisi*, 14.6% as *Trissolcus euschisti*, and 4.4% as *Trissolcus thyanta*e (Fig. 2.3a). In Switzerland, the only parasitoid species recovered from sentinel native pentatomids egg masses was *Trissolcus cultratus* (Fig. 2.3b). The only parasitoid species that successfully emerged from frozen *H. halys* egg masses were *T. euschisti* in Canada (Fig. 2.3a) and *T. cultratus* in Switzerland (Fig. 2.3b). In China, six parasitoid species successfully developed in *H. halys* egg masses across all sites, with 90% of those being *Trissolcus japonicus*, and less than 5% each of (in decreasing order) *T. cultratus*, *Anastatus* sp., *Trissolcus plautiae*, *Ooencyrtus* sp, and *Telenomus* sp. (Fig. 2.3c). No multiparasitism (i.e. more than one parasitoid species identified from the same egg mass) was detected in any of the reared egg masses.
Figure 2.3 Parasitoid adults recovered from reared sentinel egg masses of stink bugs native to each area and of *H. halys* exposed in a) Canada b) Switzerland and c) China over the 2014 and 2015 field seasons. Sample size (n) refers to the number of emerged parasitoids across all sites within each country. Emerged parasitoids from Canada and Switzerland were identified via DNA extraction and analysis, while those from China were identified taxonomically.
A total of 1124 PCR products from sentinel egg masses exposed in natural habitats in Canada (LORDC-forest) and Switzerland (Delémont) were sequenced, producing 790 high quality sequences, 730 of which were barcode-compliant (i.e. of high data quality, a minimum sequence length of 500 bp, and less than 1% ambiguous bases). Molecular analysis of Canadian sentinel egg masses from native and *H. halys* samples identified two and four unique BINs, respectively. Parasitoid DNA from native pentatomids was identified as *T. podisi* (99.6%) and BOLD:ADH9910 (0.4%) (unique BIN with no matches in BOLD) (Fig. 2.4). Parasitoid DNA from *H. halys* egg masses was identified as *T. podisi* (98.9%), *Trissolcus brochymenae* (0.23%), BOLD:ADH941 (0.69%), or BOLD:ADI0930 (0.23%) (Fig. 2.4). Multiparasitism was detected in one native egg mass (parasitized by BOLD:ADH9910 and *T. podisi*) (0.57% multiparasitism), and in four *H. halys* egg masses (one each of *T. podisi* with *T. brochymenae*, and *T. podisi* with BOLD:ADI0930, as well as two instances of *T. podisi* with BOLD:ADH9421) (1.83% multiparasitism).

Within a single natural site in Canada, species composition differed depending on the parasitism detection method used (Fig. 2.4). *Telenomus podisi* was the predominant parasitoid recovered both via rearing and molecular methods. In contrast, *Trissolcus euschisti* was not detected in molecular samples, although it successfully emerged from native pentatomid and *H. halys* egg masses at very low levels. In contrast, the molecular identification yielded additional species (*T. brochymenae*, BOLD:ADH9910, BOLD:ADH9421, and BOLD:ADI0930), not recovered with rearing methods (Fig. 2.4). Among the samples from one Swiss natural site, only one parasitoid species (*T. cultratus*) was identified based on rearing and molecular analysis (Fig. 2.5).
Figure 2.4 Parasitoid adults or DNA recovered from sentinel egg masses exposed in a natural field site in Canada (LORDC-forest) using a) rearing (identified via DNA extraction and analysis) and b) molecular methods. Sample size (n) refers to a) number of emerged parasitoids or b) the number of samples with scelionid parasitoid DNA that were barcode compliant. BOLD:ADH9910, BOLD:ADH9421, and BOLD:ADI0930 are unidentified scelionid parasitoids (i.e. no matching sequences in BOLD).
Figure 2.5 Parasitoid adults or DNA recovered from a natural field site in Switzerland (Delémont) using a) rearing (identified via DNA extraction and analysis) and b) molecular methods. Sample size (n) refers to a) number of emerged parasitoids or b) the number of samples with scelionid parasitoid DNA that were barcode compliant.
2.4 Discussion

Previous laboratory studies have shown that native scelionid egg parasitoids perform poorly on fresh *H. halys* eggs, and suggested an evolutionary trap potential of *H. halys* (Abram et al. 2014; Haye et al. 2015). Our data, obtained using molecular methods, show that the use of unsuitable *H. halys* host eggs by the native scelionid egg parasitoids also happens frequently under natural conditions, and is likely to be widespread in areas where *H. halys* is found. Therefore, egg masses of both native and exotic host species are equally likely to be attacked under field conditions, supporting the evolutionary trap potential of *H. halys* for native egg parasitoids. The likelihood and frequency of *H. halys* parasitization events by the native scelionids highlights the possible impact this may have on native parasitoid populations, unless they evolve strategies to overcome the evolutionary trap (e.g. behavioural avoidance or adaptation to the resource) (Phillips and Shine 2004; Keeler and Chew 2008).

The fact that molecular methods always gave higher egg mass parasitism estimates (at least one egg per mass) than egg parasitism (total parasitism of individual eggs per egg mass), in both native host and *H. halys* egg masses, suggests that foraging females use general cues broadly associated with the Pentatomidae when foraging for a host. Furthermore, while parasitoids attempted parasitization of many encountered egg masses, some parasitoids exploited them only partially. Similar discrepancy between parasitism at egg mass and egg levels has been previously reported based on dissections and rearing data in Pentatomidae egg masses, including *H. halys* (Koppel et al. 2009; Ogburn et al. 2016). This partial resource utilization (and subsequent successful exploitation) may vary possibly due to limited egg load, rejection of egg masses after drilling (i.e. after physical ovipositor
insertion into a host egg), or after ovipositing into several eggs in an egg mass, leading to females leaving in search of potentially more suitable resources. Some reasons to explain rejection of an egg mass might be the presence of intra- or inter-specific competitors, age of the egg, or preference for a different host species (Vinson 1976; Vinson 1998; Laumann et al. 2008; Cusumano et al. 2016)

The underestimation of parasitism in reared egg masses in both the native and introduced range of *H. halys* may be explained several ways. First, sentinel egg masses of native pentatomids in Canada and Switzerland were fresh (i.e. viable and not frozen) upon field-exposure, and most yielded host nymphs after field-recollection (Appendix B, Tables B3 and B7). Thus, it is possible that at the time of parasitization, the host embryo was sufficiently advanced in development to be unaffected (i.e. no longer suitable for parasitoid development), or that parasitization induced a host immune response resulting in neutralization of the parasitoid egg (Gross 1993; Brodeur and Vet 1995; Hoogendoorn and Heimpel 2002). If this is the case, it would be impossible to detect failed parasitism by rearing, nonetheless parasitoid DNA would be present and detectable. The second most common outcome from reared sentinel egg masses of native pentatomids was neither host nymphs nor parasitoids emerging. Interestingly, the proportion of non-emerged eggs combined with that of parasitoids actually emerged from reared egg masses, was very similar to the proportion of parasitism detected with molecular tools (39% vs. 35% in Canada and 30% vs. 36% in Switzerland) (Appendix B, Tables B3 vs. B4 for Canada and Tables B7 vs. B8 for Switzerland). Although some unhatched/unemerged eggs may have been nonviable at exposure or subsequently died due to adverse environmental conditions in the field, our results support the hypothesis that parasitoid-induced host ‘egg abortion’
(where neither the host nor the parasitoid successfully emerge) contributed to host mortality, and that some unhatched eggs were a result of failed parasitism (Abram et al. 2016).

In this study, sentinel egg masses of *H. halys* were frozen before exposure to avoid increased pest pressure and potential outbreaks in recently invaded areas. Consequently, it was not possible to assess if host egg abortion played any role in the low parasitism detected via the rearing method of *H. halys* egg masses. North American and European scelionid parasitoids rarely develop from fresh *H. halys* egg masses under laboratory conditions, but have much higher success on frozen ones (Abram et al. 2014; Haye et al. 2015). Therefore, the frozen *H. halys* egg masses used would have been suitable for development of native parasitoids. Suitability of the frozen egg masses may have declined over the 7 day exposure due to the degradation of dead host tissue, and changes in nutritional quality (both due to freezing and environmental exposure) (Skillman and Lee 2017). If the larvae of parasitoids developing in frozen *H. halys* egg masses were unable to feed on the host tissue fast enough (i.e. before decomposition and putrefaction of host material) (Strand et al. 1986; Strand et al. 1988; Quicke 1997), the insufficient amount of suitable resources within the egg could prevent complete parasitoid development.

The observed higher egg mass compared to egg parasitism, combined with the finding that egg parasitism was almost 2.5 times more likely in native hosts than in *H. halys*, indicates that native parasitoids frequently attack *H. halys* egg masses in nature, but they are less likely to utilize the entire *H. halys* egg mass. Moreover, the molecular method may underestimate parasitism at the egg level, due to false negatives or poor DNA quality. This difficulty in detecting parasitism would be especially high in eggs where parasitoids
failed to develop, leaving less DNA evidence of parasitism (in terms of quantity), with this DNA further degrading over time under natural conditions (Thomsen and Willerslev 2015). Therefore, even if parasitoids exploit egg masses only partially, parasitism at the egg level of both native and exotic hosts may be higher than estimated here.

Although we found no differences in total parasitism (i.e. parasitism by all scelionids combined) for different habitat types, species-specific differences in prevalence exist (Okuda and Yeargan 1988; Herlihy et al. 2016; Abram et al. 2017). Our molecular analysis does not account (since we only used scelionid-specific primers) for parasitization by *Anastatus* spp. (Hymenoptera: Eupelmidae) and *Ooencyrtus* spp. (Hymenoptera: Encyrtidae), which are known to attack pentatomid hosts, including *H. halys*. Parasitoids belonging to these families occur in different habitats, and in some areas represent the majority of the parasitoid community (Abram et al. 2017). Our reared samples showed no evidence of these species in the locations where sentinels were exposed, suggesting that their contribution to overall parasitism in the present study is minimal. Nonetheless, incorporating methods to detect parasitism by these other parasitoid families could provide additional information on the patterns of overall parasitism of pentatomid egg masses in different habitats. Even if different parasitoid species are present in different habitat types, our results suggest that the overall parasitism by Scelionidae would be similar (i.e. if parasitoids are present in a given area, they will find egg masses of both native pentatomids and of *H. halys*, and attempt to parasitize them).

The finding that native parasitoids locate and attack *H. halys* egg masses under field conditions highlights the possibility that there will be interactions between native parasitoids and newly introduced ones, such as *Trissolcus japonicus* (Ashmead). This
Asian native parasitoid species is capable of suppressing *H. halys* in its native range (Yang et al. 2009; Zhang et al. 2017), and has already been detected in several USA regions (Talamas et al. 2015; Milnes et al. 2016). One possibility is that native and Asian parasitoids will engage in competition for the same host egg masses, resulting in multiparasitism. Multiparasitism of stink bug egg masses by Scelionidae parasitoids has been previously documented (Orr et al. 1986, Gariepy et al., unpublished), and was also detected (although at very low level) in the present study. Since we have only sequenced a portion of all the eggs that were positive for scelionid DNA (one natural site in Canada and Switzerland each), and since not all the eggs had sufficient quality or quantity of DNA to enable species level identification, the incidence of multiparasitism was probably underestimated. Identification of species using molecular tools, and constructing species-interaction networks can provide more detailed characterization of species-level associations between pentatomid hosts and their parasitoids (Gariepy unpublished).

The results of this study also clearly show that conventional methods of estimating the incidence of attempted parasitism (e.g using rearing) in pentatomids underestimate the actual level in both egg masses and individual eggs. Parasitism estimates based on emergence from reared sentinel egg masses in Canada and Switzerland were at least 7 and 1.5 times lower, at egg mass and egg levels, respectively (both in native and exotic hosts) than those found with molecular techniques. Similarly, estimates of parasitism in reared egg masses of *H. halys* in China (native range of *H. halys*), although less pronounced than in Canada and Switzerland, were lower and suggest that failed parasitization events are not uncommon, even in a suitable host. Therefore, there is a clear advantage of using molecular methods to compliment traditional rearing, where detection of failed parasitism is often
challenging, if not impossible. Nonetheless, rearing data help put the molecular results in context, in particular, to confirm the developmental suitability of viable host eggs. Molecular identification would be particularly useful for field-collected samples, where direct observation of parasitism and subsequent assessment of developmental outcomes (as can be done under laboratory condition) is not practical.

It is possible that the use of sentinel egg masses still underestimates parasitism and parasitoid species composition. This is because preparation of sentinel egg masses can remove some important cues (e.g. in cases when eggs are washed with water during preparation), leading to a less diverse parasitoid species composition than in natural field-collected egg masses (Jones et al. 2014; Gariepy unpublished). While the use of naturally-laid egg masses may provide better estimates of the parasitoid community diversity, they are hard to find in the field. Therefore, sentinel egg masses are still useful as a method of exposing large numbers of egg masses in controlled experimental design.

Overall, our results support the idea that *H. halys* could represent an evolutionary trap for native egg parasitoids under natural conditions, as the molecular approach provided clear evidence that native parasitoids do attack egg masses of this exotic pest under natural conditions. Further research on population-level consequences of oviposition in an unsuitable host would be of interest, as it may affect the population dynamics of the parasitoids (e.g. reduced population size and bottleneck effects) as well as other pentatomids (e.g. direct competition with *H. halys* and reduced population regulation by parasitoids). Long term studies, incorporating screening for other families of parasitoids, will be necessary to examine year to year differences in the incidence of parasitism and the changes in the parasitoid communities.
2.5 References


Cusumano A, Peri E, Amodeo V, McNeil J, Colazza S. 2013. Intraguild interactions
between egg parasitoids: window of opportunity and fitness costs for a facultative hyperparasitoid. PLoS One. 8:e64768.


Hrček J, Godfray HCJ. 2015. What do molecular methods bring to host-parasitoid food


Okuda MS, Yeargan K V. 1988. Habitat partitioning by Telenomus podisi and Trissolcus
euschisti (Hymenoptera: Scelionidae) between herbaceous and woody host plants. Environ Entomol. 17:795–798.


Skillman VP, Lee JC. 2017. Nutrient content of brown marmorated stink bug eggs and


Wajnberg E, Roitberg BD, Boivin G. 2016. Using optimality models to improve the
efficacy of parasitoids in biological control programmes. Entomol Exp Appl. 158:2–16.


Chapter 3

Possible co-existence of native and exotic parasitoids and their impact on control of *Halyomorpha halys*

A version of this chapter has been published in *Journal of Pest Science* (https://www.springer.com/life+sciences/entomology/journal/10340)


3.1 Introduction

The balance of ecological interactions, shaped by shared evolutionary history, can be disrupted when a new species is intentionally or accidentally introduced into a stable ecosystem. Such introductions can result in significant ecological (community level) and evolutionary changes (Mooney and Cleland 2001; Didham et al. 2005). The type (e.g. competition, predation) and the strength of the interactions that the introduced species engages in, will determine the outcomes (neutral, positive or negative for either one or both of the interacting species).

The goal of introducing a natural enemy for biological control is to negatively impact a pest species, yet potential interactions with other species in the food web may influence the actual outcome of such introduction. The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), a highly polyphagous pest native to eastern Asia and invasive in Europe and North America, causes significant damage in many economically important crops (Hoebekke and Carter 2003; Lee, Short, et al. 2013; Rice et al. 2014; Haye, Gariepy et al. 2015). Currently, broad spectrum insecticides are used to control *H. halys*; however, IPM (integrated pest management) programmes, including the use of biological control agents, would be more desirable (Leskey et al. 2012; Lee, Wright, et al. 2013).

Parasitic wasps in the families Eupelmidae (genus *Anastatus* Motschulsky), Encyrtidae (*Ooencyrtus* Ashmead), and Scelionidae (e.g. genera *Trissolcus* Ashmead, *Telenomus* Haliday) attack *H. halys* eggs in this insect’s native range, which often results in a high incidence of parasitism (Hou et al. 2009; Yang et al. 2009; Lee, Short, et al. 2013). As these parasitoids have been effectively used to control populations of several pests,
including hemipterans (Orr 1988; Clarke 1990; Hoffmann and Davidson 1991; Tiberi et al. 1991; Alalouni et al. 2013; Choi et al. 2014), the possible use of several Asian *Trissolcus* species, especially *Trissolcus japonicus* (Ashmead), as classical biological control agents of *H. halys* in North America is being assessed (Rice et al. 2014).

Many European and North American egg parasitoids of the family Scelionidae readily attack *H. halys* eggs but generally do not complete development (Abram et al. 2014; see Chapter 4). In contrast, *Anastatus reduvii* (Howard) and *A. bifasciatus* (Geoffroy) (in North American and Europe, respectively) can successfully develop on *H. halys* eggs (Jones et al. 2014; Haye, Fisher et al. 2015; Noyes 2016). Consequently, investigation of inundative uses of *A. bifasciatus* against *H. halys* has been initiated in Europe (Stahl et al. 2018). The overall success of *Anastatus* parasitoids will not only depend on their host-searching and acceptance behaviour, but also on their ability to win intra- and interspecific competitive interactions with other egg parasitoids exploiting the same resources. Such a competitive situation would arise if *T. japonicus* is approved for release as a classical biocontrol agent against *H. halys* in Europe (or is accidently introduced, as in North America: (Milnes et al., 2016; Talamas, Herlihy et al., 2015). The potential competitive interactions between Asian and European parasitoids that would result from such introduction are largely unknown.

In this study, we determine the outcome of competitive interactions between European *A. bifasciatus* and Asian *T. japonicus*. The outcomes of such competition could impact the effectiveness of the biological control programme against this pest.
3.2 Materials and Methods

3.2.1 Insect Rearing

A *H. halys* colony was established from individuals collected in Zurich in 2012. The colony was maintained at 26 °C, 70% RH, 16L:8D photoperiod and fed a mix of beans (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.), supplemented with fresh branches of cherry (*Prunus avium* L.), buckthorn (*Rhamnus* sp.) and hazelnut (*Corylus* sp.) when available. Egg masses were collected daily from the black mesh (separation fleece for pots, Windhager AG) that served as an oviposition surface.

The colony of *A. bifasciatus* was established from sentinel *H. halys* egg masses exposed near Fully, Canton of Valais, Switzerland. A *T. japonicus* colony was initiated from naturally laid *H. halys* egg masses collected near Beijing, China. All parasitoids were provided with 10% honey water and fresh *H. halys* egg masses twice a week. The parasitized egg masses were kept separately at 26 °C, 60% humidity, and 16L:8D photoperiod. Upon the initial establishment of the laboratory colonies, specimens of *T. japonicus* and *A. bifasciatus* were taxonomically identified by E. Talamas (Smithsonian Institution, Washington, DC, USA) and L. Fusu (University of Iasi, Romania), respectively.

3.2.2 Larval Competition

Fresh (< 24 h) *H. halys* egg masses were separated into smaller clusters (12 eggs/clusters) and attached to 1 cm² pieces of flat cardboard with small amount of clear glue (Cementit, merz+benteli AG). Twenty egg clusters/treatment were exposed to randomly selected 4-day-old, mated naïve *T. japonicus* females in small (5 cm) Petri dishes, and observed until all eggs in the cluster were parasitized (as indicated by marking behaviour:
~30 min/female/egg mass). Zero, 1, 2, 3, 4, 5 or 7 days after being parasitized by *T. japonicus*, each egg cluster was exposed to a 4-day-old, mated naïve *A. bifasciatus* female in small (5 cm) Petri dish, and her parasitization attempts were observed for three hours (*A. bifasciatus* requires more time to complete parasitization than *T. japonicus*).

A similar protocol was used with the parasitization order reversed: *A. bifasciatus* was allowed to parasitize *H. halys* egg clusters for three hours (12 eggs/cluster; 20 egg masses/treatment), which were then presented to *T. japonicus* 0, 1, 2, 3, 4, 5, 9 or 14 days later. Egg clusters were observed until the female left the egg cluster for > 10 min, and the number of multiparasitized eggs was recorded (*A. bifasciatus* females never parasitized all the available eggs within the 3 h period). All egg clusters were kept for three weeks and the emerging parasitoids identified to species based on morphological characteristics (Kalina 1981; Noyes 2016; Talamas, Johnson et al. 2015). The difference of time between parasitization events in the two experiments was due to temporal differences in the development of the two species.

Fresh, unparasitized *H. halys* egg clusters were offered to *A. bifasciatus* (12 eggs/cluster; 10 egg masses) and *T. japonicus* (28 eggs/mass; 20 egg masses) as controls (to test overall acceptance and developmental outcomes) and observed for 3 and 1 h, respectively. In addition, fresh *H. halys* egg masses (n = 20) offered to *A. bifasciatus* were reared or dissected directly after parasitization to estimate actual oviposition success.

3.2.3 Adult competition

*Trissolcus japonicus* females (5-10 days old; n = 20) were placed in individual Petri dish arenas (5 cm diameter) and allowed to parasitize fresh *H. halys* egg masses (15-22 egg/mass; mean 17.7). Each female was observed until she completed parasitizing the
entire mass. Following parasitization *T. japonicus* females start egg guarding behavior (remaining on the mass post parasitization and displaying aggressive behaviour towards other parasitoids that attempt to use the egg mass). Once *T. japonicus* female competed parasitization, an *A. bifasciatus* female from the colony was introduced into the arena without removing *T. japonicus*. The interactions between the females of the two species were observed for 10 min, and the number of attempts made by *A. bifasciatus* to access the egg mass, as well as aggressive behaviour (chasing off) by *T. japonicus*, were recorded. The outcome of the adult competition was scored as unsuccessful (*A. bifasciatus* being chased off by *T. japonicus*), partially successful (*A. bifasciatus* making contact with the guarded egg mass), and successful (*A. bifasciatus* chasing *T. japonicus* off and ovipositing). Since *A. bifasciatus* does not guard eggs, the reciprocal tests of extrinsic competition were not undertaken.

### 3.2.4 Statistical analysis

The proportion of multiparasitized (parasitized by two species) eggs, and the developmental outcomes from those eggs were analyzed with $\chi^2$ tests, with values for each combination of factors calculated based on the resulting standardized residual (crosstab analysis), and compared to Bonferroni-corrected p values (indicating if the proportion of each developmental outcome was significantly different from a mean proportion of that outcome across the egg age or egg state: Beasley and Schumacker 1995; García-Pérez and Núñez-Antón 2003). All statistical analyses were carried out using SPSS (v. 23) (IBM 2016) statistical software.
3.3 Results

3.3.1 Host acceptance and larval competition

*Anastatus bifasciatus* females did not discriminate among *H. halys* egg masses of different ages previously parasitized by *T. japonicus* ($\chi^2_{(6, N = 1572)} = 21.7, p = 0.001$; 14 comparison tests, adjusted $\alpha = 3.57 \times 10^{-3}$, p of each test > adjusted $\alpha$; Fig. 3.1a). In contrast, *T. japonicus* females showed a preference for eggs recently parasitized by *A. bifasciatus*, while older ones were less acceptable ($\chi^2_{(7, N = 1692)} = 73.9, p < 0.0001$; 16 comparison tests, adjusted $\alpha = 3.13 \times 10^{-3}$; Fig. 3.1b).

In control experiments only 43.1% of all the observed parasitization events on fresh, unparasitized egg masses of *H. halys* gave rise to *A. bifasciatus* adults, whereas 96% of fresh, unparasitized *H. halys* egg masses were parasitized by *T. japonicus* and gave rise to adults. When multiparasitism of the same egg occurred, both *T. japonicus* and *A. bifasciatus* could sometimes complete development on *H. halys* egg masses previously parasitized by the other species (Fig. 3.2). The developmental outcome depended on the order of parasitization, and the time interval between the parasitization events. In all cases a proportion of multiparasitized eggs produced no parasitoids at all.

When *T. japonicus* was the first species to parasitize *H. halys* egg masses, it produced few offspring regardless of the time between initial parasitization by *T. japonicus* and subsequent exposure and attack by *A. bifasciatus* ($\chi^2_{(12, N = 651)} = 95.0, p < 0.0001$; 21 comparison tests, adjusted $\alpha = 2.38 \times 10^{-3}$). *Trissolcus japonicus* was most successful when multiparasitism occurred within 24 h, although even then it only emerged from 13% of eggs. At all time-intervals the majority of emerging parasitoids were *A. bifasciatus*, but many eggs produced no adults of either species (Fig. 3.2a).
Figure 3.1 Mean proportion (± SE) of previously parasitized *H. halys* eggs attacked by a) *A. bifasciatus* and b) *T. japonicus*. Bars with asterisks (*) indicate times after the initial parasitization that differed significantly from the mean expected proportion of eggs being multiparasitized ($\chi^2$ tests with Bonferroni corrections). For each time interval in a) and b) n=20 females.
Figure 3.2 Mean proportion of *H. halys* eggs giving rise to *T. japonicus*, *A. bifasciatus*, *H. halys* nymphs, or nothing, following multiparasitism by *T. japonicus* and *A. bifasciatus* at different time intervals (ages) between attacks: (a) parasitized by *T. japonicus* first (b) parasitized by *A. bifasciatus* first. Asterisks (*) indicate proportions of each outcome that is significantly different from a mean expected proportion of that outcome across the time after parasitization (age) ($\chi^2$ tests with Bonferroni corrections).
In contrast, when parasitization by *A. bifasciatus* was followed with parasitization by *T. japonicus* ($\chi^2_{(21, N=511)} = 84.6, p < 0.0001$; 32 comparison tests, adjusted $\alpha = 1.56 \times 10^{-3}$), *T. japonicus* successfully developed on eggs 0-4 days following exposure to *A. bifasciatus*, but failed to develop in eggs from the remaining intervals. As in the previous experiment, a larger proportion of eggs from all time-intervals produced *A. bifasciatus* rather than *T. japonicus*. However, when parasitization by *A. bifasciatus* was followed with that by *T. japonicus*, fewer *A. bifasciatus* emerged from egg masses where there was a 1-day interval between exposures to the different parasitoid species when compared to parasitization by *T. japonicus* followed by *A. bifasciatus*). A small proportion (6%) of eggs with a 4 day interval between exposures to the different parasitoid species yielded *H. halys* nymphs (Fig. 3.2b).

Although oviposition behaviour (including insertion of the ovipositor into the host egg and maintaining this posture for several minutes) was observed in each case, less than 50% of eggs parasitized only by *A. bifasciatus* yielded adults (following rearing, 43%), or contained eggs (following dissections, 36%).

### 3.3.2 Adult competition

All *A. bifasciatus* females attempted to gain access to egg masses parasitized and guarded by *T. japonicus* ($6.5 \pm 2.9$ times) within the 10 min observation period, but none were able to successfully oviposit. In all cases *T. japonicus* repeatedly ($6.0 \pm 2.7$ times) attempted to chase *A. bifasciatus* away before it could access the egg mass, but if *A. bifasciatus* females contacted the egg mass (35%) they were chased off ($1.3 \pm 0.5$ times) before they could oviposit.
3.4 Discussion

Newly-established interspecific competitive interactions between egg parasitoids can determine host population densities, and subsequently shape community structure (Cusumano et al. 2016). The degree and outcome of interspecific competition for the same limited resources will depend on whether the different parasitoids use similar host searching strategies or if the competing females differ in their host finding and dispersal abilities, as well as efficiency with which they exploit a resource once it has been located. Therefore, an understanding of both extrinsic (between adult females) and intrinsic (between larvae inside the host) competitive interactions provides a way of predicting species coexistence. Species coexistence is possible if the superior larval competitor is less efficient at locating its host, while the superior adult competitor is better at host finding or dispersal. This has been shown with *Ooencyrtus telenomicida* (Vassiliev) and *Trissolcus basalis* (Wollaston) on *Nezara viridula* (L.) (Cusumano et al. 2013), and is referred to as counterbalance competition (Zwölfer 1971; De Moraes et al. 1999). Our results suggest that counterbalance competition would happen between *T. japonicus* and *A. bifasciatus* if they occurred in sympatry as the former is a superior extrinsic competitor (due to its egg mass exploitation abilities and its aggressiveness towards other parasitoids when guarding egg masses), while the latter is a superior intrinsic competitor (as it readily accepts previously parasitized eggs and successfully develops from multiparasitized eggs of all ages).

*Anastatus bifasciatus* exhibited host feeding behaviour, a phenomenon where females feed on the exuding fluids from the stung eggs, with or without subsequent oviposition (Clausen 1940; Piek 1986). With fewer parasitized eggs and increased host
handling time (linked to lower egg load (Rosenheim and Rosen 1991; Ellers et al. 2000), the exhibited host feeding behaviour of A. bifasciatus plays an important role in this parasitoid-host interaction. The fact that in the absence of multiparasitism, less than half of the H. halys eggs contained A. bifasciatus eggs or gave rise to A. bifasciatus adults (as determined via dissections directly after parasitization by A. bifasciatus only), suggests that many of the observed attacks only involved host feeding. If that were the case with eggs previously exploited by A. bifasciatus, then T. japonicus larvae developed successfully only in eggs previously used exclusively for host feeding by A. bifasciatus, and consequently were not subject to interspecific competition. However, under such conditions, higher than actually observed numbers of T. japonicus adults (Fig. 3.2b) would be expected.

There are a number of factors that may contribute to this reduction in T. japonicus development. First, it is possible that venom or other substances injected by A. bifasciatus females during oviposition interfere with the development of T. japonicus. Changes in host egg physiology can be triggered by substances injected by the female during oviposition, or by the developing larva itself. Species of Trissolcus are known to inject arrestment factors that stop embryonic development of the host, while their eggs release ooplasm-altering teratocytes upon hatching (Dahlman 1991; Volkoff and Colazza 1992). Similarly, Eupelmid parasitoids (e.g. Eupelmus orientalis or Euplectrus sp.) use venom to paralyze or arrest the development of their hosts (Doury et al. 1997; Nakamatsu and Tanaka 2003). Further research on the presence and function of venom in Anastatus would be useful in determining its role(s) and impact on the development of in-host competitors. Second, host feeding by A. bifasciatus might reduce the amount or quality of the finite and limiting food
resources inside the eggs (Slansky 1986), making them less suitable for *T. japonicus* development. Finally, the mechanical damage caused by *A. bifasciatus* probing the egg might either kill the developing parasitoid larva directly, or expose it to external mortality factors. Scabs on eggs of several stink bug species after natural and artificial oviposition wounds take up to 24 h to be formed (Koppel et al. 2011), which might cause substantial loss of the limited food resources for the developing larva, or provide enough time for movement of external agents (e.g. pathogens) into the eggs.

Exotic species introduction can result in negative impacts on native species (Rodriguez 2006) through direct (e.g. competition) and indirect (trophic interactions) novel selection pressures (Berthon 2015). Ideally, the introduction of an exotic biological control agent would result in coexistence with native species and efficient control of the target pest (Schulthess et al. 2001). While this coexistence would have to be confirmed with subsequent field trials, the results of our laboratory experiments suggest that the introduction of *T. japonicus* as a biological control agent would not have a significantly negative impact on *A. bifasciatus* as most of the eggs parasitized by the latter (both previously unparasitized and parasitized) would yield *A. bifasciatus* adults. In fact, *T. japonicus* appears to co-exist with *Anastatus gastropachae* Ashmead in Japan (Arakawa and Namura 2002). The presence of *T. japonicus* and *A. bifasciatus* could have a positive and potentially synergistic effect on the control of *H. halys* (Fig. 3.3). If and when *T. japonicus* is used in Europe, the numbers released at different locations should be determined taking existing *A. bifasciatus* populations into account, in order to maximise the synergistic effect of both species in controlling the pest.
Figure 3.3 General schematic showing development outcomes from *H. halys* eggs parasitized by a) *T. japonicus* b) *A. bifasciatus* c) *T. japonicus* followed by *A. bifasciatus* and d) *A. bifasciatus* followed by *T. japonicus*. 
3.5 References


Chapter 4

An exotic parasitoid provides an invasional lifeline for native parasitoids

A version of this chapter has been published in *Ecology and Evolution* (https://onlinelibrary.wiley.com/journal/20457758).

4.1 Introduction

Invasive species may pose major threats to the biodiversity of native species at the same or higher trophic levels due to competition, predation, or facilitation (Simberloff 1981; Schmitz and Simberloff 1997; Elton 2000; Pimentel et al. 2000; Rodriguez 2006). Furthermore, the establishment of one exotic species can facilitate the establishment and spread of additional non-indigenous species, potentially leading to synergistic, negative effects, referred to as “invasional meltdown” (Simberloff and Von Holle 1999; Simberloff 2006). In contrast, the establishment of an invasive species may benefit a native one in different ways, such as being a “trophic subsidy” by providing a new exploitable resource for native species at a higher trophic level (Rodriguez 2006).

Indigenous natural enemies will benefit from the presence of an exotic host if their survival and/or reproduction is enhanced by exploiting exotic species as a trophic subsidy. Conversely, if previously reliable cues are no longer associated with adaptive outcomes following the invasion of non-indigenous species, this could result in an evolutionary trap that reduces the fitness and reproductive success of the native organism (Schlaepfer et al. 2002; Schlaepfer et al. 2005; Berthon 2015). If a native parasitoid accepts an invasive species as a host, but the offspring fails to complete development, then the host becomes an evolutionary trap for the native species (Schlaepfer et al. 2002; Abram et al. 2014). However, an evolutionary trap is not necessarily inescapable (Berthon 2015) and may be circumvented if the organism learns avoidance behaviour, or develops mechanical and/or physiological adaptations that permit it to exploit the host (Phillips and Shine 2004; Keeler and Chew 2008).
The potential of an evolutionary trap exists with the widespread establishment of the invasive pest, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in Europe and North America because *H. halys* eggs are readily attacked by native *Trissolcus* Ashmead and *Telenomus* Haliday parasitoid wasp species (Hymenoptera: Scelionidae), but they are unsuitable for development of larvae of these parasitoids (Abram et al. 2014; Haye et al. 2015). The impact of this evolutionary trap for native parasitoids may be further exacerbated by the proposed introduction of the Asian parasitoid (*Trissolcus japonicus* (Ashmead)) as a classical biological control agent against *H. halys* in countries where it has been detected (Rice et al. 2014). In fact, two adventive introductions into the USA already occurred (Talamas, Herlihy et al. 2015; Milnes et al. 2016).

Interspecific interactions between parasitoids can influence community structure, which is important, not only from an ecological but also from a practical (biological control) perspective (Boivin and Brodeur 2006). A thorough understanding of these interactions is critical to estimate the potential direct and indirect effects, positive or negative, associated with the introduction of the exotic parasitoid against an exotic pest. Although in-host competition between parasitoid larvae can have negative consequences (e.g. death, reduced fitness) (Godfray 1994; Boivin and Brodeur 2006), there may be benefits in the form of interspecific facilitation (Poelman et al. 2014; Cusumano et al. 2016). For example, secretions injected by heterospecific or conspecific parasitoid females ovipositing into the same host may act synergistically to overcome the host immune response, or teratocytes released by one species may provide additional nutritional resources for the other species, thereby facilitating the development of the competitively superior parasitoid.
There is little information regarding interspecific interactions between native and exotic egg parasitoids associated with Pentatomidae, so in this study we determined the outcomes of larval competitive interactions between the exotic *T. japonicus* and the native *Trissolcus cultratus* (Mayr), which might naturally occur following either an intentional introduction of *T. japonicus* as a biological control agent for *H. halys* in Europe, or an accidental one. This host-parasitoid model system presents an excellent opportunity to assess the possibility of different interspecific interactions (e.g., competition, facilitation), between a native and exotic parasitoid on an invasive host (*H. halys*), and to evaluate the impacts of these interactions from basic and applied perspectives.

### 4.2 Materials and Methods

#### 4.2.1 Stink bug rearing

A colony of *H. halys* was established from individuals collected in Zurich, Switzerland, in 2012 and maintained in continuous rearing on a diet of organic beans (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) at 26 °C, 70% RH, 16L:8D photoperiod in Bug Dorms (45×45×45, Mega View). Fresh-cut branches of cherry (*Prunus avium* L.), buckthorn (*Rhamnus* sp.) and hazelnut (*Corylus* sp.) were also provided when available. Strips of black mesh (separation fleece for pots, Windhager AG) were provided as oviposition substrate. Eggs were collected daily and maintained under the same conditions.

#### 4.2.2 Parasitoid rearing

*Trissolcus japonicus*, originally collected around Beijing (China), were maintained on fresh *H. halys* egg masses. *Trissolcus cultratus*, collected from frozen, sentinel *H. halys* egg masses in the Delémont valley (Canton Jura, Switzerland) were maintained on frozen
(at -80 °C then thawed 30 min prior to use) *H. halys* egg masses. Parasitoids (mated, ≥ 2 days old) were held in in a clear plastic containers (10 cm diameter, 5 cm height) with 10% honey water solution as a food source and 8-10 fresh or frozen *H. halys* egg masses provided twice a week. Parasitized egg masses were kept at 26 °C, 60% RH, and 16L:8D photoperiod.

Upon the initial establishment of the laboratory colonies of *T. japonicus* and *T. cultratus*, specimens were sent to E. Talamas (Smithsonian Institution, Washington, DC, USA) and M. C. Bon (USDA-ARS European Biological Control Laboratory, Montferrier sur Lez, France) for morphological and molecular identification, respectively.

### 4.2.3 Time-course developmental study

All assays were carried out in round plastic Petri plates (5×1 cm) into which females were introduced for the duration of the experiment, and observations were made (using a stereomicroscope) between 0800 and 1300 h at 26 °C, 60% RH, and 16L:8D photoperiod. Haphazardly selected *T. japonicus* females (4 days old, mated, naïve) were offered fresh (< 24 h) *H. halys* egg clusters (12 eggs/ mass) and observed until all the eggs in the cluster were parasitized (as indicated by marking behaviour) or until the female left the egg cluster for > 10 min. *Trissolcus cultratus* females (4 days old, mated, naïve) were provided fully parasitized egg clusters (*n* = 20 per time interval) that were attacked by *T. japonicus* 0 h, 1, 2, 3, 4, or 5 days earlier and observed until they had parasitized the entire egg cluster, or left the cluster for > 10 min.

As controls, fresh and frozen *H. halys* egg clusters (12 eggs/ mass) of the corresponding ages were offered to *T. cultratus* females to determine the effect of host age and host necrosis on acceptance and suitability for development (*n*=10 per time interval).
For frozen egg controls, the eggs were reared for 0, 1, 2, 3, 4, and 5 days prior to being frozen and exposed to the parasitoid. This approach ensured that parasitoid was provided hosts at different stages of embryonic development. The number of eggs attempted (drilled) and parasitized (marked) by each *T. cultratus* female was recorded. Egg masses were kept for three weeks and all adult parasitoids obtained were identified to species based on morphological characteristics including facial striae and clypeal setae (Talamas, Johnson et al. 2015).

4.2.4 Egg mass (patch) guarding behavior

To determine how long *T. japonicus* females guard parasitized egg masses, 20 egg masses (28±1 eggs/ egg mass) were exposed to randomly selected 4 days old, mated, naïve females in small round (5×1 cm) plastic Petri plates inside large round Petri dish arenas (15 cm diameter × 2 cm depth). Once the egg mass was parasitized, a second small Petri plate with a fresh egg mass was placed in the arena and the female’s guarding behaviour was verified every six hours until she left the parasitized egg mass.

4.2.5 Statistical analysis

The proportions of eggs drilled and marked were arcsin transformed (to ensure data were normally distributed) and analyzed with a two-way ANOVA followed by Tukey post-hoc tests. In cases where the main effects of egg mass age and/or state were significant, further one-way ANOVA analyses with Tukey post-hoc tests were performed. The developmental outcomes from parasitized eggs for each egg state were analyzed using separate $\chi^2$ tests, with the p-value for each cell calculated based on resulting residuals, and compared to Bonferroni-corrected p-values (indicating if the proportion of each
developmental outcome was significantly different from a mean proportion of that outcome across the egg age or egg state). All statistical analyses were carried out using SPSS (v.23) (IBM 2016) statistical software.

4.3 Results

4.3.1 Host acceptance behavior

*Trissolcus cultratus* females readily attacked and marked *H. halys* egg masses, but the relative proportions of drilled and marked eggs varied based on age and state (i.e., parasitized, fresh, frozen) of the egg masses (Fig. 4.1).

The proportion of control *H. halys* egg masses that *T. cultratus* females drilled (fresh: \(F_{(5,59)} = 1.56, p = 0.19\); frozen: \(F_{(5,59)} = 1.51, p = 0.20\)) or marked (fresh: \(F_{(5,59)} = 0.60, p = 0.70\); frozen: \(F_{(5,59)} = 0.86, p = 0.51\)) did not differ as a function of egg mass age, averaging more than 93% in all cases. A similar trend was observed when females were provided 0, 3, 4, and 5 day old parasitised *H. halys* eggs (Fig. 4.1). Significantly fewer egg masses parasitized by *T. japonicus* one and two days prior to testing were drilled (\(F_{(5,119)} = 37.1, p < 0.001\); Fig. 4.1a) and marked (\(F_{(5,117)} = 26.4, p < 0.001\); Fig 4.1b) by *T. cultratus* females.

4.3.2 Developmental success and interspecific larval competition

The proportion of *T. cultratus* larvae successfully developing in fresh \(\chi^2_{(10, N=707)} = 76.2, p < 0.001\) and frozen \(\chi^2_{(5, N=663)} = 169.1, p < 0.001\) *H. halys* eggs varied as a function of egg mass age. Less than 1% of <24 h old eggs supported the development of *T. cultratus* while no parasitoids emerged from those >24 h (Fig. 4.2a), despite observed drilling and
Figure 4.1 Mean proportion (±SE) of (unparasitized) fresh or frozen H. halys egg masses, as well as fresh egg masses previously parasitized by T. japonicus which were a) drilled and b) marked by T. cultratus as a function of age/time since parasitized by T. japonicus. Bars not sharing the same letters are statistically different based on Tukey's post hoc test. For each time interval, n=20.
Figure 4.2 Mean proportion of *T. japonicus*, *T. cultratus*, *H. halys* nymphs or nothing emerging from different aged *H. halys* eggs that were a) fresh, b) frozen or c) multiparasitized by *T. japonicus* and *T. cultratus* at different time intervals (ages). Asterisks (*) indicate proportions of each outcome for fresh, frozen, and multiparasitized eggs that is significantly different from a mean proportion of that outcome across the egg age ($\chi^2$ tests with Bonferroni corrections).
marking behaviours (Fig. 4.1a and b). However, parasitoid attack did reduce the number of *H. halys* that emerged. In contrast, some *T. cultratus* (4-54%) successfully developed in 0-4 day-old frozen egg masses although the proportion declined with age of the egg mass (Fig. 4.2b).

In contrast to the pattern seen with fresh, unparasitized *H. halys* eggs (Fig. 4.2a), some *T. cultratus* were able to successfully complete development when exploiting eggs that had previously been parasitized by the exotic *T. japonicus* (Fig. 4.2c). The relative developmental success of both parasitoids was influenced by the time elapsed between *T. japonicus* and *T. cultratus* females ovipositing ($\chi^2_{(10, N=942)} = 746.8$, $p < 0.001$). If the time delay was two days or less, then at least 75% of the hosts gave rise to *T. japonicus* while less than 15% gave rise to *T. cultratus*. A similar pattern was observed when the time delay between oviposition was 5 days, but if there was a 3-day difference then the majority of parasitized host eggs produced *T. cultratus*. When there was a 4-day time difference between oviposition very few parasitoids of either species emerged, whereas in the 5-day interval treatment a high proportion of *T. japonicus* adults emerged (Fig. 4.2c).

### 4.3.3 Egg mass guarding behavior

Following parasitization of fresh *H. halys* egg masses (28 eggs/ mass), 90% of *T. japonicus* females guarded the masses for less than 6 or 12 h, and were found either parasitizing a second egg mass, or exploring Petri plates in search of alternate resources. Only a single female remained on the parasitized egg mass for 24 h.
4.4 Discussion

The intertrophic level effects of introducing a novel potential host into an ecosystem will depend in part on the decision making behaviours of parasitoids that are based on the use of reliable cues during host habitat location, host location, host acceptance and host suitability (Vinson 1976) to maximise reproductive success (Williams and Nichols 1984; Schlaepfer et al. 2005). Thus, if a new host is unsuitable for development this could result in an evolutionary trap for parasitoids unless at some stage in the foraging process females avoid unsuitable hosts (Phillips and Shine 2004) or can overcome the defensive barriers of the host (Keeler and Chew 2008). Our results support the hypothesis that the introduction of *H. halys* represents a potential evolutionary trap that could impact the diversity and population dynamics of native Pentatomidae and their scelionid parasitoids (Abram et al. 2014), given that native parasitoids accept *H. halys* as a host but fail to complete development in healthy eggs (Abram et al. 2014; Haye et al. 2015). It has also been observed that introduced species often facilitate one another’s establishment, resulting in an “invasional meltdown” (Simberloff and Von Holle 1999; Simberloff 2006), and even the introduction of a beneficial species for biological control may interact with other introduced species in a way that negatively impacts native species (Howarth 1985; Schellhorn et al. 2002; Messing et al. 2006). The facilitation of native species by non-indigenous ones has been largely overlooked (Rodriguez 2006) as has interspecific facilitation among parasitoids (Cusumano et al. 2016).

Our results indicate that the introduction of *T. japonicus* as a biological control agent against *H. halys* may provide native *Trissolcus* species a partial escape from the evolutionary trap created by the presence of *H. halys* because, under certain conditions, *T.*
cultratus may successfully develop in H. halys eggs previously attacked by T. japonicus (Fig. 4.2c). To our knowledge, this is the first example of interspecific facilitation among egg parasitoids, and the first example of a secondary invader (T. japonicus) potentially facilitating the use of a primary invader (H. halys) as host by a native species (T. cultratus). The time window for this “invasional lifeline” will be quite narrow for several reasons (Fig. 4.3). For example, during the first 24 hours following the initial parasitization, T. cultratus will have limited access to the parasitized egg masses due to egg guarding by T. japonicus (behavioural inaccessibility). Furthermore, competitive inaccessibility will prevent development in eggs parasitized 1 and 2 days earlier, while physical or physiological inaccessibility will limit development in eggs with fully developed pupae of the competitor (i.e. 4 and 5 days). Therefore the “invasional lifeline” will exist only where T. japonicus becomes well established in areas where H. halys are found.

We assume that factors injected by T. japonicus females to overcome host defences, allow T. cultratus to exploit H. halys as a primary parasitoid in a small percentage of attacks that occur immediately after T. japonicus has oviposited (see day 0 in Fig. 4.2c). However, as T. japonicus females remained on the egg mass for 6-12 h following oviposition (patch guarding) the likelihood of T. cultratus females finding recently parasitized, unguarded H. halys egg masses would be low under natural conditions.

The fact that T. cultratus females attacked and marked significantly fewer H. halys eggs 1 and 2 days after they had been previously attacked by T. japonicus when compared to day 0 (Figure 4.1c), suggested that they are responding to the presence of the developing T. japonicus larvae rather than the presence of a marking pheromone. Avoiding eggs at this
Figure 4.3 General schematic showing the temporal pattern of suitability of parasitized and unparasitized *H. halys* eggs for *T. cultratus* as a function of their age (D = days).
stage of *T. japonicus* development would be adaptive, as few or no *T. cultratus* adults emerged from those that were attacked (Fig. 4.2c), suggesting that the early *T. japonicus* larval instars, equipped with sickle-shaped mandibles, are superior competitors and effectively eliminate eggs or newly hatched *T. cultratus* larvae. In contrast, *T. cultratus* was successful when attacking *H. halys* eggs containing fully grown *T. japonicus* larvae (e.g. three days following the initial parasitization by *T. japonicus*; Fig. 4.2c). In this case *T. cultratus* acts as a facultative hyperparasitoid on *T. japonicus*, a tactic used by some egg parasitoids when in an environment with a limited unexploited resource (Brodeur 2000). For example, the generalist egg parasitoid, *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae), is capable of exploiting pentatomid egg masses either as a primary parasitoid or as a facultative hyperparasitoid when eggs have already been parasitized by *Trissolcus basalis* (Wollaston) (Cusumano et al. 2013).

Even though *T. cultratus* females readily accept and mark eggs attacked 4 and 5 days previously by *T. japonicus* (Fig. 4.1a and b) they are no longer suitable hosts (Fig. 4.2c). This developmental insuitability may be due to the salivary secretions produced by parasitoid larvae just prior to pupation that solidify to provide protection from foreign bodies (Safavi 1968) and the subsequent sclerotization of the pupal exoskeleton (Volkoff and Colazza 1992). This is similar to the situation observed when *T. cultratus* females were provided *H. halys* eggs that had been frozen 3-5 days after being laid (Fig. 4.2b); while eggs were attacked and marked (Fig. 4.1) very few progeny were produced (Fig. 4.2) suggesting that the advanced nymphal stages of *H. halys* were unsuitable for *T. cultratus* larvae. This effect of host developmental stage has been observed in other *Trissolcus* species, with females attacking all developmental stages of host eggs and successful
parasitism decreasing significantly in eggs > 3 days old (Awadalla 1996; Kivan and Kilic 2005).

As noted, facultative hyperparasitism has not been extensively studied and it is possible that many scelionid parasitoids associated with stinkbugs use this strategy. Thus, *T. japonicus* may also develop as a facultative hyperparasitoid on congeneric species and could exploit native scelionids developing within other pentatomid species. Intraguild predation in the form of facultative hyperparasitism may reduce the success of a biological control program (Messing et al. 2006; Boivin and Brodeur 2006) or promote conservation and stabilization of natural communities (Müller and Brodeur 2002). Consequently, we suggest that the possibility of facultative hyperparasitism should be investigated when assessing risk factors to native species following the introduction of exotics for classical biological control.

The actual impact (at least short term) of this potential evolutionary trap will depend on a number of other factors, such as (i) the relative densities of the introduced and indigenous host egg masses and (ii) the degree to which native parasitoids prefer native hosts over introduced ones, as the impact would be less if females of different species discriminate in their selection of oviposition sites. Furthermore, as *T. japonicus* is a generalist parasitoid of Pentatomidae, it could exploit native stinkbug egg masses following its introduction as a classical biological control agent against *H. halys* (Talamas, Herlihy et al. 2015). If this is the case, interspecific competition could result in a significant decline in native parasitoid populations, especially if, under field conditions, indigenous species readily accept *H. halys* eggs. As noted above for native species, the outcome will also depend on whether *T. japonicus* preferentially oviposit in *H. halys* egg masses.
Clearly, the preference of foraging native parasitoids and *T. japonicus* must be evaluated in order to assess the real impact that *H. halys* will have on food webs following its introduction into new ecosystems. In the long-term, it is possible that native parasitoids successfully developing on *H. halys* eggs previously parasitized by *T. japonicus* will adapt to recognize and seek out cues indicative of both the acceptability and developmental suitability of *H. halys* as a host. It is therefore essential that all potential interactions between native and exotic egg parasitoids be investigated as this information will be vital to predict potential ecological outcomes, including the efficacy of biological control programmes against *H. halys*.

### 4.5 References


Cusumano A, Peri E, Colazza S. 2016. Interspecific competition/facilitation among insect
Pimentel D, Lach L, Zuniga R, Morrison D. 2000. Environmental and economic costs of
Talamas EJ, Herlihy M V., Dieckhoff C, Hoelmer KA, Buffington M, Bon M-C, Weber


Chapter 5

Understanding the mismatch between behaviour and development in a novel host-parasitoid association

A version of this chapter has been accepted for publication in *Scientific Reports* (https://www.nature.com/srep/)

Konopka JK, Poinapen D, Gariepy T, McNeil JN.
5.1 Introduction

The preference-performance hypothesis postulates that female insects will oviposit in or on hosts most suitable for their offspring’s development (Gripenberg et al. 2010). This preference should be most evident when the progeny are physically constrained to a limited resource, such as a parasitoid female laying in/on a host that will serve as the sole food source for her progeny. The general behaviours of foraging egg parasitoid females culminating in oviposition have been well described (Bin et al. 1993; Quicke 1997; Vinson 1998; Fatouros et al. 2008). The first steps, finding a suitable habitat and searching for a host, involve the chemical cues emitted by the hosts and/or those associated with the host (e.g. host plants, infochemicals left by an ovipositing herbivore) (Mumm and Dicke 2010; Fatouros et al. 2012; Hilker and Fatouros 2015; Ponzio et al. 2016). Once a female locates a host egg mass, she assesses the suitability of this host by antennation and ovipositor probing (Mattiacci et al. 1993; Borges et al. 2003; Silva et al. 2006; Laumann et al. 2009; Michereff et al. 2016). If the host is found suitable, she then oviposits, also and leaves an oviposition deterrent pheromone (Roitberg and Mangel 1988; Hofsvang 1990; Bin et al. 1993; Colazza et al. 1996).

At any step in this behavioural sequence, the female’s decision will be influenced by several factors. These factors include the female’s biological and physiological state (e.g. age, egg load, nutritional or mating status, previous oviposition experience), or the perceived quality of the egg mass or individual eggs within the mass (e.g. age, size, shape, chemical cues, parasitized state or presence of a competitor) (Strand and Vinson 1983; Minkenberg et al. 1992; Weber et al. 1996; Hirose et al. 2003; Boivin et al. 2004; Cusumano et al. 2012). Other factors such as genetic variability of the parasitoids (e.g.
differences in receptor sensitivity or activity levels), status of the host eggs (i.e. if the host eggs are fertilized or viable), or environmental stochasticity, can also affect the female’s decision (Kivan and Kilic 2005; Abram et al. 2015; Yang et al. 2016).

The introduction of a new (exotic) host species into the system can add a further dimension to the complexity of host acceptance. An introduced new host may disrupt the link between reliability of cues and the subsequent adaptive outcome, possibly leading to an evolutionary trap, especially if previously reliable cues are no longer indicative of developmental success of the progeny (Schlaepfer et al. 2002; Schlaepfer et al. 2005; Berthon 2015). Subsequently, the developmental outcome (performance) cannot be reliably predicted by the female’s choice of the host (preference).

In North America and Europe, native scelionid egg parasitoids (Telenomus and Trissolcus), are faced with this problem following the introduction of Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) from Asia. These native parasitoids readily oviposit in H. halys egg masses, but their offspring rarely complete development (Abram et al. 2014; Haye et al. 2015). So far, no clear explanation or mechanism has been proposed for this mismatch between behavioural acceptance by these females and lack of larval development, even though significant efforts are being made to characterize and understand the impact H. halys has on the native parasitoid communities following its introduction (Abram et al. 2017).

The surface chemicals, age and physical state (fresh or frozen) of the egg, as well as the presence of intrinsic or extrinsic competitors, are known to influence the female acceptance behaviours when encountering H. halys egg masses (Haye et al. 2015; Tognon et al. 2016, Chapters 3 and 4). Although such studies provide valuable information about
this host-parasitoid system, they usually focus on evaluating one or two factors at a time. Yet, it is important to understand the relative importance and reliability of each cue at different steps leading up to oviposition and marking. An ideal approach would involve testing multiple factors simultaneously for better understanding of their relative importance in the different decision making steps taken by foraging females (i.e. what those decisions are being influenced by the most), and the subsequent development of their progeny. A full factorial design incorporating all factors that may influence parasitoid behaviour and development is tedious and time consuming, and makes meaningful interpretation of the results difficult.

Therefore, a systematic and unbiased approach to screen and rank the most influential factors affecting measured outcomes (in this case parasitoid behaviour and development), while simultaneously reducing experimental variability is needed. An orthogonal array (OA) design (Taguchi 1986; Taguchi 1987) is ideal to overcome many of the challenges posed by the host-parasitoid system involving *H. halys*. This method allows the determination of critical factors and their optimal combination that affect (maximize or minimize) measured response variables (e.g. behaviour and development), and the ranking of their relative importance, as successfully applied in molecular biology, agriculture, biotechnology, and more recently in insect behaviour (Cobb and Clarkson 1994; Rao et al. 2008; Poinapen et al. 2013; Poinapen et al. 2017).

We used the OA method to assess and rank the influence of several factors characterizing the host resource (host species, age of the eggs, status of the eggs, and presence of egg surface chemicals). We regarded these factors as critical to understand the discrepancy between the host acceptance behaviour of a common North American egg
parasitoid, *Trissolcus euschisti* (Ashmead) (Hymenoptera: Scelionidae), exploiting stink bug host egg masses (including *H. halys*), and the ability of the parasitoids to complete development in those egg masses.

5.2 Materials and Methods

5.2.1 Insect colonies

Eggs of three stink bug species were used, obtained from colonies (26 °C, 70% RH, 16L:8D photoperiod), established using field collected adults from Hamilton and London (ON, Canada) in 2012, and restocked annually. Two native species, the polyphagous *Euschistus variolarius* (Palisot de Beauvois) and the predatory *Podisus maculiventris* (Say), and the exotic *H. halys* were selected. Adults of *H. halys* and *E. variolarius* were held in BugDorm mesh cages (45×45×45 cm; ~ 50 adults/ cage) and fed an organic diet (to avoid pesticide residue) consisting of romaine lettuce, carrots, apples, dry peanuts and soybeans, supplemented with zucchini, celery, and green beans when available. Adults of *P. maculiventris* were kept in plastic buckets (height = 15 cm; diameter = 15 cm) and fed *Tenebrio molitor* L. larvae. Stink bug egg masses were collected daily from the cheesecloth provided as an oviposition substrate.

Colonies of *T. euschisti* and *Telenomus podisi* (Ashmead) were established from parasitoid adults emerging from sentinel egg masses exposed in London in 2016, and maintained at 24 °C, 50% RH, 16L:8D photoperiod. Colony parasitoids were kept in clear plastic cups (height= 4 cm; diameter= 9 cm), provided with a honey water solution and fresh *P. maculiventris* egg masses once a week for oviposition. Parasitized colony egg masses were kept separately until adult emergence and experimental females were selected upon emergence from those egg masses. Experimental female parasitoids were collected
daily and held in the presence of males but no host egg masses, until being used in the experiments.

5.2.2 Orthogonal array

We employed an L9 (3^4) (four factors at three levels) orthogonal array (OA) design (Table 5.1) to determine the relative importance of different cues on oviposition decision and development of *T. euschisti* (Table 5.2), focusing on main effects only (i.e. no interactions). This method allows us to not only determine which factors have an effect on parasitoid behaviour and development, but also to rank the relative importance of those factors (i.e. their relative influence on the measured response variables). The added advantage of OA design compared to full factorial design is the reduced number of experiments to run (9 for an L9 OA design vs. 81 for a full factorial with the same number of tested factors). Since different interactions can exist between tested factors for each measured outcome variable, we decided to use OA design that puts less emphasis on those interactions. We focused on main effects only because our interest was in the overall effect of each factor on several different measured outcomes associated with female behaviour and progeny development assessed simultaneously, as opposed to in isolation.

In our design (Tables 5.2 and 5.3), the factors chosen to investigate the effect of the host egg mass on the parasitoid choice included: host species (*P. maculiventris, E. variolarius*, and *H. halys*), age of the eggs (1, 2 and 3 d), status of the eggs (parasitized, fresh, and frozen), and surface solvent wash (70 % acetone, dH_2O, and no wash). Since
Table 5.1 L9 standard orthogonal array indicating combination of factors (4 factors each with 3 levels) and experiments (9 independent ones) to be performed.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Factor A</th>
<th>Factor B</th>
<th>Factor C</th>
<th>Factor D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 5.2 Factors (and their respective levels) for the no-choice tests of *Trissolcus euschisti* (Hymenoptera: Scelionidae) host acceptance and development on stink bug (Hemiptera: Pentatomidae) egg masses.

<table>
<thead>
<tr>
<th>FACTORS</th>
<th>Host Species</th>
<th>Age of eggs (days)</th>
<th>Status of eggs</th>
<th>Surface wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEVELS</td>
<td><em>Podisus maculiventris</em></td>
<td>3</td>
<td>parasitized</td>
<td>acetone</td>
</tr>
<tr>
<td></td>
<td><em>Halyomorpha halys</em></td>
<td>2</td>
<td>frozen</td>
<td>water</td>
</tr>
<tr>
<td></td>
<td><em>Euschistus variolarius</em></td>
<td>1</td>
<td>fresh</td>
<td>none</td>
</tr>
</tbody>
</table>
Table 5.3 An L9 orthogonal design with factor combination for the no-choice tests of *Trissolcus euschisti* (Hymenoptera: Scelionidae) host acceptance and development on stink bug (Hemiptera: Pentatomidae) egg masses.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Host species</th>
<th>Age of eggs</th>
<th>Status of eggs</th>
<th>Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Podisus maculiventris</em></td>
<td>3</td>
<td>parasitized</td>
<td>acetone</td>
</tr>
<tr>
<td>2</td>
<td><em>Podisus maculiventris</em></td>
<td>2</td>
<td>frozen</td>
<td>water</td>
</tr>
<tr>
<td>3</td>
<td><em>Podisus maculiventris</em></td>
<td>1</td>
<td>fresh</td>
<td>none</td>
</tr>
<tr>
<td>4</td>
<td><em>Halyomorpha halys</em></td>
<td>3</td>
<td>frozen</td>
<td>none</td>
</tr>
<tr>
<td>5</td>
<td><em>Halyomorpha halys</em></td>
<td>2</td>
<td>fresh</td>
<td>acetone</td>
</tr>
<tr>
<td>6</td>
<td><em>Halyomorpha halys</em></td>
<td>1</td>
<td>parasitized</td>
<td>water</td>
</tr>
<tr>
<td>7</td>
<td><em>Euschistus variolarius</em></td>
<td>3</td>
<td>fresh</td>
<td>water</td>
</tr>
<tr>
<td>8</td>
<td><em>Euschistus variolarius</em></td>
<td>2</td>
<td>parasitized</td>
<td>none</td>
</tr>
<tr>
<td>9</td>
<td><em>Euschistus variolarius</em></td>
<td>1</td>
<td>frozen</td>
<td>acetone</td>
</tr>
</tbody>
</table>
colony parasitoids had been reared on *P. maculiventris* eggs, egg masses of this species (fresh, 1 d old, unrinsed) served as a control (Experiment 3, Table 5.3). Therefore, we expected this treatment to give the best performance both by foraging females and developing progeny.

The goal of the OA analysis is to identify factors that reduce variability in the outcome variables, by minimizing the effect of noise factors (factors which are not easily controlled), to subsequently identify optimal factor settings. This process was done by calculating a signal to noise ratio (S/N ratio) for each factor and level, to determine which of the four factors (host species, age of eggs, status of eggs, and surface wash) reduced the variability in the outcome variables (behaviour and development). The calculated difference between maximum and minimum S/N ratios for each response variable (Delta (Δ)) provides a quantification of the effect of each factor on each outcome variable, and is used to rank the influence of each factor on each response variable.

For egg mass acceptance, patch exploitation (number of eggs drilled, marked, and superparasitized), and progeny development, calculation of the S/N ratios were done to maximize the response (larger the better) using equation 1 (higher values indicate higher host attractiveness and resource utilization). The response was minimized (smaller the better) for S/N of patch residence (time on egg mass, time drilling, time drilling/egg) using equation 2 (lower values indicate more efficient use of time on a resource),

\[
S/N_i = -10 \log \left( \frac{\sum (\bar{y}^2)}{N_i} \right)
\]  
\[
S/N_i = -10 \log \left( \frac{\sum (1/\bar{y}^2)}{N_i} \right)
\]

where \(\bar{y}_i = \frac{1}{N_i} \sum_{u=1}^{N_i} y_{i,u}\) is the mean; \(i=\) experiment number, \(u=\) trial number, and \(N_i=\) number of trails for experiment \(i\).
5.2.3 *Experimental set up*

Fresh stink bug egg masses were collected daily from cheesecloth (oviposition substrate), separated into smaller clusters (12 eggs/mass), attached with the substrate to square pieces of white cardboard (~1×1 cm) with clear non-toxic glue (Ross®, Canada), and held in individual small round (5×1 cm) Petri dishes until needed. For each age category, egg masses were subjected to different treatments before being assayed using the OA design (Table 5.3):

(i) egg masses were either fresh, or frozen for 5 min at -80 °C approximately 30-60 min before testing.

(ii) to obtain the ‘parasitized’ egg state, individual fresh egg masses were exposed to *T. podisi* females (1 female/egg mass) from the colony 24 h before the assay. All exposures were carried out under a stereomicroscope to ensure all the eggs were parasitized.

(iii) egg masses were left unrinse or were immersed three times (10 s each wash) in approximately 1.5 mL of either 70% acetone (removal of cues with kairomonal activity) or distilled water (partial removal of water soluble cues) (Bin et al. 1993; Borges et al. 1999; Michereff et al. 2016). The rinsed eggs were allowed to dry completely before use. Surface washes are meant to remove host-deposited chemical cues, and parasitoid marking chemicals (in the case of “parasitized” egg status).

Fifteen, 4 day old, mated, naïve *T. euschisti* were tested for each of the nine experiments (i.e. n= 15 females for each experiment) (Table 5.3), and each individual was used only once. All assays were performed in individual Petri dishes at 24±1 °C, 50% RH.
during the first 8 h of the scotophase (active period of parasitoids). Each parasitoid female was observed under a stereomicroscope, and was removed once all eggs were parasitized, or when the female showed no further interest in the egg mass (i.e. no antennation or exhibition of egg mass guarding behaviour).

The following parameters were measured: i) if the female accepted the egg mass (as indicated by arrestment behaviour and substrate drumming followed by oviposition), (ii) patch residence (time from the first contact until the female finished marking); iii) total time spent drilling and time drilling per egg as measures of decision making once on a resource; iv) number of eggs drilled (those into which female inserted her ovipositor and marked (those on which a female dragged her ovipositor following oviposition) reflecting host attractiveness and perceived suitability for development of the progeny; (v) the incidence of superparasitism, where a female oviposits and marks an egg twice, and vi) host suitability (offspring survival to adulthood from the different egg masses).

5.2.4 Statistical analysis

Patch residence data (time on mass, time drilling, and time/egg) were checked for normality using Shapiro-Wilks and Levene’s tests, and then analyzed by one-way ANOVA with Tukey’s post hoc test following a logarithmic transformation. All other data (proportion of egg masses accepted, proportion of eggs drilled, marked and superparasitized) were analysed by χ² tests, with values for each cell calculated based on standardized residuals (crosstab analysis) and compared with Bonferroni-corrected p values. Statistical analyses of qualitative and quantitative data were carried out using SPSS (v. 24) (IBM, 2016) statistical software. The impact factors (host species, egg age, egg
status, and surface solvent wash) were ranked based on the L9 OA designed and analyzed in Minitab statistical software (v. 18 Minitab, Coventry, UK).

5.3 Results

5.3.1 Behaviour

*Trissolcus euschisti* females accepted *H. halys* and *E. variolarius* egg masses washed in acetone (Experiments 5 and 9 respectively), 53% and 40% less often as oviposition sites, respectively \((\chi^2 (8, N=135) = 56.84, p < 0.001)\) than all other egg masses, including the control (Experiment 3; freshly laid non washed *P. maculiventris* eggs) (Fig. 5.1). Surface chemical cues and host species were the two most important factors affecting host acceptance (Table 5.4). Surface chemicals were 2.5 times more important than egg age, which was ranked last.

The estimates of patch residence, total time spent on an egg mass \((F(8, 105) = 6.16, p<0.001; \text{Fig. 5.2a})\), the total time spent drilling \((F(8, 104) = 7.22, p<0.001; \text{Fig. 5.2b})\), and the time to drill each egg \((F(8, 104) = 10.52, p<0.001; \text{Fig. 5.2c})\), followed similar patterns. The majority of time spent on an egg mass was allocated to oviposition (as indicated by drilling), and the remainder to unsuccessful parasitization attempts and surface antennation (those behaviours accounted for the time not spent drilling). Thus, it is not surprising that the times spent on control egg masses (Experiment 3) were the shortest, while the longest times were observed on old, surface-washed eggs of native stinkbug species. Age ranked as the most important factor for all three estimates of patch residence (i.e. total time, total time drilling, and total time drilling per egg), followed by surface chemistry (Table 5.4).
Figure 5.1 Proportion of host egg masses accepted by *T. euschisti* females in nine experiments based on L9 (3⁴) orthogonal array (OA) design. Bars with asterisks (*) indicate experiments where the acceptance was significantly lower (p < 0.05) based on χ² test. For each experiment, n=15 females.
Table 5.4 Ranking of factor based on influence on *T. euschisti* egg parasitoid host acceptance, patch residence, patch exploitation, and progeny development from stink bug host egg masses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Signal to noise ratio of factors (Δ S/N)</td>
<td>Host species</td>
<td>Egg age</td>
<td>Egg status</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acceptance</td>
<td>Number of egg masses accepted</td>
<td>3.55 (2)</td>
<td>1.75 (4)</td>
<td>2.71 (3)</td>
</tr>
<tr>
<td>Patch residence</td>
<td>Total time on egg mass</td>
<td>0.60 (4)</td>
<td>1.99 (1)</td>
<td>1.59 (2)</td>
</tr>
<tr>
<td></td>
<td>Total time drilling</td>
<td>1.23 (3)</td>
<td>2.25 (1)</td>
<td>0.75 (4)</td>
</tr>
<tr>
<td></td>
<td>Time drilling/egg</td>
<td>1.02 (3)</td>
<td>1.85 (1)</td>
<td>0.66 (4)</td>
</tr>
<tr>
<td>Patch exploitation</td>
<td>Eggs drilled</td>
<td>0.10 (1)</td>
<td>0.09 (2)</td>
<td>0.04 (4)</td>
</tr>
<tr>
<td></td>
<td>Eggs marked</td>
<td>1.48 (1)</td>
<td>1.21 (2)</td>
<td>0.56 (4)</td>
</tr>
<tr>
<td></td>
<td>Eggs superparasitized</td>
<td>12.09 (3)</td>
<td>1.61 (4)</td>
<td>17.60 (1)</td>
</tr>
<tr>
<td>Progeny development</td>
<td>Proportion of <em>T. euschisti</em> emerged</td>
<td>10.00 (2)</td>
<td>6.05 (4)</td>
<td>20.52 (1)</td>
</tr>
</tbody>
</table>

Note: Delta (Δ) represents the difference between the maximum and minimum S/N for each factor at 3 levels (values represent the Δ, followed by a relative rank in brackets (ranks are assigned for each row individually). Factors with higher Δ have greater influence on a given measured variable (1= most influence; 4= least influence).
Figure 5.2 Mean (± SE) a) time spent on egg mass b) total time spent drilling, and c) time spent drilling per egg (for successful parasitization attempts) by *T. euschisti* females in nine experiments based on L9 \((3^4)\) orthogonal array (OA) design, summarizing behaviour of the parasitoid on host egg masses. Bars with the same lower case letter within each panel are not significantly different based on Tukey’s post hoc test \((\alpha = 0.05)\). For each experiment, \(n=15\) females.
Age was up to 3 times more influential than host species for total time on egg mass and egg status for time drilling.

The proportion of eggs drilled and marked (on each prepared egg mass), and the incidence of superparasitism (a reflection of patch exploitation) did not vary among most treatments (Figure 5.3). However, the response to parasitized and frozen, surface-washed, *P. maculiventris* egg masses (Experiments 1 and 2) differed significantly from all other treatments. The proportion of those eggs drilled was ~20% lower in Experiment 2 ($\chi^2(8, N=1380) = 118.18, p < 0.001$), while the proportion marked was up to 30% lower in both Experiments 1 and 2 ($\chi^2(8, N=1380) = 135.57, p < 0.001$). Furthermore, the proportion of superparasitism observed was up to 13% higher in Experiment 1 compared with all other experiments ($\chi^2(8, N=1295) = 92.61, p < 0.001$). For both egg drilling and marking, host species and egg age were the two highest ranking factors (host species was 2.5 times more important than egg status), while egg status and surface cues were most important for superparasitism (egg status was 1.5 and 11 times more important than host species and egg age, respectively) (Table 5.4).

**5.3.2 Development**

The proportion of each developmental outcome (i.e. *T. euschisti*, *T. podisi*, host nymphs, or no development) from parasitized egg masses differed among the treatments ($\chi^2(24, N=1247) = 2230.7, p < 0.001$). Stink bug nymphs emerged only from fresh and parasitized *H. halys* egg masses. In most experiments, there was a proportion of eggs (2-66%) that produced no parasitoids or hosts (Fig. 5.4). *Trissolcus euschisti* development depended on host species and status of the eggs ($\chi^2(8, N=1248) = 722.30, p < 0.001$), with 70-90% emerging from fresh (Experiments 3 and 7) or frozen (Experiments 2 and 9)
Figure 5.3 Mean (± SE) proportion of eggs drilled, marked, and superparasitized by *T. euschisti* females in nine experiments based on L9 \( (3^4) \) orthogonal array (OA) design, summarizing behaviour of the parasitoid on host egg masses. Bars with asterisks (*) indicate experiments where the means are significantly different \( (p < 0.05) \), among the different experiments based on \( \chi^2 \) tests for each measured outcome. For each experiment, \( n=15 \) females.
Figure 5.4 Mean proportion of *Trissolcus euschisti*, *Telenomus podisi*, host nymphs (*Podisus maculiventris*, *Halyomorpha halys*, or *Euschistus variolarius*) or nothing emerging from egg masses parasitized by *T. euschisti* females in nine experiments based on L9 (3⁴) orthogonal array (OA) design, summarizing development of the parasitoid on host egg masses. Asterisks (*) indicate proportions of each outcome that is significantly different from a mean proportion of that outcome across each of the nine experiments (based on χ² tests with Bonferroni corrections).
native host eggs, and *P. maculiventris* being most suitable host for development (Fig. 5.4). However, *T. euschisti* completed development in < 6% of native hosts previously parasitized by *T. podisi* (Experiments 1 and 8), with most multiparasitized egg masses producing *T. podisi*.

*Halyomorpha halys* eggs are clearly less suitable than native pentatomid species as < 40% of frozen *H. halys* egg masses (Experiment 4) yielded *T. euschisti*, compared with 70-90% from frozen native host eggs (Experiments 2 and 9). Similarly, in both fresh *H. halys* eggs and those parasitized by *T. podisi* (Experiments 5 and 6), most eggs yielded stinkbug nymphs and < 10% gave rise to parasitoids. Interestingly, only a small proportion of multiparasitized *H. halys* eggs resulted in parasitoid adults (mainly *T. euschisti*); in contrast multiparasitized eggs of native hosts (Experiments 1 and 8) yielded > 95% parasitoid adults (mainly *T. podisi*). The most important factor for successful development of *T. euschisti* was egg status, followed by host species. Egg status was 2 times more influential than host species, and 3 times more important than both surface wash, and egg age (Table 5.4).

5.4 Discussion

All cues that were investigated provided foraging *T. euschisti* females with information leading up to oviposition, but their relative importance changed at different steps in the sequence of behaviours. The chemical cues from the egg surface and host species (undoubtedly inter-related) were the most important in the decision to approach and investigate a potential host. Without these cues (i.e. likely at least partially removed when the eggs were washed in acetone), a reduction in arrestment and substrate drumming
was observed. The amount of time spent on the host egg mass and time spent drilling mainly depended on the age of the host eggs. Nevertheless, the actual decision to drill and mark the eggs following ovipositor probing was influenced by the host species identity. Following oviposition into the host, the likelihood of successful development of the parasitoid larvae was dependent on the egg status or viability of the host (fresh or frozen) and presence of competitors (parasitized egg state) (Table 5.5).

Our study demonstrated that the behaviour and development of *T. euschisti* egg parasitoids are influenced by separate and distinct critical factors associated with the host egg resource. Although the predominant factor influencing parasitoid female behaviour once the female accepted the resource and started ovipositing was the age of host eggs, this factor was ranked as least important for the successful development of progeny. Similarly, the predominant factor influencing development of progeny (egg status) was ranked last among factors influencing behaviour. This reversed ranking of critical factors represents a mismatch between behaviour and development, and questions the reliability and relative importance of different cues for the foraging parasitoid females. The fact that parasitoid development, but not the behaviour of the foraging females, was mostly influenced by the presence of competitors and viability of the host (i.e. status of the egg), indicates that native egg parasitoids are making a maladaptive decision to oviposit in an unsuitable host. This decision is linked to a mismatch between the cues females are able to detect, and the subsequent expected adaptive outcome of their choice.

To maximize fitness gain, foraging parasitoid females can either increase the time spent in high quality patches (by spending more time assessing the suitability of a resource), or decrease the time spent per individual host within the patch once it is deemed
Table 5.5 Summary of decision making of foraging egg parasitoid (*Trissolcus euschisti*) on stink bug host egg masses based on the ranking of critical factors.

<table>
<thead>
<tr>
<th>Behaviour/outcome</th>
<th>Mode of resource assessment</th>
<th>Decision to make</th>
<th>Decision most influenced by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptance</td>
<td>Host searching</td>
<td>- Is this a potential host?</td>
<td>Surface chemical cues present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Should this resource be approached and investigated further?</td>
<td></td>
</tr>
<tr>
<td>Patch residence</td>
<td>Antenntion of the eggs</td>
<td>- How much time to spend on this patch?</td>
<td>Age of the host eggs</td>
</tr>
<tr>
<td>Patch exploitation</td>
<td>Ovipositor probing and ovipoition</td>
<td>- Should more eggs be drilled?</td>
<td>Host species</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Should eggs be marked?</td>
<td></td>
</tr>
<tr>
<td>Progeny development</td>
<td>Post oviposition</td>
<td>- Can host defences be overcome?</td>
<td>Host viability and presence of competitors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Are there competitors present?</td>
<td></td>
</tr>
</tbody>
</table>
acceptable (Wajnberg 2006). This time-allocation was observed when *T. euschisti* was provided young, fresh, intact *P. maculiventris* eggs (see Fig. 5.2 and Fig. 5.3, Experiment 3). Our results show that the age of the eggs is a very important cue for patch residence time (total time, time drilling) and patch exploitation (drilling and marking), indicating that information obtained once oviposition has been initiated affects the time spent on the egg mass. The finding that females spent the most time drilling the oldest eggs (as seen in Experiments 4, 5, and 7) of the native hosts with which they were familiar, is linked to the general decline in host quality with age, and relatively higher risk associated with parasitizing an older host (e.g. possible immune response of the developing host, difficulty in probing for small yolk volume, or not enough resources for completion of the development) (Strand et al. 1986; Vinson 1998; Hirose et al. 2003).

Our findings provide some explanation why eggs of exotic *H. halys* are accepted by native parasitoids as a host, even though they are unsuitable for progeny development. A reduction in host recognition and acceptance by *T. euschisti* was observed in acetone-washed *H. halys* and *E. variolarius* egg masses, but not in those of *P. maculiventris*, suggesting that in the latter case some chemical cues were not removed by the solvent used. This finding indicates that there might be species-specific differences in volatiles mediating parasitization of pentatomid eggs (Tognon et al. 2016), and thus a chemical similarity with a successfully-exploited native species could explain why *H. halys* eggs are perceived by foraging native parasitoid females as patches worth exploiting. Furthermore, once on the patch, the age of the eggs was almost as important as host species with respect to oviposition. Thus, there are sufficient cues to stimulate oviposition even though the host may be unsuitable for development of the progeny. Although Tognon et al. (2017)
suggested surface chemicals from *H. halys* eggs inhibited parasitization by native North American egg parasitoids, our behavioural data do not support this idea, at least for *T. euschisti*. In fact, *T. euschisti* females are quite willing to lay eggs in viable *H. halys*, but are unable to complete development, just like their European counterparts (Haye et al. 2015, Chapter 4). Since we did not investigate or assess the actual chemical components from the host eggs, further research is needed to identify the infochemicals present and determine their relative importance.

Based on data from the current study and in the literature, *H. halys* is not a high quality host once an egg has been laid, with the viability of the host and presence of the competitors being the highest ranked factors for the successful development of the parasitoid. Freeze-killed eggs increase the developmental suitability for North American and European egg parasitoids on *H. halys*, compared to fresh eggs (Haye et al. 2015, Chapter 4). This improved developmental success did not occur in eggs of native species like *P. maculiventris*, where freezing provides no additional advantage to *T. euschisti*. Yet, the number of progeny produced from frozen *H. halys* eggs was much lower, compared with frozen eggs of native hosts, suggesting that *H. halys* is inherently less suitable. Therefore, the presence of developing host embryo, is not the only factor preventing parasitoid development in fresh *H. halys* eggs. The difference in the suitability of *H. halys* and native host eggs for parasitoid development could be related to factors such as the physiological environment and/or the quality and quantity of available resources. For example, the initial egg content composition, as well as the changes in nutritional quality might differ between *H. halys* (Skillman and Lee 2017) and native hosts following freezing. Furthermore, in larger *H. halys* eggs, parasitoid larvae may be unable to consume the host
tissue fast enough, such that the decomposition of frozen host tissues results in an unsuitable environment.

Although European *T. cultratus* is capable of producing some progeny from fresh *H. halys* egg masses, by acting as a facultative hyperparasitoid on *T. japonicus* when in interspecific competition (Chapter 4), this phenomenon was not observed when *T. euschisti* was offered stink bug eggs previously parasitized by *T. podisi*. In the case of *H. halys*, lack of facultative hyperparasitism might occur because *T. podisi* is unable to develop or kill the eggs of this exotic host, and future research should explore the potential of *T. euschisti* (or other native North American parasitoids) to develop as facultative hyperparasitoids on *H. halys* eggs following successful exploitation by *T. japonicus*. For native stink bugs (*P. maculiventris* and *E. variolarius*) (Experiment 1 and 8, Fig. 5.4), *T. podisi* was the dominant parasitoid reared from the egg masses, suggesting that *T. euschisti* do not act as a facultative hyperparasitoid on *T. podisi*. Since facultative hyperparasitism usually occurs during a very specific time window in the developmental process (Van Alphen and Thunnissen 1982; Strand and Vinson 1984; Sullivan 1987; Cusumano et al. 2013), we did not observe it, given the short time interval between the first and second parasitizations in the current study.

The influence of host choice on parasitoid fitness is based on the premise that females make host selection using reliable cues that indicate past success, thus making their choice adaptive. From an ecological perspective, parasitoids are time- and egg-limited. The time and resources that parasitoids allocate to a resource patch will affect the number of progeny they are able to produce, and hence their fitness. The inability of the egg parasitoids to recognize hosts unsuitable for development of their progeny, whether this
unsuitability is due to the host species (e.g. an exotic species, such as *H. halys*) or the egg status of a known host (e.g. parasitized *P. maculiventris*), results in wasted reproductive effort, both in terms of time (time sink) and resources (egg sink) (Hoogendoorn and Heimpel 2002; Heimpel et al. 2003; Abram et al. 2014). A subsequent reduction in parasitoid populations could have both direct (e.g. increase in populations of native stink bugs) and indirect (e.g. increased apparent or direct competition) effects on trophic interactions between the stink bug and natural enemy communities (Holt 1977; Kenis et al. 2009).

Therefore, from an evolutionary perspective, selective pressure to avoid unsuitable hosts (Phillips and Shine 2004), or to overcome barriers to development in those hosts (Keeler and Chew 2008) is likely high for parasitoids (Abram et al. 2014; Abram et al. 2017). In the case of native parasitoids (including *T. euschisti*), there is considerable evidence that they do not avoid *H. halys* egg masses, in part because they provide cues similar to the native pentatomids with which they have co-evolved. The inability of parasitoids to discriminate between a suitable and unsuitable host resource would make *H. halys* an evolutionary trap (Abram et al. 2014), unless the native parasitoids are able to overcome the barrier to successful development. While *H. halys* has been present in North America only since the 1990s (Hoebeke and Carter 2003), three egg parasitoid genera (including *Telenomus*) have been recorded to have at least partial success on fresh *H. halys* egg under field conditions (Jones et al. 2014; Cornelius, Dieckhoff, Vinyard, et al. 2016). This partial success suggests that native parasitoids may have already started adapting to this novel *H. halys* host (Cornelius, Dieckhoff, Hoelmer, et al. 2016; Abram et al. 2017). This progressive adaptation of North American parasitoids to *H. halys* could possibly be
due to ecological and phylogenetic similarity of *H. halys* to the hosts that the parasitoids normally attack, thus shortening the time required for natural enemy recruitment (Vindstad et al. 2013).

From a biological control perspective, native parasitoids may provide limited control of *H. halys* via egg abortion (Abram et al. 2016), as seen in the reduction in emergence following parasitization by *T. euschisti* (either alone or with *T. podisi*), even though the parasitoids were unable to complete development. However, in a short term, a classical biological control approach using Asian egg parasitoids (e.g. *Trissolcus japonicus*) that have co-evolved with *H. halys* (Rice et al. 2014) or native eupelmid parasitoids (e.g. genus *Anastatus*) that are frequently reported from field collected egg masses (Jones et al. 2014; Haye et al. 2015) would be more effective. In a long term, the interspecific interactions between introduced and resident parasitoids could change as native species adapt to the exotic host.

While the OA approach reduces the time and resources required to screen and determine relative importance (i.e. ranking) of critical factors, it has some limitations. First, the L9 design used here tests main effects only, not accounting for interactions of the factors. There is no doubt that interactions do exist (Abram et al. 2017; Chapter 4), so further experiments can be carried out using a full factorial design once the most important factors have been determined as in the current experiments for each measured outcome (i.e. female behaviour and progeny development). Therefore, in our study, the OA serves as an initial screening method to minimize the amount of time and resources required to reach similar conclusion when using other methods (Rao et al. 2008). Additionally, there are some constraints associated with specific levels of factors used in our study, which might
affect the relative influence of those factors on the measured variables. For example, if wider range of host egg age was used, this factor might have stronger influence on female host acceptance.

Although this approach for determining the causes of the mismatch between behaviour and development in egg parasitoids is unconventional, our study enabled the ranking of certain critical factors. While individual factors may affect behaviour (acceptance, patch time allocation, host resource exploitation) and development of the parasitoids, it is the relative importance of individual factors when assessed simultaneously (as opposed to in isolation) that influences the decision making of the parasitoids. If the goal is to minimize host emergence and maximize natural enemy emergence (as would be necessary in a biological control program against *H. halys* using egg parasitoids), then utilizing the OA approach can significantly reduce the time and effort required to obtain critical information on the key factors affecting parasitoid behaviour and development. Consequently, this approach could provide a new way of interpreting host-parasitoid interactions (both from an ecological and evolutionary perspective), and enable more efficient screening of biological control agents, and their impacts on target and non-target organisms.

5.5 References


Poinapen D, Brown DCW, Beeharry GK. 2013. Seed orientation and magnetic field strength have more influence on tomato seed performance than relative humidity and duration of exposure to non-uniform static magnetic fields. J Plant Physiol. 170:1251–1258.


Schlaepfer MA, Sherman PW, Blossey B, Runge MC. 2005. Introduced species as


Vinson SB. 1998. The general host selection behavior of parasitoid Hymenoptera and a


Chapter 6

Timing of failed parasitoid development in *Halyomorpha halys* eggs

A version of this chapter will be submitted for publication in *Biological Invasions* (https://link.springer.com/journal/10530)

Konopka JK, Poinapen D, Holdsworth DW, Gariepy T, McNeil, JN.
6.1 Introduction

Shared evolutionary history shapes species interactions, with the number and diversity of interactions providing resilience to ecosystems (Vermeij 1994; Brockhurst et al. 2014). The introduction of new species can drastically modify the structure and stability of a food web (David et al. 2017). The early stages of interactions between native and exotic species are often characterized by rapid evolution of all species involved (Brockhurst et al. 2014; David et al. 2017). To persist, native species can either tolerate the presence of the exotic ones or exploit the invaders (Berthon 2015; David et al. 2017). For example, native parasitoids could use an exotic species as an alternative host resource, but this could result in serious constraints if females readily accept a new host that is unsuitable for the development of their progeny. These constraints may result from insufficient time (due to a lack of shared co-evolutionary history) required for the parasitoid populations to adjust their host-utilization preferences over evolutionary time in response to the offspring performance (or lack thereof) in this new host (Camara 1997; Feldman and Haber 1998; Janz 2002).

North American egg parasitoids (Hymenoptera: Scelionidae) encountering the invasive exotic *Halyomorpha halys* (Hemiptera: Pentatomidae) are no exception to such constraint. The results of previous experiments (outlined in Chapters 2-5) show that while native parasitoids parasitize eggs of *H. halys* under both field (Chapter 2) and laboratory conditions (Chapter 5), their progeny rarely develops in fresh eggs of this host (Chapters 4-5). As the acceptance of *H. halys* at least partially reduces reproductive effort (in terms of time and resources), native parasitoids should be under selection pressure to either avoid
*H. halys* as a host, or develop a way to overcome the barrier to development posed by this unsuitable host (Chapters 3-4).

Currently, we have little knowledge about the factors that limit native scelionid parasitoid development in *H. halys* eggs. No late-stage parasitoid larvae are detected following dissection of parasitized live *H. halys* eggs under laboratory conditions (Abram et al. 2014). Field surveys generally report low parasitoid emergence, but a small number of undeveloped (partially developed) parasitoids have been dissected out of sentinel or naturally laid *H. halys* egg masses (Cornelius, Dieckhoff, Vinyard, et al. 2016; Cornelius, Dieckhoff, Hoelmer, et al. 2016; Herlihy et al. 2016; Ogburn et al. 2016; Abram et al. 2017; Dieckhoff et al. 2017). Egg parasitoids develop rapidly at 27 °C reaching pupal stage about 5 days following parasitization (Volkoff and Colazza 1992). Since fully developed parasitoid larvae are never found in dissected *H. halys* eggs, larval development must stop soon after parasitization.

Host and parasitoid development times, combined with evidence of poor performance of native scelionids on fresh eggs of *H. halys*, suggest that the developmental barrier presented by *H. halys* eggs likely occurs within the first 5 days following oviposition, prior to host development and emergence. To elucidate the actual occurrence of events that might lead to failed development, the first step is to identify a precise time window when normal development stops. However, obtaining this information through dissection is challenging due to the small size of host eggs (diameter of ~1.3 mm for *H. halys* and ~0.88 mm for *Podisus maculiventris*) (Bundy and McPherson 2000; Hoebeke and Carter 2003).
Therefore, in this chapter, we use a DNA-based molecular method (with parasitoid- and host-specific primers of COI gene) to assess the development of North American native *Trissolcus euschisti* egg parasitoid at different time points (0-120 h post parasitization) in suitable (*P. maculiventris*) and unsuitable (*H. halys*) fresh and frozen eggs. Unparasitized eggs of both host species are used as a developmental baseline of the host. We further corroborate the findings with *in-situ* micro-computed tomography (micro-CT) imaging of intact parasitized and non-parasitized eggs.

### 6.2 Materials and Methods

#### 6.2.1 Egg mass parasitization and processing

Freshly laid (≤ 2 h) egg masses of *H. halys* and *P. maculiventris*, obtained from stink bug colonies housed at Agriculture and Agri-Food Canada (London, ON, Canada), were either frozen for 5 min at -80 °C or left unprocessed. Fresh and frozen egg masses (≥ 15 eggs per mass) were exposed to randomly selected *T. euschisti* females from a laboratory colony (≤ 4 females per egg mass), and observed using a stereomicroscope to ensure parasitization. Immediately after all eggs in the mass were parasitized, three eggs were taken at regular intervals (0, 6, 24, 48, 72, 96, and 120 h) and frozen at -80 °C for subsequent molecular analysis. The same process was followed for freshly laid egg masses that were not exposed to parasitoids to obtain a baseline for normal host development at the same intervals. All egg masses were kept at 24 °C and 60% RH between time point collections. The experiment was repeated three times for both *H. halys* and *P. maculiventris*, and samples from each time point were combined (n=9 eggs for each time point from fresh parasitized, frozen parasitized, and fresh non-parasitized).
A similar set of experiments (with changes described below) was performed to prepare samples for visualizing the longitudinal (i.e. temporal) development using micro-CT, a proven method for insect imaging (Metscher 2009; Lowe et al. 2013; Poinapen et al. 2017). Following parasitization (from fresh parasitized and frozen parasitized) or collection from the colony (for fresh non-parasitized), entire egg masses were placed in Bouin’s fluid (Electron Microscopy Sciences, Hatfield, PA, USA) for fixation overnight for *P. maculiventris*, or up to 48 h for *H. halys* (due to difference in egg size) for each time point (0, 6, 24, 48, 72, 96, and 120 h), with additional time points (168, 216, and 264 h) for fresh and frozen parasitized *P. maculiventris* (to capture full development of the parasitoid in a suitable host). Bouin’s fluid was used because this fixative is recommended for embryos (Rolls 2013). Following fixation, egg masses were rinsed in 0.1 M phosphate buffer (1.8% sucrose, pH 7.2) (3 times, 10 min each), and serially dehydrated in 70, 80, 90, 95, and 100% ethanol (2×10 min each) following a modified protocol of Lipke and Michalik (2016). Finally, fully dehydrated samples were critical point dried (CPD) using Samdri PVT-3B Critical Point Dryer (Tousimis, Rockville, MD, USA) (Pandithage 2012).

6.2.2 DNA extraction and amplification

DNA from individual frozen eggs from each developmental time point (non-parasitized, fresh parasitized, and frozen parasitized) was extracted using a Chelex DNA extraction method (Walsh et al. 1991) modified for use in 96-well plates (Gariepy et al. 2014), as described in Chapter 2. Parasitoid adults were used as positive controls in each plate, and egg shells from hatched pentatomid eggs were incorporated to later correct the amount of DNA for the hosts (i.e. DNA of the shell alone was subtracted from all samples).
PCRs targeting the cytochrome c oxidase subunit I (CO1) of scelionid and pentatomid mitochondrial DNA were performed in 25 µL volumes as described in Chapter 2. For PCRs targeting pentatomid DNA, the same protocol and reagents as for scelionid DNA were used, with Pent-F2 (5′-TATTGAATAGGACAACCTGGAAG-3′) (Gariepy et al. 2014) instead of SCEL_F1 primer, and annealing temperature of the thermal cycle profile modified to 53 °C. In contrast to method used in Chapter 2, the PCR products were not subjected to re-PCR.

All PCR products were examined using a QIAxel Advanced (Qiagen Sciences, Germantown, MD, USA) multicapillary electrophoresis instrument and visualized using QIAxel ScreenGel software (v. 1.2.0). Samples of the correct size peaks (600-800 bp for Scelionidae and Pentatomidae) with ≥ 0.1 relative fluorescence units (RFUs) were scored as positive, and the DNA concentration (ng/µL) was recorded for each sample.

6.2.3 Sample preparation for micro-CT data acquisition

For each time point, 3-5 eggs from each sample were carefully separated and placed in micro-wells made in expanded polystyrene foam disks (diameter= 1 cm). Three disks (each containing not parasitized, fresh parasitized, and frozen parasitized eggs of the same time point) were placed in 1.2 mL cryogenic vials (Corning Incorporated, Corning, NY, USA). Time points were grouped together into two groups and scanned for each host species: 0, 6, and 24 h, and 48, 72, and 96 h. All remaining time points were grouped together for scanning. About 200 eggs were scanned in total.

Prepared vials containing eggs were scanned in a GE eXplore Locus SP X-ray micro-CT system (GE Healthcare, London, Canada). The acquisition parameters were: 90 kVp; 70 µA, with 1200 views (10 frames per view), and 3000 ms exposure with a 0.254
mm aluminium filter. X-ray projections were acquired at 0.3° increment angle over 360° rotation around the sample. A plastic microtube containing water and air was included in each vial for Hounsfield Units (HU) calibration.

6.2.4 Image reconstruction and 3D rendering

The acquired 2D projections were reconstructed at 5.7 µm isotropic voxel spacing into a 3D image using the Feldkamp filtered-back projection algorithm (Feldkamp et al. 1984) (RTK v1.3; freely available at https://www.openrtk.org/) (Rit et al. 2014). The reconstructed images were rendered in Volview (v3.4, Kitware Inc). Before rendering, a median filter (VTK) of isotropic kernel size of 3 was applied for denoising. A representative volume of each sample at each time point was selected for illustration.

6.2.5 Statistical analysis

The data on the amount of parasitoid and host DNA were analyzed by 2-way ANOVAs (egg state and time as factors), followed by Tukey’s post hoc tests (α=0.001) to determine main effects among time points and among egg states. The data were subsequently analyzed by 1-way ANOVA for each time point followed by Tukey’s post hoc tests (α=0.05), to determine specific differences among egg states for each time point. Statistical analyses were carried out using SPSS (v. 24) (IBM 2016) statistical software.

6.3 Results

6.3.1 DNA analysis

The concentration of scelionid PCR products in *H. halys* and *P. maculiventris* parasitized eggs increased with time, but varied among the egg states (Fig. 6.1). The concentration of PCR products from fresh and frozen parasitized *P. maculiventris* eggs
followed the same increasing trends, with non-parasitized eggs remaining at 0 ng/µL (Fig. 6.1a). Conversely, frozen parasitized *H. halys* eggs had up to 6-fold higher concentration of DNA than either fresh parasitized or non-parasitized eggs (especially evident after 24 h). There was an increase in the concentration of scelionid PCR products in fresh parasitized *H. halys* eggs compared to non-parasitized eggs after 72 h of development (Fig. 6.1b).

The concentration of pentatomid PCR products in *P. maculiventris* parasitized and non-parasitized eggs remained constant with time. Conversely, in *H. halys* eggs (Fig. 6.2) this amount was variable. There were no differences in the concentration of PCR products among the egg states, with no change for individual egg states with time (i.e. no interaction). The only difference in the concentration of DNA among the egg states was observed towards the end of the tested period, at 96 and 120 h of development. The concentration of DNA dropped for both fresh parasitized and frozen parasitized eggs of *P. maculiventris* (Fig. 6.2a) and *H. halys* (Fig. 6.2b) at those time points, compared to non-parasitized eggs.

### 6.3.2 Micro-CT 3D reconstruction of development

In non-parasitized egg of both *P. maculiventris* (Fig. 6.3a and b) and *H. halys* (Fig. 6.3c and d), normal hemipteran host development was observed. Starting at 6 h, a differentiation of the outer layer was observed, leading to blastoderm and germ band formation. The progressive reduction in egg content density was likely caused by breakdown of the yolk, culminating in fully grown nymph at 120 h (Fig. 6.3b and d).
Figure 6.1 Temporal pattern of COI parasitoid DNA (ng/µL; mean ±SE) following parasitization by *Trissolcus euschisti* in fresh parasitized, frozen parasitized, and non-parasitized stink bug eggs of a) *Halyomorpha halys* and b) *Podisus maculiventris*. N= not parasitized, F= parasitized while fresh, Z= frozen prior to parasitization, P= *Podisus maculiventris*, B = *Halyomorpha halys*. Capital letters indicate differences among time points, and lower case letters indicate differences among egg states (i.e. same letters indicate no difference). Asterisks (*) indicate differences from the non-parasitized (N) stink bug eggs within each time point. Significance assigned based on Tukey’s post hoc tests (α=0.001 and α=0.05 for 2-way and 1-way ANOVAs, respectively). Sample size for each egg state at each time point is n=9).
Figure 6.2 Temporal pattern of COI host DNA (ng/µL; mean ±SE) following parasitization by *Trissolcus euschisti* in fresh, frozen, and non-parasitized stink bug eggs of a) *Halyomorpha halys* and b) *Podisus maculiventris*. N = not parasitized, F = parasitized while fresh, Z = frozen prior to parasitization, P = *P. maculiventris*, B = *H. halys*. Capital letters indicate differences among time points, and lower case letters indicate differences among egg states (i.e. same letters indicate no difference). Asterisks (*) indicate differences from the non-parasitized (N) stink bug eggs within each time point. Significance assigned based on Tukey’s post hoc tests (α=0.001 and α=0.05 for 2-way and 1-way ANOVAs, respectively). Sample size for each egg state at each time point is n=9).
When frozen eggs of *P. maculiventris* (Fig. 6.4a and b) and *H. halys* (Fig. 6.4c and d) were parasitized by *T. euschisti* (i.e. when the host was freeze-killed), normal parasitoid development ensued. Parasitoid eggs inside individual stink bug eggs were visible at 0-24 h in *P. maculiventris* (Fig. 6.4a) and 0-6 h in *H. halys* (Fig. 6.4c). Parasitoid eggs appeared to hatch between 24-48 h post-parasitization, with clearly distinguishable larvae visible at 48 h. These larvae increased in size with each subsequent time point in both *P. maculiventris* and *H. halys* until they reached late 3rd instar hymenopteriform larvae/prepupae at 120 h post parasitization. At 120 h, parasitoid larvae were surrounded by what might be a peripupal membrane, with expelled meconium at the bottom of the egg (Fig. 6.4). The development of parasitoids proceeded for the next 6 days, with further changes and metamorphosis during the pupal stage (presented for *P. maculiventris* eggs in Appendix C, Fig. C1), eventually giving rise to adults approximately 14 days following parasitization.

In fresh *P. maculiventris* eggs, there were no traces of the host left at 48-120 h post parasitization by *T. euschisti*, and only developing parasitoid larvae, similar to those in frozen eggs, were observed (Fig. 6.5a and b). In contrast, *T. euschisti* development in fresh *H. halys* eggs was limited, and no parasitoid larvae of comparable size or developmental stage were located in most eggs at 48-120 h post parasitization (Fig. 6.5c and d). Simultaneously, differentiation of the outer layer, characteristic of the host development (Fig. 6.3) was visible in some 24-96 h *H. halys* eggs (Fig. 6.5c and d) but not *P. maculiventris* (Fig. 6.5a and b) eggs. Several different developmental outcomes were spotted at 120 h for *T. euschisti* developing in fresh *H. halys* eggs, including no detectable host or parasitoid and fully grown parasitoid larva (Appendix C, Fig. C2).
Figure 6.3 2D slices and 3D-CT volume reconstruction at 5.7 µm isotropic voxel spacing of non-parasitized eggs of *Podisus maculiventris* (PN) (a and b) and *Halyomorpha halys* (BN) (c and d) over 120 h of development. The scale bars in 0 h panels apply to all images. The color scale in 3D-CT reconstructed images refers to Hounsfield Units (HU), representing relative material density (brown= least dense, white = most dense). Arrows in 2D slices point to location of developing host (green). See Videos 1 and 2 for *P. maculiventris* and *H. halys*, respectively.
Figure 6.4 2D slices and 3D-CT volume reconstruction at 5.7 µm isotropic voxel spacing of parasitized frozen eggs of *Podisus maculiventris* (PZ) (a and b) and *Halyomorpha halys* (BZ) (c and d) over 120 h of development (from the time of parasitization). The scale bars in 0 h panels apply to all images. The color scale in 3D-CT reconstructed images refers to Hounsfield Units (HU), representing relative material density (brown = least dense, white = most dense). Arrows in 2D slices point to location of eggs (white), larvae (pink), and possible teratocytes (blue). Breaks and irregularities in host egg shells are due to processing and handling, not due to parasitization. See Videos 3 and 4 for *P. maculiventris* and *H. halys*, respectively.
Figure 6.5 2D slices and 3D-CT volume reconstruction at 5.7 μm isotropic voxel spacing of parasitized fresh eggs of *Podisus maculiventris* (PF) (a and b) and *Halyomorpha halys* (BF) (c and d) over 120 h of development (from the time of parasitization). The scale bars in 0 h panels apply to all images. The color scale in 3D-CT reconstructed images refers to Hounsfield Units (HU), representing relative material density (brown = least dense, white = most dense). Arrows in 2D slices point to location of eggs (white), larvae (pink), host (green), and possible teratocytes (blue). Breaks and irregularities in host egg shells are due to processing and handling, not due to parasitization. See Videos 5 and 6 for *P. maculiventris* and *H. halys*, respectively.
We also observed a clear difference in cellular growth represented by “cloudiness” or clumps of cells within fresh and frozen parasitized *P. maculiventris* eggs, as well as frozen parasitized *H. halys*, but not in fresh parasitized *H. halys* (Fig. 6.4 and 6.5). This cell matter accumulated on the periphery of the host eggs, and first appeared at 6-24 h post parasitization.

### 6.4 Discussion

To successfully exploit a host, egg parasitoids maximize their chances of success by preferentially ovipositing in young hosts (i.e. freshly laid eggs), preventing or delaying host development, and/or developing faster (Vinson 1998). The relative importance of these strategies varies depending on host-parasitoid system, with host development modification being most common and efficient way of ensuring developmental success of the parasitoid offspring (Piek 1986; Strand 2014). Strategies that are typically successful in a co-evolved host-parasitoid association may be ineffective on marginal or novel hosts, as observed for native scelionid parasitoids attacking eggs of the invasive *H. halys*.

The fact that parasitoid eggs are observed in both fresh and frozen eggs of both pentatomid hosts indicates that egg deposition proceeds normally in *H. halys*. However, our results indicate that normal parasitoid development of *T. euschistii* in fresh *H. halys* eggs stops early (~24-48 h post oviposition), coinciding with egg hatch or early larval development (Volkoff and Colazza 1992). The DNA evidence and the intact 3D visualization show that the parasitoid development in fresh and frozen *P. maculiventris* eggs follows the same trajectory over time. In contrast, the patterns observed in fresh and frozen *H. halys* start to diverge 24 h post parasitization. This pattern suggests that the barrier to development in fresh *H. halys* may be associated with the failure of *T. euschistii*...
eggs to hatch or with the death of larvae soon after hatching, which would match previous findings based on dissections (Abram et al. 2014).

There are several possible reasons for *T. euschisti* not being able to develop in freshly laid eggs of *H. halys*. First, the substances that *T. euschisti* females (and those of other native parasitoids) inject when ovipositing (e.g. venom, accessory glad secretions) (Vinson and Iwantsch 1980; Doury et al. 1997; Pennacchio and Strand 2006), which provide a more suitable environment for the larval parasitoid, or protective coatings applied to the eggs during oviposition (Salt 1965; Salt 1968) may be ineffective against *H. halys*, or deposited in an insufficient amount. Hosts similar in size to *H. halys* (e.g. *Nezara viridula*) are successfully parasitized by scelionids (Corrêa-Ferreira and Moscardi 1995; Sujii et al. 2002), indicating that parasitoids are capable of injecting sufficient amount of substances during oviposition to overcome defences of larger hosts. Consequently, as only some parasitoid larvae were observed at 120 h post oviposition as shown by the 3D image volumes of fresh *H. halys* eggs, the effectiveness of female-injected substances might be species-specific, and not suitable against *H. halys*.

Alternatively, *H. halys* may have a defence reaction that is not present in the normally encountered hosts such as *P. maculiventris*. Eggs are thought to lack an immune defence (Godfrey 1994), at least until hemocyte formation (Abrams et al. 1993; Strand and Pech 1995). Consequently, as *H. halys* eggs were only few hours old when parasitized, any defence reaction would have to occur at a cellular level prior to hemocyte formation as reported in two other host-egg parasitoid interactions. When the eucalyptus borer (*Phoracantha recurva*) is attacked by the encyrtid parasitoid, *Avetianella longoi*, there is a two-phase defence response within 24 h post parasitization. There is a general wound
response to mechanical damage to the host egg serosal membrane during oviposition, which is followed by a cellular encapsulation. Interestingly, the second phase was observed in *P. recurva* eggs only, but not in those of *P. semipunctata* (Reed et al. 2007). Following parasitisation by *Trichogramma evanescens*, there is enhanced transcription of immune related genes in *Manduca sexta* eggs (Abdel-Latief and Hilker 2008).

Similar immune responses could exist in *H. halys* eggs as the inhibition of parasitoid development occurs over a similar period as the events in *P. recurva*. If true, the biochemical or physiological interaction would differ in *H. halys* compared to suitable hosts, and *H. halys* eggs could be an unacceptable environment for normal parasitoid development. While cellular encapsulation in *P. recurva* caused parasitoids to die before hatching, this defence reaction of the host was not always predictable and some parasitoid eggs were able to hatch and partially complete development (Reed et al. 2007). Such variability in effectiveness of the cellular encapsulation could be the reason for the lack of distinguishable parasitoid remains in majority of unhatched *H. halys* eggs (Abram et al. 2014), while some eggs contain various stages of partially developed parasitoids (Cornelius, Dieckhoff, Vinyard, et al. 2016; Cornelius, Dieckhoff, Hoelmer, et al. 2016; Herlihy et al. 2016; Ogburn et al. 2016; Abram et al. 2017; Dieckhoff et al. 2017). Investigation of immune-related gene expression in *H. halys* upon parasitization, would provide evidence to support or refute the presence of such immune response.

Another event that coincides with the timing of the failed development of native parasitoids in *H. halys* relates to the release of teratocytes. Teratocytes are derived from extra-embryonic tissues (e.g. serosa) surrounding the parasitoid embryo (i.e. the membrane dissociates into individual or clumps of cells) when the egg hatches (Salt 1968; Dahlman
1990; Dahlman 1991; Quicke 1997; Strand 2014). These cells get distributed throughout the host (Salt 1968), can accumulate on the periphery of the host (Volkoff and Colazza 1992), and are thought to have trophic, immunosuppressive, and secretory (e.g. digestive enzymes) functions that help the developing larva overcome the host (Dahlman 1990; Dahlman 1991; Volkoff and Colazza 1992; Hotta et al. 2001; Nakamatsu et al. 2002).

While the function and number of teratocytes are species-specific, all Scelionidae produce teratocytes (Vinson and Iwantsch 1980), and some parasitoids (e.g. *Trissolcus basalis*, *Telenomus heliothidis*) need them to complete development (Strand et al. 1988; Cônsoli et al. 2001).

If the more pronounced peripheral cell growth observed in fresh and frozen parasitized *P. maculiventris* and frozen *H. halys* represents teratocytes (Fig. 6.4 and 6.5), there are two possible explanations for their absence in fresh *H. halys* eggs. First, host immune response could inhibit parasitoid egg hatching and thus no teratocytes are released. Alternatively, if the parasitoid egg hatches and teratocytes are released, the environment inside *H. halys* eggs may not be appropriate for the proper dissociation and growth of teratocytes, resulting in subsequent death of the early stage parasitoid larva.

Teratocytes do not divide, but they increase in size and their number declines as the parasitoid develops (Pennacchio et al. 1994; Barratt and Sutherland 2001; Hotta et al. 2001). Disrupted teratocyte growth pattern in some host-parasitoid assemblages, including exotic marginal hosts, may reflect the unsuitability of a host for parasitoid development (Alleyne et al. 2001; Barratt and Sutherland 2001; Firlej et al. 2007). Lower successful parasitism and delayed development in unsuitable (or marginal) hosts have been associated with a lack or low number of teratocytes in Crambidae-Braconidae and Coccinellidae-
Braconidae host parasitoid systems (Alleyne et al. 2001; Firlej et al. 2007). A low number of teratocytes could be the result of improper cleavage of serosal membrane when the egg hatches (Strand et al. 1985). Furthermore, since teratocytes need host-derived components (Cônsoli et al. 2001), the lack or inaccessibility of those components in *H. halys* eggs could make them unsuitable for normal teratocyte growth. This disrupted growth pattern could affect nutrient absorption, the ability to suppress host immune response or the amount of secretory digestive enzymes, resulting in the low success of native parasitoids in the exotic host.

Additionally, the injection of teratocytes into a non-parasitized host can cause delayed development or cause malformation by disrupting hormone-regulation (e.g. juvenile hormone) of ecdysis (Vinson 1970; Zhang and Dahlman 1989). It has been noted that very few fresh parasitized *H. halys* eggs give rise to parasitoids, but they also do not produce host nymphs (see Chapters 4 and 5). Thus, this host ‘egg abortion’ (Abram et al. 2016) could be caused by teratocytes (even if in low number) preventing proper host development. Investigation of teratocyte growth pattern and activity in *H. halys* compared to native pentatomid hosts attacked by scelionid egg parasitoids could determine if abnormal teratocyte number or growth pattern is responsible for failed development in this exotic host.

In conclusion, we were able to distinguish and observe host and parasitoid development *in situ* and separate lines of evidence (DNA and 3D *in situ* visualization using micro-CT) provided clear indication that if any development of native parasitoids occurs in *H. halys*, it stops early in development (the first 24-48 h post parasitization). As this lack of development did not occur in frozen *H. halys* eggs (or occurred to a lesser extent; see
Chapter 5), the possibility of host cellular immune response and/or disrupted parasitoid teratocyte growth pattern in \textit{H. halys} eggs merits further attention to identify the actual mechanisms inhibiting native parasitoid development in this exotic host.

6.5 References


Bundy CS, McPherson RM. 2000. Morphological examination of stink bug (Heteroptera:


Volkoff N, Colazza S. 1992. Growth patterns of teratocytes in the immature stages of *Trissolcus basalis* (Woll.) (Hymenoptera : Scelionidae), an egg parasitoid of *Nezara*


Chapter 7

General discussion
7.1 Ecological interactions

The resilience of ecological communities and food webs is partially determined by the intra- and inter-trophic interactions among many species in a given ecosystem. Invasive species can destabilize ecological communities by becoming novel hosts, pray, predators or competitors for native species, and they can have lasting effects on population dynamics, especially if they reduce the fitness of native individuals (Schmitz and Simberloff 1997; Elton 2000; Pimentel et al. 2000). An exotic invasive species can reduce the fitness of a native species when it acts as an evolutionary trap due to misdirected resource use (Schlaepfer et al. 2002; Schlaepfer et al. 2005). For example, an invasive species can appear as a suitable alternative prey or host for a native one, but it may in fact be unsuitable for consumption or development of the native’s progeny (i.e. previously reliable cues are no longer associated with an expected adaptive outcome of behaviour). Insects are frequent participants in such interactions either as invaders or affected local species (Kenis et al. 2009; Bezemer et al. 2014).

The introduction and spread of *Halyomorpha halys*, an East Asia native, in both Europe and North America has triggered a concerted effort to identify and characterize the multitrophic interactions affected by this exotic species. Despite current progress (Lee 2015; Abram et al. 2017), the difficulty in characterizing ecological interactions between *H. halys* and native parasitoids is partly due to the prevailing limited understanding of Pentatomidae-Scelionidae host-parasitoid system. Furthermore, the techniques used to estimate mortality and the identification of immature parasitoid stages from unemerged eggs have a number of shortcomings and remain challenging.
Nevertheless, it is clear that *H. halys* can act as an evolutionary trap for native parasitoid wasps under laboratory conditions as demonstrated by several studies (Abram et al. 2014; Haye et al. 2015). Yet, this phenomenon has not been confirmed under field conditions. Additionally, the mismatch between behavioural acceptance of an unsuitable host and the lack of development in this host still does not have a concrete explanation in terms of the cues used by parasitoids, as well as their relative importance in the decision-making process. Furthermore, the barriers to successful development of native parasitoids in *H. halys* eggs remains unidentified, and the stage(s) affected by this barrier remain unclear. Lastly, approaches to facilitate an improved understanding of parasitoid development in this invasive host, as well characterize possible competitive interactions on this host have not been addressed.

With the increasing interest in *H. halys* and the use of natural enemies (including parasitoids) as biological control agents, advances with new recommendations for pest management have been made. Building on this newly gained knowledge and understanding of this particular host-parasitoid system presents an excellent opportunity for discovering trophic interactions involving parasitoids, from perspectives beyond applied pest management, and examining these processes from a fundamental perspective. Therefore, the overall objective of this thesis was to enhance the understanding of Pentatomidae-Scelionidae host-parasitoid interactions from a behavioural and trophic ecology perspectives, rather than from a biological control viewpoint, focusing on factors for successful host use by egg parasitoid wasps associated with stink bugs.
Towards enhanced understanding of Pentatomidae-Scelionidae host parasitoid interactions: summary of results

Several behavioural, molecular, and imaging approaches were undertaken to elucidate the trophic and competitive interactions of native egg parasitoids interacting with an exotic invasive stink bug host (\textit{H. halys}), and an interspecific competitor (exotic \textit{Trissolcus japonicus} egg parasitoid).

First, exposure of sentinel stink bug egg masses in different habitats in introduced and native ranges of \textit{H. halys} was carried out, followed by the use of a DNA-based approach to detect parasitism in those egg masses (see Chapter 2). This approach resulted in the detection and identification of interactions between \textit{H. halys} and native egg parasitoids under natural conditions in areas where \textit{H. halys} has recently established. Perhaps most importantly, this method permitted the detection of unsuccessful parasitization attempts based on presence of parasitoid DNA in \textit{H. halys} eggs, which is otherwise difficult to quantify. Egg masses of both native and exotic stink bugs were equally likely to be parasitized regardless of the location, type of habitat, or date of exposure. The molecular detection and identification of parasitism clearly demonstrated that the lack of parasitoid emergence from \textit{H. halys} egg masses was not because females of native species rejected the exotic host, but rather because of the failure of parasitoid larvae to complete development. The lack of avoidance behaviour by foraging females clearly supported the idea of the evolutionary trap, and our data showed for the first time that this phenomenon actually happens under field conditions.

Second, the finding that native parasitoids parasitize sentinel \textit{H. halys} egg masses in the field led to the laboratory investigation of possible competitive interactions between
native parasitoids and exotic parasitoids being considered for introduction as biological control agents of *H. halys*, to assess the likelihood of their coexistence. Direct behavioural observations were used to characterize outcomes of intrinsic (between larvae) and extrinsic (between adults) competitive interaction between exotic *T. japonicus* and native *Anastatus bifasciatus* (see Chapter 3) and between *T. japonicus* and native *Trissolcus cultratus* (see Chapter 4) parasitoid pairings. The results from the direct observations, coupled with the assessment of developmental outcomes from multiparasitized egg masses, demonstrated that coexistence of native *A. bifasciatus* and exotic *T. japonicus* egg parasitoids is possible as a result of counterbalance competition. This conclusion was reached after establishing that: i) *T. japonicus* is a superior extrinsic (adult stage) competitor as characterized by egg guarding and aggressiveness; ii) *A. bifasciatus* is a superior intrinsic (larval stage) competitor as it can develop on previously parasitized eggs of all ages; and iii) parasitization and/ or host feeding by *A. bifasciatus* can interfere with development of *T. japonicus*. This study has also led to unexpected finding of time-dependent facultative hyperparasitisation of exotic *T. japonicus* parasitoid by the native *T. cultratus*, which could reduce the impact of the evolutionary trap posed by *H. halys*. This facultative hyperparasitism is the first example of: (1) population level interspecific facilitation (i.e. interaction benefiting at least one participant) between two egg parasitoid wasp species during parasitization of a stink bug host (*H. halys*), and (2) facilitation by an exotic parasitoid (secondary invader), enabling a native parasitoid species to develop on previously unsuitable exotic host (primary invader).

Combined, these two approaches provided strong evidence that there are sufficient cues to initiate parasitization of *H. halys* eggs by native parasitoids, even if the eggs are
unsuitable for development. This realization has led to the investigation of the mechanism behind the mismatch between the behavioural acceptance and the lack of development in terms of cues used by foraging females, and the relative importance of those cues (see Chapter 5). The orthogonal array (OA) experimental design was used to quantify the influence of parameters characterizing host egg mass resource (host species, age of the eggs, status of the eggs, and presence of egg surface chemicals) on parasitoid behaviour and development. The finding that different aspects of behaviour and development of the egg parasitoids are influenced by separate and distinct critical factors associated with the host egg resource, means that the maladaptive decision to oviposit in an unsuitable host is a result of a mismatch between the cues females use, and the expected outcome of this choice. Therefore the mismatch is a result of a disconnect between the parasitoid female’s perception of host suitability and the performance of her offspring in this host. In addition to the investigation of multiple factors affecting parasitoid behaviour on the host resource, we were able to rank them in their relative importance, leading to a more systematic understanding of the steps involved in decision making of egg parasitoids. Although unconventional, the use of the orthogonal array approach in ecology merits further attention as it provides a new way of investigating species interactions, and can significantly reduce the time and effort required to obtain critical information on factors affecting any process under investigation (including fast and effective screening in biological systems).

The behavioural studies presented in this thesis revealed which factors egg parasitoids use to make decisions to parasitize potential hosts, and why hosts unsuitable for larval development are still accepted for oviposition, but not when or why larval development stopped. Consequently, as a first step in identifying the barrier(s) to
successful development, temporal pattern of the timing of failed parasitoid development in *H. halys* was examined using DNA-based approach (PCR) coupled with *in situ* 3D visualization by X-ray micro-computed tomography (micro-CT). Examination and comparison of parasitoid development in suitable and unsuitable host eggs revealed that parasitoid development in fresh *H. halys* eggs fails soon after parasitization, either at egg hatching or shortly after during early-stage larval development. In light of these findings several proposed mechanisms for arrested parasitoid development were suggested: host cellular immune response, and the role the teratocyte growth pattern plays in parasitoid development, both of which merit further investigation.

### 7.3 General discussion

Currently, *H. halys* eggs are perceived as suitable oviposition sites by European and North American female native scelionid egg parasitoids but they are physiologically unsuitable for the larval development. However, the failure to exploit a novel resource that is ecologically suitable (i.e. attractive and abundant in the environment) could be temporary, with *H. halys* eventually becoming part of the host range of native scelionid parasitoids.

The presence of invasive species can impose selection pressures on the life history traits of native species, altering their behaviour, morphology and/or physiology (Strauss et al. 2006; Berthon 2015). The actual evolutionary response will depend on the strength of selective pressure and the adaptive capacity of the native species (Berthon 2015). The presence of *H. halys* at high densities, which is expected for this polyphagous species, likely exerts strong selection pressure on native scelionid parasitoids. Native parasitoids can thus either respond via phenotypic plasticity (if their adaptation capacity is low), or
adaptively (if their adaptation capacity is high) (Strauss et al. 2006; Berthon 2015; Turcotte and Levine 2016). Since native scelionid parasitoids do not recognize *H. halys* as an unsuitable host (as indicated by their acceptance of this host under both laboratory and field conditions), this introduced species currently acts as an evolutionary trap. This maladaptive host choice can increase the selection pressure on other traits, ultimately allowing escape from the evolutionary trap (Carroll et al. 1997; Philips and Shine 2006; Lau et al. 2008; Berthon 2015).

Therefore, rather than learning to avoid the unsuitable host, scelionids are more likely to overcome the barriers currently inhibiting normal development in *H. halys*. In fact, there is evidence that some native scelionids that have been in contact with *H. halys* in North America for > 15 years, are completing development in this host increasingly more often, and thus already adapting to this previously unsuitable host (Cornelius et al. 2016; Abram et al. 2017). Artificial selection and release of parasitoids capable of development in *H. halys* can further accelerate this process and would be especially beneficial for small populations with low genetic diversity in areas where *H. halys* is an important component of the stink bug community. Although introduction and establishment of *H. halys* presents an ecological demand of evolutionary change in the native community, in reality the fitness landscape continuously changes in a dynamic way (Kokko et al. 2017). Therefore the changes in parasitoid community subsequently impose selection pressure on *H. halys*.

Additional complexity will be introduced by presence of *T. japonicus*, as it is a competitor for native egg parasitoids, especially as it can exploit both *H. halys* and native hosts. Some native scelionids could co-exist with *T. japonicus* under certain conditions,
but this co-existence may not be sustainable. For example, we reported that *T. cultratus* could develop on *T. japonicus* through facultative hyperparasitism, but if this does not occur frequently under natural conditions, the introduced parasitoid would serve only as a competitor, and would not provide a long term ‘evolutionary lifeline’ for the native species. Consequently, the recent detection of *T. japonicus* populations in USA (Talamas, Herlihy et al. 2015; Milnes et al. 2016), together with plans for its release as a biological control agent in North America, native egg parasitoids including scelionids, will be faced with additional selection pressure from a novel competitor.

Local extinctions of native scelionids are possible (due to inability to overcome developmental barrier posed by *H. halys*, or due to competitive exclusion by *T. japonicus*). Over time, the equilibrium of ecological interactions will bring the system to a stable state. Only in the unlikely event of no alternative native hosts, will native parasitoid populations not persist. The results reported in this thesis highlight a number of possible outcomes, so this stink bug-parasitoid system offers an excellent opportunity to conduct long term field studies to document the patterns observed in different ecosystems.

### 7.4 Limitations and future work

The results from the experiments presented here have provided enhanced understanding of pentatomid-scelionid host-parasitoid interactions for successful or unsuccessful host use by egg parasitoids associated with native and exotic stink bugs. Nonetheless, there are still many aspects of host-parasitoid interactions involving *H. halys* that require further consideration. First, the findings based on experiments conducted under laboratory conditions cannot be easily extrapolated to field conditions. For example, although facultative hyperparasitism occurrence as a possible ‘invasional lifeline’ for
native parasitoids competing with exotic *T. japonicus* was proposed here, the presence of facultative hyperparasitism among Scelionidae (both in native-native and native-exotic parings) under field conditions needs to be confirmed. This investigation could be challenging as it would first require detailed characterization or meta-analysis of many different intra- and inter-specific parasitoid interactions under laboratory conditions, followed by closely monitored exposure of parasitized egg masses in the field.

Second, females in all laboratory experiments were naïve (i.e. with no prior oviposition experience), and parasitoids were tested in no-choice scenarios (i.e. not presented with alternative hosts). Future studies should incorporate aspects of true choice (Martel and Boivin 2011) using both naïve and experienced females to determine if preference exists when parasitoids are simultaneously presented with more than one host that is perceived to be acceptable (regardless of the actual suitability for development). Such tests can include the Y-tube olfactometry for chemical cues or experiments in a modified arena set up with multiple egg masses offered at the same time. These experiments can allow determination of search effort, time allocation on each resource patch, and the actual preference for one resource over the other (if any).

While the focus of this thesis was on the effect of host eggs on the parasitoid behaviour and development, the incorporation of factors associated with females themselves (e.g. age, previous experience) would provide additional information on the interactions with a potential host. Combining cues associated with host egg masses with factors related to female biological and physiological state would help determine the importance of each factor, as well as their interactions, on female foraging behaviour.
Further investigation into the underlying cause of failed development of native parasitoids in fresh *H. halys* eggs is also needed. The results of experiments investigating developmental differences in stink bug egg masses (using both molecular and imaging methods) provided some evidence of timing of the important events that may be involved in developmental arrestment. Histology or metabolomics/proteomics of the parasitized eggs would provide additional insight into the timing of failed development. Investigation of the mechanism behind failed development could involve determining the role of teratocytes in developmental success or failure of parasitoids in stink bugs, as well as genetic, immunological, and metabolic profiling of parasitized and non-parasitized eggs, to identify pathways that may be involved in developmental arrest of native parasitoids in *H. halys* eggs.

### 7.5 Conclusion

This thesis has expanded our knowledge of Pentatomidae-Scelionidae host-parasitoid interactions using several novel approaches to investigate trophic and competitive interactions of egg parasitoids with their hosts, and with each other, under field and laboratory conditions. The findings have provided direct evidence of the decision making process during host selection by egg parasitoids, and have added significantly to our understanding of the mismatch between parasitization behaviour and lack of development in an exotic host species. Although the work presented in this thesis focuses on the ecological interactions, the findings are relevant and applicable in the development of biological control strategy, as part of the integrated pest management approach for *H. halys*.
7.6 References


Lee D-H. 2015. Current status of research progress on the biology and management of


Appendices
Appendix A

Field site locations and sentinel egg mass preparation and exposure
Figure A1. Locations of natural (red), agricultural (green), and urban (blue) field sites in Canada used for exposure of native and exotic (*H. halys*) stink bugs (Pentatomidae: Hemiptera) sentinel egg masses during 2014 field seasons.
**Figure A2.** Locations of natural (red), agricultural (green), and urban (blue) field sites in Switzerland used for exposure of native and exotic (*H. halys*) stink bugs (Pentatomidae: Hemiptera) sentinel egg masses during 2015 field seasons.
Figure A3. Locations of natural (red), and agricultural (green) field sites in China used for exposure of native and exotic (*H. halys*) stink bugs (Pentatomidae: Hemiptera) sentinel egg masses during 2014 field seasons.
**Figure A4.** Set up of sentinel egg masses indicating a) prepared sentinel egg masses prior to field exposure, and b) and c) individual egg masses indicating stink bug species, date of exposure, site number (here S2 refers to the City Orchard natural site in Canada), number of eggs in the mass, as well as number (indicating paired egg masses) and type of egg mass (letters A and B following the egg mass number indicate if the egg mass is going to be reared or frozen upon recollection).
Figure A5. Exposure of sentinel egg masses in the field, demonstrating a) attachment of an egg mass to the underside of a leaf, b) placement of clip cage over the egg mass, and c) deployed egg mass with a clip cage on a host plant (here apple tree).
Appendix B

Seasonal parasitism based on rearing and molecular analysis of sentinel egg masses from Canada, Switzerland, and China
Table B1. Number of exposed and recollected *H. halys* egg masses (frozen prior to exposure) and mean seasonal parasitism in six sites in Canada over June-September 2014 field season, based on recorded emergence from reared egg masses.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/recollected</th>
<th># eggs exposed/recollected</th>
<th># parasitoids emerged</th>
<th>% parasitism</th>
<th># egg masses with parasitoids emerged</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>London</td>
<td>Natural</td>
<td>June</td>
<td>14/14</td>
<td>354/348</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td>July</td>
<td>25/25</td>
<td>649/635</td>
<td>2</td>
<td>0.31</td>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td>August</td>
<td>20/20</td>
<td>516/503</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Σ</td>
<td>59/59</td>
<td>1519/1486</td>
<td>2</td>
<td>0.13</td>
<td>1</td>
<td>1.69</td>
</tr>
<tr>
<td>London</td>
<td>Natural</td>
<td>June</td>
<td>12/12</td>
<td>319/305</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td>July</td>
<td>20/20</td>
<td>541/528</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td>August</td>
<td>25/23</td>
<td>689/613</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Σ</td>
<td>57/55</td>
<td>1549/1446</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blenheim</td>
<td>Agricultural</td>
<td>June</td>
<td>8/8</td>
<td>229/212</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blenheim</td>
<td></td>
<td>July</td>
<td>16/15</td>
<td>429/399</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blenheim</td>
<td></td>
<td>August</td>
<td>16/16</td>
<td>419/404</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blenheim</td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Σ</td>
<td>44/43</td>
<td>1077/1015</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hamilton</td>
<td>Urban</td>
<td>June</td>
<td>6/6</td>
<td>170/170</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hamilton</td>
<td></td>
<td>July</td>
<td>27/27</td>
<td>695/683</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hamilton</td>
<td></td>
<td>August</td>
<td>20/20</td>
<td>531/509</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hamilton</td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Σ</td>
<td>53/53</td>
<td>1396/1362</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>London</td>
<td>Agricultural</td>
<td>June</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td>July</td>
<td>9/9</td>
<td>223/222</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td>August</td>
<td>12/12</td>
<td>309/293</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>London</td>
<td></td>
<td>September</td>
<td>6/6</td>
<td>169/169</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Σ</td>
<td>27/27</td>
<td>701/684</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table B1 continued. Number of exposed and recollected *H. halys* egg masses (frozen prior to exposure) and mean seasonal parasitism in six sites in Canada over June-September 2014 field season, based on recorded emergence from reared egg masses.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/recollected</th>
<th># eggs exposed/recollected</th>
<th># parasitoids emerged</th>
<th>% parasitism</th>
<th># egg masses with parasitoids emerged</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>London</td>
<td>Agricultural</td>
<td>June</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(ESW2)</td>
<td></td>
<td>July</td>
<td>7/7</td>
<td>179/164</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>12/12</td>
<td>319/318</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>8/8</td>
<td>216/212</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>∑</strong></td>
<td></td>
<td></td>
<td><strong>27/27</strong></td>
<td><strong>714/694</strong></td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
<td><strong>0</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>263/260</strong></td>
<td><strong>6956/6687</strong></td>
<td><strong>2</strong></td>
<td><strong>0.03</strong></td>
<td><strong>1</strong></td>
<td><strong>0.38</strong></td>
</tr>
</tbody>
</table>

*Note: Number of eggs exposed and recollected does not include eggs damaged and/or predated (both pre and post exposure).*
Table B2. Number of exposed and recollected *H. halys* egg masses (frozen prior to exposure) and mean seasonal parasitism in in six sites in Canada over June-September 2014 field season based on molecular analysis targeting COI barcoding region of Scelionidae.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/recollected</th>
<th># eggs exposed/recollected</th>
<th># eggs analysed</th>
<th># eggs with scelionid DNA</th>
<th>% egg parasitism</th>
<th># egg masses with scelionid DNA</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>London (LORDC)</td>
<td>Natural</td>
<td>June</td>
<td>14/14</td>
<td>370/369</td>
<td>364</td>
<td>122</td>
<td>33.5</td>
<td>11</td>
<td>78.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>25/25</td>
<td>675/671</td>
<td>671</td>
<td>342</td>
<td>51.0</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>20/19</td>
<td>520/466</td>
<td>466</td>
<td>253</td>
<td>54.3</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>∑</td>
<td>59/58</td>
<td>1565/1506</td>
<td>1501</td>
<td>47.8</td>
<td>55</td>
<td>94.8</td>
</tr>
<tr>
<td>London (City Orchard)</td>
<td>Natural</td>
<td>June</td>
<td>12/12</td>
<td>327/320</td>
<td>309</td>
<td>151</td>
<td>48.9</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>20/20</td>
<td>550/550</td>
<td>550</td>
<td>89</td>
<td>16.2</td>
<td>18</td>
<td>90.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>25/25</td>
<td>668/665</td>
<td>665</td>
<td>92</td>
<td>13.8</td>
<td>21</td>
<td>84.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>∑</td>
<td>57/57</td>
<td>1545/1535</td>
<td>1524</td>
<td>21.8</td>
<td>51</td>
<td>89.5</td>
</tr>
<tr>
<td>Blenheim</td>
<td>Agricultural</td>
<td>June</td>
<td>8/8</td>
<td>231/229</td>
<td>228</td>
<td>11</td>
<td>4.83</td>
<td>2</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>16/16</td>
<td>441/440</td>
<td>440</td>
<td>48</td>
<td>10.9</td>
<td>11</td>
<td>68.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>16/16</td>
<td>429/426</td>
<td>426</td>
<td>97</td>
<td>22.8</td>
<td>14</td>
<td>87.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>∑</td>
<td>40/40</td>
<td>1101/1095</td>
<td>1094</td>
<td>14.3</td>
<td>27</td>
<td>67.5</td>
</tr>
<tr>
<td>Hamilton</td>
<td>Urban</td>
<td>June</td>
<td>6/6</td>
<td>164/164</td>
<td>162</td>
<td>12</td>
<td>7.41</td>
<td>5</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>27/26</td>
<td>728/666</td>
<td>666</td>
<td>143</td>
<td>21.5</td>
<td>21</td>
<td>80.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>20/20</td>
<td>514/522</td>
<td>522</td>
<td>57</td>
<td>10.9</td>
<td>17</td>
<td>85.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>∑</td>
<td>53/52</td>
<td>1406/1352</td>
<td>1350</td>
<td>15.7</td>
<td>43</td>
<td>82.7</td>
</tr>
<tr>
<td>London (LORDC)</td>
<td>Agricultural</td>
<td>June</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>9/9</td>
<td>221/220</td>
<td>219</td>
<td>84</td>
<td>38.4</td>
<td>8</td>
<td>88.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>12/12</td>
<td>310/310</td>
<td>310</td>
<td>52</td>
<td>16.8</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>6/6</td>
<td>161/161</td>
<td>160</td>
<td>22</td>
<td>13.8</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>∑</td>
<td>27/27</td>
<td>692/691</td>
<td>689</td>
<td>22.9</td>
<td>26</td>
<td>96.3</td>
</tr>
</tbody>
</table>
Table B2 continued. Number of exposed and recollected *H. halys* egg masses (frozen prior to exposure) and mean seasonal parasitism in in six sites in Canada over June-September 2014 field season based on molecular analysis targeting COI barcoding region of Scelionidae.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/recollected</th>
<th># eggs exposed/recollected</th>
<th># eggs analysed</th>
<th># eggs with scelionid DNA</th>
<th>% egg parasitism</th>
<th># egg masses with scelionid DNA</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>London</td>
<td>Agricultural</td>
<td>June</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(ESW)</td>
<td></td>
<td>July</td>
<td>7/7</td>
<td>195/193</td>
<td>193</td>
<td>31</td>
<td>16.1</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>12/12</td>
<td>314/301</td>
<td>298</td>
<td>17</td>
<td>5.71</td>
<td>6</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>8/8</td>
<td>220/220</td>
<td>220</td>
<td>4</td>
<td>1.82</td>
<td>3</td>
<td>37.5</td>
</tr>
<tr>
<td><strong>∑</strong></td>
<td></td>
<td></td>
<td><strong>27/27</strong></td>
<td><strong>729/714</strong></td>
<td><strong>711</strong></td>
<td><strong>52</strong></td>
<td><strong>7.31</strong></td>
<td><strong>16</strong></td>
<td><strong>59.3</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>263/261</strong></td>
<td><strong>7038/6893</strong></td>
<td><strong>6869</strong></td>
<td><strong>1627</strong></td>
<td><strong>23.7</strong></td>
<td><strong>218</strong></td>
<td><strong>83.5</strong></td>
</tr>
</tbody>
</table>
Table B3. Number of exposed and recollected egg masses of native pentatomids (*P. maculiventris*, *E. variolarius*, *E. servus*, *E. tristigmus*, and *T. custator*) and mean seasonal parasitism in six sites in Canada over June-September 2014 field season, based on recorded emergence from reared egg masses.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/recollected</th>
<th># egg masses exposed/recollected</th>
<th># nymphs</th>
<th>no emergence</th>
<th># parasitoids emerged</th>
<th>% parasitism</th>
<th># egg masses with parasitoids emerged</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>London (LORDC)</td>
<td>Natural</td>
<td>June</td>
<td>12/12</td>
<td>260/260</td>
<td>132</td>
<td>45</td>
<td>83</td>
<td>31.9</td>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>28/28</td>
<td>501/437</td>
<td>245</td>
<td>102</td>
<td>90</td>
<td>20.6</td>
<td>6</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>22/21</td>
<td>412/367</td>
<td>278</td>
<td>69</td>
<td>20</td>
<td>5.4</td>
<td>1</td>
<td>4.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>62/61</td>
<td>1173/1064</td>
<td>655</td>
<td>216</td>
<td>193</td>
<td>18.1</td>
<td>9</td>
<td>14.8</td>
</tr>
<tr>
<td>London (City Orchard)</td>
<td>Natural</td>
<td>June</td>
<td>12/12</td>
<td>260/257</td>
<td>162</td>
<td>91</td>
<td>4</td>
<td>1.6</td>
<td>1</td>
<td>8.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>20/20</td>
<td>422/395</td>
<td>242</td>
<td>153</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>26/25</td>
<td>489/453</td>
<td>226</td>
<td>227</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>58/57</td>
<td>1171/1015</td>
<td>630</td>
<td>471</td>
<td>4</td>
<td>0.36</td>
<td>1</td>
<td>1.75</td>
</tr>
<tr>
<td>Blenheim</td>
<td>Agricultural</td>
<td>June</td>
<td>4/4</td>
<td>46/45</td>
<td>27</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>8/8</td>
<td>143/84</td>
<td>50</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>8/8</td>
<td>156/154</td>
<td>117</td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>20/20</td>
<td>345/283</td>
<td>194</td>
<td>89</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hamilton</td>
<td>Urban</td>
<td>June</td>
<td>4/4</td>
<td>68/68</td>
<td>52</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>20/19</td>
<td>384/359</td>
<td>125</td>
<td>175</td>
<td>59</td>
<td>16.4</td>
<td>2</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>16/15</td>
<td>266/241</td>
<td>174</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>40/38</td>
<td>718/688</td>
<td>351</td>
<td>258</td>
<td>59</td>
<td>8.6</td>
<td>2</td>
<td>5.26</td>
</tr>
<tr>
<td>London (LORDC)</td>
<td>Agricultural</td>
<td>June</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>9/9</td>
<td>186/178</td>
<td>115</td>
<td>35</td>
<td>28</td>
<td>15.7</td>
<td>1</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>12/12</td>
<td>219/218</td>
<td>143</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>6/6</td>
<td>118/118</td>
<td>86</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>27/27</td>
<td>523/514</td>
<td>344</td>
<td>142</td>
<td>28</td>
<td>5.4</td>
<td>1</td>
<td>3.70</td>
</tr>
</tbody>
</table>
Table B3 continued. Number of exposed and recollected egg masses of native pentatomids (*P. maculiventris, E. variolarius, E. servus, E. tristigmus*, and *T. custator*) and mean seasonal parasitism in six sites in Canada over June-September 2014 field season, based on recorded emergence from reared egg masses.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/recollected</th>
<th># eggs exposed/recollected</th>
<th># nymphs</th>
<th>no emergence</th>
<th># parasitoids emerged</th>
<th>% parasitism</th>
<th># egg masses with parasitoids emerged</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>London (ESW) Agricultural</td>
<td>June</td>
<td>0/0</td>
<td>0/0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>10/10</td>
<td>200/199</td>
<td>166</td>
<td>24</td>
<td>9</td>
<td>2.6</td>
<td>1</td>
<td>10.0</td>
<td>5.79</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>20/19</td>
<td>381/351</td>
<td>202</td>
<td>149</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>10/10</td>
<td>199/197</td>
<td>127</td>
<td>70</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>∑</strong></td>
<td></td>
<td></td>
<td>40/39</td>
<td>780/747</td>
<td>495</td>
<td>243</td>
<td>9</td>
<td>1.2</td>
<td>1</td>
<td>2.56</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>247/242</td>
<td>4710/4401</td>
<td>2669</td>
<td>1419</td>
<td>293</td>
<td>6.66</td>
<td>14</td>
<td>5.79</td>
</tr>
</tbody>
</table>

*Note: Number of eggs exposed and recollected does not include eggs damaged and/or predated (both pre and post exposure).*
Table B4. Number of exposed and recollected egg masses of native pentatomids and mean seasonal parasitism in six sites in Canada over June-September 2014 field season, based on molecular analysis targeting COI barcoding region of Scelionidae.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date</th>
<th># egg masses exposed/recollected</th>
<th># eggs exposed/recollected</th>
<th># eggs analysed</th>
<th># eggs with scelionid DNA</th>
<th>% parasitism</th>
<th># egg masses with scelionid DNA</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>London</td>
<td>Natural</td>
<td>June</td>
<td>12/12</td>
<td>228/228</td>
<td>228</td>
<td>121</td>
<td>53.1</td>
<td>9</td>
<td>75.0</td>
</tr>
<tr>
<td>(LORDC)</td>
<td></td>
<td>July</td>
<td>28/28</td>
<td>485/421</td>
<td>421</td>
<td>208</td>
<td>49.4</td>
<td>25</td>
<td>89.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>22/22</td>
<td>392/356</td>
<td>314</td>
<td>76</td>
<td>24.2</td>
<td>15</td>
<td>68.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Sum</td>
<td></td>
<td></td>
<td>62/62</td>
<td>1105/1005</td>
<td>963</td>
<td>405</td>
<td>42.1</td>
<td>49</td>
<td>79.0</td>
</tr>
<tr>
<td>London</td>
<td>Natural</td>
<td>June</td>
<td>12/12</td>
<td>245/244</td>
<td>155</td>
<td>16</td>
<td>10.3</td>
<td>7</td>
<td>58.3</td>
</tr>
<tr>
<td>(City Orchard)</td>
<td></td>
<td>July</td>
<td>20/20</td>
<td>431/389</td>
<td>340</td>
<td>55</td>
<td>16.2</td>
<td>12</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>26/25</td>
<td>475/403</td>
<td>304</td>
<td>19</td>
<td>6.25</td>
<td>11</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Sum</td>
<td></td>
<td></td>
<td>58/57</td>
<td>1151/1036</td>
<td>799</td>
<td>90</td>
<td>11.3</td>
<td>30</td>
<td>55.6</td>
</tr>
<tr>
<td>Blenheim</td>
<td>Agricultural</td>
<td>June</td>
<td>4/4</td>
<td>50/50</td>
<td>25</td>
<td>4</td>
<td>16.0</td>
<td>1</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>8/8</td>
<td>136/136</td>
<td>70</td>
<td>24</td>
<td>34.3</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>8/7</td>
<td>167/160</td>
<td>73</td>
<td>39</td>
<td>53.4</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Sum</td>
<td></td>
<td></td>
<td>20/19</td>
<td>353/346</td>
<td>168</td>
<td>67</td>
<td>39.9</td>
<td>12</td>
<td>85.7</td>
</tr>
<tr>
<td>Hamilton</td>
<td>Urban</td>
<td>June</td>
<td>4/4</td>
<td>54/53</td>
<td>7</td>
<td>3</td>
<td>42.9</td>
<td>2</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>20/20</td>
<td>381/359</td>
<td>332</td>
<td>146</td>
<td>44.0</td>
<td>16</td>
<td>84.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>16/16</td>
<td>276/273</td>
<td>167</td>
<td>101</td>
<td>60.5</td>
<td>13</td>
<td>86.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Sum</td>
<td></td>
<td></td>
<td>40/40</td>
<td>711/685</td>
<td>506</td>
<td>250</td>
<td>49.4</td>
<td>31</td>
<td>83.8</td>
</tr>
<tr>
<td>London</td>
<td>Agricultural</td>
<td>June</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>(LORDC)</td>
<td></td>
<td>July</td>
<td>9/9</td>
<td>172/162</td>
<td>102</td>
<td>44</td>
<td>41.1</td>
<td>5</td>
<td>71.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>12/12</td>
<td>217/201</td>
<td>127</td>
<td>78</td>
<td>61.4</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>6/6</td>
<td>109/109</td>
<td>108</td>
<td>29</td>
<td>26.9</td>
<td>5</td>
<td>83.3</td>
</tr>
<tr>
<td>Sum</td>
<td></td>
<td></td>
<td>27/27</td>
<td>498/472</td>
<td>342</td>
<td>151</td>
<td>44.2</td>
<td>22</td>
<td>88.0</td>
</tr>
</tbody>
</table>
Table B4 continued. Number of exposed and recollected egg masses of native pentatomids and mean seasonal parasitism in six sites in Canada over June-September 2014 field season, based on molecular analysis targeting COI barcoding region of Scelionidae.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/recollected</th>
<th># eggs exposed/recollected</th>
<th># eggs analysed</th>
<th># eggs with scelionid DNA</th>
<th>% parasitism</th>
<th># egg masses with scelionid DNA</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>London (ESW)</td>
<td>Agricultural</td>
<td>June</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>10/10</td>
<td>194/165</td>
<td>114</td>
<td>23</td>
<td>20.2</td>
<td>7</td>
<td>87.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>20/20</td>
<td>383/380</td>
<td>221</td>
<td>38</td>
<td>17.2</td>
<td>13</td>
<td>76.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>September</td>
<td>10/10</td>
<td>187/184</td>
<td>183</td>
<td>66</td>
<td>36.1</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>40/40</td>
<td>764/729</td>
<td>518</td>
<td>127</td>
<td>24.5</td>
<td>30</td>
<td>85.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>247/245</td>
<td>4582/4273</td>
<td>3296</td>
<td>1090</td>
<td>35.2</td>
<td>174</td>
<td>79.6</td>
</tr>
</tbody>
</table>

Note: Egg masses where stink bug nymphs hatched prior to freezing were not analyzed molecularly, and were excluded from the percent egg parasitism calculation.
**Table B5.** Number of exposed and recollected *H. halys* egg masses (frozen prior to exposure) and mean seasonal parasitism in three sites (Switzerland) over May-August 2015 field season, based on recorded emergence from reared egg masses.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/ recollected</th>
<th># eggs exposed/ recollected</th>
<th># parasitoids emerged</th>
<th>% egg parasitism</th>
<th># egg masses with parasitoids emerged</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delémont</td>
<td>Natural</td>
<td>May 14/12</td>
<td>384/324</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June 20/19</td>
<td>556/528</td>
<td>12</td>
<td>2.27</td>
<td>1</td>
<td>5.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>July 16/16</td>
<td>427/416</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August 16/16</td>
<td>428/391</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>66/63</td>
<td>1795/1659</td>
<td>12</td>
<td>0.72</td>
<td>1</td>
<td>1.59</td>
</tr>
<tr>
<td>Basel</td>
<td>Urban</td>
<td>May 15/14</td>
<td>405/374</td>
<td>26</td>
<td>7.0</td>
<td>1</td>
<td>7.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>June 19/19</td>
<td>466/466</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July 25/25</td>
<td>696/682</td>
<td>27</td>
<td>4.0</td>
<td>1</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>August 20/19</td>
<td>543/508</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>79/77</td>
<td>2110/2030</td>
<td>53</td>
<td>2.61</td>
<td>2</td>
<td>2.60</td>
</tr>
<tr>
<td>Courtemelon</td>
<td>Agricultural</td>
<td>May 10/9</td>
<td>275/247</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June 20/20</td>
<td>541/539</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July 20/20</td>
<td>552/546</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August 20/20</td>
<td>536/515</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>70/69</td>
<td>1904/1847</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>215/209</td>
<td>5809/5536</td>
<td>65</td>
<td>1.17</td>
<td>3</td>
<td>1.44</td>
</tr>
</tbody>
</table>

*Note: Number of eggs exposed and recollected does not include eggs damaged and/or predated (both pre and post exposure).*
Table B6. Number of exposed and recollected *H. halys* egg masses (frozen prior to exposure) and mean seasonal parasitism in three sites (Switzerland) over May-August 2015 field season based on molecular analysis targeting COI barcoding region of Scelionidae.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/recollected</th>
<th># eggs exposed/recollected</th>
<th># eggs analysed</th>
<th># eggs with scelionid DNA</th>
<th>% parasitism</th>
<th># egg masses with scelionid DNA</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delémont</td>
<td>Natural</td>
<td>May</td>
<td>14/13</td>
<td>382/349</td>
<td>349</td>
<td>58</td>
<td>16.6</td>
<td>8</td>
<td>61.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>20/19</td>
<td>551/524</td>
<td>524</td>
<td>69</td>
<td>13.2</td>
<td>9</td>
<td>47.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>16/14</td>
<td>433/374</td>
<td>374</td>
<td>32</td>
<td>8.56</td>
<td>13</td>
<td>92.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>16/15</td>
<td>432/418</td>
<td>409</td>
<td>4</td>
<td>0.98</td>
<td>4</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>∑</strong></td>
<td>66/61</td>
<td>1798/1665</td>
<td>1656</td>
<td>163</td>
<td>9.84</td>
<td>34</td>
<td>55.7</td>
</tr>
<tr>
<td>Basel</td>
<td>Urban</td>
<td>May</td>
<td>15/15</td>
<td>394/394</td>
<td>394</td>
<td>21</td>
<td>5.33</td>
<td>9</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>19/19</td>
<td>494/494</td>
<td>494</td>
<td>140</td>
<td>28.3</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>25/25</td>
<td>698/693</td>
<td>693</td>
<td>61</td>
<td>8.80</td>
<td>22</td>
<td>88.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>20/20</td>
<td>560/547</td>
<td>547</td>
<td>111</td>
<td>20.3</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>∑</strong></td>
<td>79/79</td>
<td>2124/2128</td>
<td>2128</td>
<td>333</td>
<td>15.6</td>
<td>70</td>
<td>88.6</td>
</tr>
<tr>
<td>Courtemelon</td>
<td>Agricultural</td>
<td>May</td>
<td>10/10</td>
<td>272/270</td>
<td>270</td>
<td>21</td>
<td>7.78</td>
<td>5</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>20/19</td>
<td>543/515</td>
<td>515</td>
<td>74</td>
<td>14.4</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>20/20</td>
<td>551/539</td>
<td>539</td>
<td>115</td>
<td>21.3</td>
<td>19</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>20/20</td>
<td>543/531</td>
<td>531</td>
<td>147</td>
<td>27.7</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>∑</strong></td>
<td>70/69</td>
<td>1909/1855</td>
<td>1855</td>
<td>357</td>
<td>19.2</td>
<td>63</td>
<td>91.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>215/209</td>
<td>5831/5648</td>
<td>5639</td>
<td>853</td>
<td>15.1</td>
<td>167</td>
<td>79.9</td>
</tr>
</tbody>
</table>
Table B7. Number of exposed and recollected egg masses of native pentatomids (*C. fuscispinus*, *E. ornatum*, and *G. lineatum*) and mean seasonal parasitism in three sites (Switzerland) over May-August 2015 field season, based on recorded emergence from reared egg masses.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/recollected</th>
<th># eggs exposed/recollected</th>
<th># nymphs</th>
<th>no emergence</th>
<th># parasitoids emerged</th>
<th>% parasitism</th>
<th># egg masses with parasitoids emerged</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delémont</td>
<td>Natural</td>
<td>May</td>
<td>9/7</td>
<td>144/119</td>
<td>94</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>10/10</td>
<td>111/111</td>
<td>74</td>
<td>25</td>
<td>12</td>
<td>10.8</td>
<td>1</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>3/3</td>
<td>45/45</td>
<td>30</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>22/20</td>
<td>300/275</td>
<td>198</td>
<td>65</td>
<td>12</td>
<td>4.36</td>
<td>1</td>
<td>5.0</td>
</tr>
<tr>
<td>Basel</td>
<td>Urban</td>
<td>May</td>
<td>12/12</td>
<td>171/171</td>
<td>98</td>
<td>73</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>13/13</td>
<td>167/152</td>
<td>116</td>
<td>22</td>
<td>14</td>
<td>9.21</td>
<td>3</td>
<td>23.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>1/1</td>
<td>8/8</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>7/7</td>
<td>85/85</td>
<td>81</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>33/33</td>
<td>431/416</td>
<td>302</td>
<td>100</td>
<td>14</td>
<td>3.37</td>
<td>3</td>
<td>9.09</td>
</tr>
<tr>
<td>Courtemelon</td>
<td>Agricultural</td>
<td>May</td>
<td>6/6</td>
<td>97/97</td>
<td>69</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>11/11</td>
<td>139/123</td>
<td>80</td>
<td>33</td>
<td>10</td>
<td>8.13</td>
<td>2</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>1/1</td>
<td>12/12</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>4/4</td>
<td>50/28</td>
<td>13</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>22/22</td>
<td>298/260</td>
<td>174</td>
<td>76</td>
<td>10</td>
<td>3.85</td>
<td>2</td>
<td>9.09</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>77/75</td>
<td>1029/951</td>
<td>674</td>
<td>241</td>
<td>36</td>
<td>3.79</td>
<td>6</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Note: Number of eggs exposed and recollected does not include eggs damaged and/or predated (both pre and post exposure).
Table B8. Number of exposed and recollected egg masses of native pentatomids (*C. fuscispinus*, *E. ornatum*, and *G. lineatum*) and mean seasonal parasitism in three sites (Switzerland) over May-August 2015 field season, based on molecular analysis targeting COI barcoding region of Scelionidae.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/ recollected</th>
<th># eggs exposed/ recollected</th>
<th># eggs analysed</th>
<th># eggs with scelionid DNA</th>
<th>% parasitism</th>
<th># egg masses with scelionid DNA</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delémont</td>
<td>Natural</td>
<td>May</td>
<td>9/7</td>
<td>143/120</td>
<td>120</td>
<td>75</td>
<td>62.5</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>10/10</td>
<td>105/105</td>
<td>89</td>
<td>50</td>
<td>56.2</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>0/0</td>
<td>0/0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>3/3</td>
<td>39/39</td>
<td>12</td>
<td>5</td>
<td>41.7</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>∑</strong></td>
<td>22/20</td>
<td>287/264</td>
<td>221</td>
<td>130</td>
<td>58.8</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>Basel</td>
<td>Urban</td>
<td>May</td>
<td>12/12</td>
<td>183/182</td>
<td>179</td>
<td>60</td>
<td>33.5</td>
<td>11</td>
<td>91.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>13/13</td>
<td>165/163</td>
<td>108</td>
<td>18</td>
<td>16.7</td>
<td>6</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>1/1</td>
<td>6/6</td>
<td>6</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>7/7</td>
<td>82/78</td>
<td>14</td>
<td>1</td>
<td>7.1</td>
<td>1</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>∑</strong></td>
<td>33/33</td>
<td>436/429</td>
<td>307</td>
<td>79</td>
<td>25.7</td>
<td>18</td>
<td>64.3</td>
</tr>
<tr>
<td>Courtemelon</td>
<td>Agricultural</td>
<td>May</td>
<td>6/6</td>
<td>92/92</td>
<td>92</td>
<td>18</td>
<td>19.6</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>11/11</td>
<td>143/129</td>
<td>115</td>
<td>40</td>
<td>34.8</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>1/1</td>
<td>12/12</td>
<td>4</td>
<td>2</td>
<td>50.0</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>4/4</td>
<td>42/38</td>
<td>21</td>
<td>3</td>
<td>14.3</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>∑</strong></td>
<td>22/22</td>
<td>289/269</td>
<td>232</td>
<td>63</td>
<td>27.2</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td><strong>∑</strong></td>
<td>77/75</td>
<td><strong>1012/962</strong></td>
<td>760</td>
<td><strong>272</strong></td>
<td><strong>35.8</strong></td>
<td><strong>52</strong></td>
<td><strong>69.3</strong></td>
</tr>
</tbody>
</table>

*Note: Egg masses where stink bug nymphs hatched prior to freezing were not analyzed molecularly, and were excluded from the percent egg parasitism calculation*
<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/recollected</th>
<th># eggs exposed/recollected</th>
<th># nymphs</th>
<th>no emergence</th>
<th># parasitoids emerged</th>
<th>% parasitism</th>
<th># egg masses with parasitoids emerged</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bai Wang Mountain</td>
<td>Natural</td>
<td>May</td>
<td>6/1</td>
<td>153/28</td>
<td>25</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>7/7</td>
<td>189/187</td>
<td>38</td>
<td>16</td>
<td>133</td>
<td>71.1</td>
<td>5</td>
<td>71.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>7/6</td>
<td>175/153</td>
<td>0</td>
<td>18</td>
<td>135</td>
<td>88.2</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>5/5</td>
<td>112/112</td>
<td>0</td>
<td>24</td>
<td>88</td>
<td>78.6</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25/19</td>
<td>629/480</td>
<td>63</td>
<td>61</td>
<td>47.2</td>
</tr>
<tr>
<td>Beianhe village</td>
<td>Agricultural</td>
<td>May</td>
<td>8/7</td>
<td>223/195</td>
<td>154</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>8/8</td>
<td>208/200</td>
<td>0</td>
<td>32</td>
<td>168</td>
<td>84.0</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>3/2</td>
<td>79/51</td>
<td>0</td>
<td>1</td>
<td>50</td>
<td>98.0</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>1/1</td>
<td>26/26</td>
<td>0</td>
<td>1</td>
<td>25</td>
<td>96.2</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Σ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20/18</td>
<td>536/472</td>
<td>154</td>
<td>75</td>
<td>51.2</td>
</tr>
<tr>
<td>Fragrant Hills</td>
<td>Natural</td>
<td>May</td>
<td>5/2</td>
<td>136/54</td>
<td>28</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>6/6</td>
<td>165/165</td>
<td>93</td>
<td>72</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>6/6</td>
<td>160/154</td>
<td>45</td>
<td>67</td>
<td>45</td>
<td>29.2</td>
<td>3</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>4/4</td>
<td>91/91</td>
<td>0</td>
<td>13</td>
<td>78</td>
<td>85.7</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Σ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21/18</td>
<td>552/464</td>
<td>166</td>
<td>180</td>
<td>26.5</td>
</tr>
<tr>
<td>Lengquan village</td>
<td>Agricultural</td>
<td>May</td>
<td>19/12</td>
<td>516/320</td>
<td>171</td>
<td>77</td>
<td>72</td>
<td>22.5</td>
<td>3</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>15/13</td>
<td>413/354</td>
<td>77</td>
<td>147</td>
<td>130</td>
<td>36.7</td>
<td>7</td>
<td>53.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>26/25</td>
<td>670/647</td>
<td>170</td>
<td>203</td>
<td>274</td>
<td>42.3</td>
<td>14</td>
<td>56.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>18/18</td>
<td>434/433</td>
<td>49</td>
<td>126</td>
<td>258</td>
<td>59.6</td>
<td>15</td>
<td>83.0</td>
</tr>
<tr>
<td>Σ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>78/68</td>
<td>2033/1754</td>
<td>467</td>
<td>553</td>
<td>41.8</td>
</tr>
<tr>
<td>Shujiatuo village</td>
<td>Agricultural</td>
<td>May</td>
<td>8/7</td>
<td>209/183</td>
<td>28</td>
<td>55</td>
<td>100</td>
<td>54.6</td>
<td>5</td>
<td>71.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>8/7</td>
<td>219/190</td>
<td>88</td>
<td>77</td>
<td>25</td>
<td>13.2</td>
<td>1</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>4/4</td>
<td>115/105</td>
<td>27</td>
<td>37</td>
<td>41</td>
<td>39.0</td>
<td>2</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Σ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20/18</td>
<td>543/478</td>
<td>143</td>
<td>169</td>
<td>34.7</td>
</tr>
</tbody>
</table>
Table B9 continued. Number of exposed and recollected fresh *H. halys* egg masses and mean seasonal parasitism in six sites in China over May-August 2014 field season based on recorded emergence from reared egg masses.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/recollected</th>
<th># eggs exposed/recollected</th>
<th># nymphs</th>
<th>no emergence</th>
<th># parasitoids emerged</th>
<th>% parasitism</th>
<th># egg masses with parasitoids emerged</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yangtaishan</td>
<td>Natural</td>
<td>May 7/6</td>
<td>196/162</td>
<td>34</td>
<td>120</td>
<td>8</td>
<td>4.94</td>
<td>2</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>June 6/4</td>
<td>150/108</td>
<td>50</td>
<td>31</td>
<td>27</td>
<td>25.0</td>
<td>1</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>July 4/3</td>
<td>101/82</td>
<td>0</td>
<td>56</td>
<td>26</td>
<td>31.7</td>
<td>1</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>August</td>
<td></td>
<td>1/1</td>
<td>17/17</td>
<td>14</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>∑</strong></td>
<td></td>
<td></td>
<td>464/369</td>
<td>98</td>
<td>210</td>
<td>61</td>
<td>16.5</td>
<td>4</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>182/155</td>
<td>4757/4017</td>
<td>1091</td>
<td>1248</td>
<td>1683</td>
<td>41.9</td>
<td>85</td>
<td>54.8</td>
</tr>
</tbody>
</table>
Table B10. Number of exposed and recollected fresh *H. halys* egg masses and mean seasonal parasitism in six sites in China over May-August 2014 field season based on molecular analysis targeting COI barcoding region of Scelionidae.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/collected</th>
<th># eggs exposed/recollected</th>
<th># eggs analysed</th>
<th># eggs with scelionid DNA</th>
<th>% egg parasitism</th>
<th># egg masses with scelionid DNA</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bai Wang Natural</td>
<td>May</td>
<td>4/4</td>
<td>107/107</td>
<td>107</td>
<td>53</td>
<td>49.5</td>
<td>2</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>7/7</td>
<td>214/207</td>
<td>207</td>
<td>118</td>
<td>57.0</td>
<td>5</td>
<td>71.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>4/4</td>
<td>110/103</td>
<td>103</td>
<td>100</td>
<td>97.1</td>
<td>4</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>4/3</td>
<td>101/77</td>
<td>77</td>
<td>77</td>
<td>100</td>
<td>3</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>SUM</td>
<td></td>
<td></td>
<td>19/18</td>
<td>532/494</td>
<td>494</td>
<td>348</td>
<td>70.4</td>
<td>14</td>
<td>77.8</td>
</tr>
<tr>
<td>Beianhe village Agricultural</td>
<td>May</td>
<td>7/7</td>
<td>191/190</td>
<td>190</td>
<td>92</td>
<td>48.4</td>
<td>7</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>8/8</td>
<td>204/201</td>
<td>201</td>
<td>199</td>
<td>99.0</td>
<td>8</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>3/3</td>
<td>84/84</td>
<td>84</td>
<td>82</td>
<td>97.6</td>
<td>3</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>1/1</td>
<td>28/28</td>
<td>28</td>
<td>27</td>
<td>96.5</td>
<td>1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>SUM</td>
<td></td>
<td></td>
<td>19/19</td>
<td>507/503</td>
<td>503</td>
<td>400</td>
<td>79.5</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td>Fragrant Hills Natural</td>
<td>May</td>
<td>3/3</td>
<td>84/75</td>
<td>75</td>
<td>1</td>
<td>1.33</td>
<td>1</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>7/7</td>
<td>195/195</td>
<td>195</td>
<td>1</td>
<td>0.51</td>
<td>1</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>6/6</td>
<td>159/131</td>
<td>131</td>
<td>89</td>
<td>67.9</td>
<td>5</td>
<td>83.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>5/5</td>
<td>101/96</td>
<td>96</td>
<td>44</td>
<td>45.8</td>
<td>3</td>
<td>60.0</td>
<td></td>
</tr>
<tr>
<td>SUM</td>
<td></td>
<td></td>
<td>21/21</td>
<td>539/497</td>
<td>497</td>
<td>135</td>
<td>27.2</td>
<td>10</td>
<td>47.6</td>
</tr>
<tr>
<td>Lengquan village Agricultural</td>
<td>May</td>
<td>12/12</td>
<td>307/294</td>
<td>294</td>
<td>60</td>
<td>20.4</td>
<td>7</td>
<td>58.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>15/14</td>
<td>392/387</td>
<td>387</td>
<td>322</td>
<td>83.2</td>
<td>14</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>27/26</td>
<td>704/656</td>
<td>656</td>
<td>360</td>
<td>54.9</td>
<td>19</td>
<td>73.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>19/19</td>
<td>494/494</td>
<td>494</td>
<td>378</td>
<td>76.5</td>
<td>14</td>
<td>73.7</td>
<td></td>
</tr>
<tr>
<td>SUM</td>
<td></td>
<td></td>
<td>73/71</td>
<td>1897/1831</td>
<td>1831</td>
<td>1120</td>
<td>61.2</td>
<td>54</td>
<td>76.1</td>
</tr>
<tr>
<td>Shujiatuo village Agricultural</td>
<td>May</td>
<td>6/6</td>
<td>167/167</td>
<td>165</td>
<td>103</td>
<td>62.4</td>
<td>4</td>
<td>66.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>8/8</td>
<td>209/209</td>
<td>208</td>
<td>39</td>
<td>18.8</td>
<td>3</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>4/4</td>
<td>111/111</td>
<td>101</td>
<td>47</td>
<td>46.5</td>
<td>2</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SUM</td>
<td></td>
<td></td>
<td>18/18</td>
<td>487/487</td>
<td>474</td>
<td>189</td>
<td>39.9</td>
<td>9</td>
<td>50.0</td>
</tr>
</tbody>
</table>
Table B10 continued. Number of exposed and recollected fresh *H. halys* egg masses and mean seasonal parasitism in six sites in China over May-August 2014 field season based on molecular analysis targeting COI barcoding region of Scelionidae.

<table>
<thead>
<tr>
<th>Site</th>
<th>Type of site</th>
<th>Exposure date (month)</th>
<th># egg masses exposed/ recollected</th>
<th># eggs exposed/ recollected</th>
<th># eggs analysed</th>
<th># eggs with scelionid DNA</th>
<th>% egg parasitism</th>
<th># egg masses with scelionid DNA</th>
<th>% egg mass parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yangtaishan</td>
<td>Natural</td>
<td>May</td>
<td>7/5</td>
<td>196/125</td>
<td>125</td>
<td>110</td>
<td>88.0</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>2/2</td>
<td>48/48</td>
<td>48</td>
<td>40</td>
<td>83.3</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>4/4</td>
<td>107/107</td>
<td>107</td>
<td>54</td>
<td>50.5</td>
<td>3</td>
<td>75.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>2/0</td>
<td>41/0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>∑</td>
<td>15/11</td>
<td>392/280</td>
<td>280</td>
<td>204</td>
<td>72.9</td>
<td>10</td>
<td>90.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>165/158</td>
<td>4354/4092</td>
<td>4082</td>
<td>2396</td>
<td>58.7</td>
<td>116</td>
<td>73.4</td>
</tr>
</tbody>
</table>
Appendix C

Parasitoid development in fresh and frozen *P. maculiventris* eggs (7-11 days) and in fresh *H. halys* at 120 h (5 days)
Figure C1. 2D slices and 3D-CT volume reconstruction of a) and b) frozen parasitized and c) and d) fresh parasitized *Podisus maculiventris* eggs from 168 to 264 h (7-11 days) post parasitization at 48 h (2 day) intervals. PZ= frozen parasitized *P. maculiventris*; PF= fresh parasitized *P. maculiventris*. The images were obtained at 5.7 μm isotropic voxel spacing. The scale bars in top left corners of the 168 h panels apply to all images. The color scale in 3D-CT reconstructed images refers to Hounsfield Units (HU), representing relative electron density (increasing from dark to light).
Figure C2. Three observed outcomes at 120 h post parasitization of fresh *H. halys* eggs represented as a) 2D slices and b) 3D-CT volume reconstruction at 5.7 µm isotropic voxel spacing. The scale bar in top left corner applies to all images. The color scale in 3D-CT reconstructed images refers to Hounsfield Units (HU), representing relative material density (brown = least dense, white = most dense). Breaks and irregularities in host egg shells are due to processing and handling, not due to parasitization.
# Curriculum Vitae

**Name:** Joanna K. Konopka  
**Post-secondary Education and Degrees:** The University of Western Ontario  
**Location:** London, Ontario, Canada  
**MSc:** 2011-2013  
**Honours and Awards:**  
- Ontario Graduate Scholarship (OGS)  
- Queen Elizabeth II Graduate Scholarship in Science and Technology  
- 2016-2017  
- Natural Science and Engineering Research Council (NSERC) Doctoral Post Graduate Scholarship (PGSD3)  
- 2014-2016  
- Ontario Graduate Scholarship (OGS)  
- 2014 - 2015 (declined)  
- Natural Science and Engineering Research Council (NSERC) Alexander Graham Bell Canada Graduate Scholarship (CGS M)  
- 2012-2013  
- Ontario Graduate Scholarship (OGS)  
- 2012 - 2013 (declined)  
- Entomological Society of America (ESA) Student Transition and Early Professionals (STEP) Travel Award  
- 2016  
- Entomological Society of Canada (ESC) Graduate Research Travel Scholarship  
- 2015
 Related Work

Experience:
Teaching Assistant in Biology
The University of Western Ontario
2011-2018

Research Assistant
The University of Western Ontario
Metal Tolerance and Toxicity/ Behavioural Ecology of Insects
May- Aug. 2011

Insect Molecular Biology Intern
Agriculture and Agri-Food Canada
Jan. - Aug. 2010

Insecticide Toxicology Intern
Agriculture and Agri-Food Canada
May- Dec. 2009

Publications:

Poinapen D*, Konopka JK*, Umoh JU, Norley C, McNeil JN, Holdsworth DW. 2017
Micro-CT imaging of live insects using carbon dioxide gas-induced hypoxia as
anesthetic with minimal impact on certain subsequent life history traits. BMC

temperature, determines age of sexual maturation in Striacosta albicosta (Noctuidae)
J Insect Physiol. 103:86-90.

Konopka JK, Haye T, Gariepy T, McNeil JN. 2017. Possible coexistence of native and
4:1119-1125.

parasitoid provides an invasional lifeline for native parasitoids. Ecol Evol. 7: 277-
284.

Konopka JK, McNeil JN. 2015. Mating status regulates post-mating refractory period in

to cadmium depends on their feeding strategy? J Chem Ecol. 39: 546-554

of Cydia pomonella (Lepidoptera: Tortricidae). J Econ Entomol. 105: 872-877.

Tolman JH, Vernon RS, Konopka J, McPherson B. 2010. Small plot field evaluation of
treatments for control of cabbage maggot attacking late season rutabaga in mineral