

The parasitoid community associated with the invasive leek moth, *Acrolepiopsis assectella* (Zeller) (Lepidoptera: Acrolepiidae): can conservation biological control benefit an introduced classical biological control agent in North America?

By

Jacob H Miall

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science in Biology

**Carleton University,
Ottawa, Ontario, Canada**

©2018, Jacob H Miall

Abstract

The leek moth is a European pest of *Allium* spp. established near Ottawa, Ontario, Canada in the early 1990's. Its spread throughout eastern Canada and into the northeastern United States represents the potential for significant economic losses to *Allium* growers. Using a life-table approach, I aimed to determine if resource supplementation would influence the species composition, relative abundance and host-killing ability of indigenous parasitic wasps in the leek moth system and the classical biological control agent, *Diadromus pulchellus*. In greenhouse cages, adding buckwheat to a sugar-deprived system increased the longevity, and parasitism levels of *D. pulchellus*; however, a similar effect was measured on the facultative hyperparasitoid, *Conura albifrons*. In the field, the parasitoid species composition was the same in both buckwheat-supplemented and standard leek plots; however, the relative abundance of the parasitoid community differed between the standard and resource-enriched plots. These studies provide insight into how conservation biological control may affect classical biological control.

Acknowledgements

Firstly, I would like to thank my co-supervisors Drs. Peter Mason and Naomi Cappuccino for their positive attitudes and helpful guidance, support and patience during this project. Secondly to my good friend and colleague Dr. Paul Abram, who provided a constant supply of optimism to this project and my abilities, and who spent countless hours helping me work through the statistical analyses and earlier drafts of this thesis. I would also like to thank my external supervisor, Dr. Jessica Forrest, for providing helpful comments and insight in assisting with the design of this project. Drs. Andrew Bennett (Ichneumonidae), Gary Gibson (Chalcididae), and José Fernández-Trinia (Braconidae) provided identifications on many of the parasitoids discussed in this thesis.

I also wish to thank everyone I have worked with in the Mason and Cappuccino labs throughout this project, which could not have been completed without their help in the laboratory, greenhouse, and field: Chelsey Blackman, Hannah Clouthier, Christine Cock, Stephanie Coghlan, Tina Dancau, Ana Maria Farmakis, Melanie Lacroix, Connor Lee, Noah Marion, and Christina Thomsen for reviewing earlier versions of the thesis. This list would be incomplete without me extending my gratitude to my friend and colleague Andrea Brauner for her support, from start to finish. Andrea's ability to recognize when I was in need of encouragement, "space", or simply a good laugh, was a constant reminder of just how lucky I am to have had the opportunity to pursue this degree doing something I absolutely love. I won't ever forget that.

I am grateful to all the farmers I have had the privilege of meeting and collaborating with over the years, and who have happily dedicated portions of their farmland for various components of this project: John Wise (Wise Acres Farm,

Centreville, Ontario); Jack Hinton and Glennis Harwig (Hawkwood Farm, Almonte, Ontario); and Peter Smith (Fair Weather Farm, Osgoode, Ontario).

Thank you to my parents, Jerry and Sue, who both possess a remarkable energy for life that has been and continues to be an inspiration to me. Lastly, I would like to thank Martha and our two amazing kids, Jonah and Tessa. Not a day went by where I didn't feel their love, enthusiasm, and encouragement for me in all aspects of life.

Preface

The work presented in my thesis is part of an ongoing project on the classical biological control of leek moth led by Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, Ottawa, Ontario, Canada. My thesis contributes to a better understanding of what happens after a biological control agent is released, in terms of how to improve the impact of the agent on the target host and how resident parasitoids may be affecting the introduced agent. I designed and implemented the experiments, collected the data, and wrote all chapters of this thesis. Each member of my supervisory committee provided valuable guidance with respect to all of these components. Data organization and statistical analyses for Chapters 2 and 3 were performed by me with guidance from Dr. Paul Abram at AAFC Agassiz, British Columbia, Canada. Chapters 2 and 3 are being prepared for submission to refereed journals; Chapter 2 is co-authored by Paul Abram, Naomi Cappuccino, and Peter Mason, Chapter 3 is co-authored by Paul Abram, Naomi Cappuccino, Andrew Bennett and Peter Mason. This work will help to expand the published record on this pest system.

Table of Contents

Abstract	ii
Acknowledgements	iii
Preface	v
Table of Contents	vi
List of Tables	ix
List of Figures	x
List of Appendices	xiii
Appendix 1: Aerial images of 2015 and 2016 buckwheat and no-buckwheat life table study field site.	xiii
Appendix 2: Complete stage-specific life tables for second generation leek moth in 2015 and 2016 caged and uncaged, buckwheat and no-buckwheat treatments.	xiii
Acronyms	xiii
Glossary	1
Chapter 1: General Introduction	3
Biological control of insect pests	3
Nectar-feeding requirements of hymenopteran parasitoids	6
Effects of nectar provisioning on biological control.....	7
Improved crop yield in the presence of nectar	9
Natural enemy guilds and trophic interactions	10
Effects of nectar provision on other trophic levels	12
Experimental approach and study system.....	13
<i>Use of life tables to measure success of biological control agents, and natural enemy impacts</i>	13
<i>Study system</i>	15
<i>Leek moth</i>	16
<i>Diadromus pulchellus</i>	17
<i>Conura albifrons</i>	17
Figures	22

Chapter 2: Conservation meets classical: testing the effect of floral enrichment on the efficacy of an introduced parasitoid of leek moth and their facultative hyperparasitoid.	24
Introduction.....	24
Materials & Methods	29
<i>Insect colonies</i>	29
<i>Plants used as nectar sources</i>	31
<i>Plant maintenance</i>	32
<i>Experimental procedure</i>	33
<i>Statistical analyses</i>	37
Results.....	38
<i>Offspring production and host killing</i>	38
<i>Female longevity</i>	40
<i>Offspring sex ratio</i>	41
Discussion.....	42
Figures.....	46
 Chapter 3: Evaluating the effect of floral enrichment on generational mortality and the parasitoid community of leek moth in the field.	 54
Introduction.....	54
Materials & Methods	58
<i>Leek moth culture</i>	58
<i>Host plant maintenance</i>	59
<i>Plant selection</i>	59
<i>Field plot</i>	60
<i>Experimental procedure</i>	61
<i>Statistical analyses</i>	67
Results.....	68
<i>Stage-specific mortality</i>	68
<i>Generational mortality</i>	69
<i>Parasitism levels and effects on leek moth population growth</i>	70

<i>Parasitoid composition</i>	71
Discussion	72
Tables	79
Figures	81
Chapter 4: General Discussion	90
Summary of findings	90
Opportunities and limits for applying CBC techniques to the leek moth system in Canada	92
General potential and limits for combining classical and conservation biological control strategies	95
Future research	97
Figures	100
References	101
Appendix	121
Appendix 1. Aerial images of 2015 and 2016 buckwheat and no-buckwheat life table study field site.	121
Appendix 2. Complete stage-specific life tables for second generation leek moth in 2015 and 2016 caged and uncaged, buckwheat and no-buckwheat treatments.....	123

List of Tables

Table 1. Contribution of each leek moth, <i>Acrolepiopsis assectella</i> , life stage to generational mortality for caged and uncaged, buckwheat and no-buckwheat leek plots in 2015 and 2016. Resulting net reproductive rate (R_0) is tabulated for each treatment in 2015 and 2016.....	79
Table 2. Influence of parasitism on leek moth, <i>Acrolepiopsis assectella</i> , reproductive output (R_0) for each larval, pupal and combined stages for cage and uncaged, buckwheat and no-buckwheat leek plots in 2015 and 2016.	80

List of Figures

- Figure 1.** Leek moth, *Acrolepiopsis assectella*, **A.** Adult, **B.** Late-instar larvae causing extensive feeding damage to a garlic, *Allium sativum*, plant. (Photos: A.M. Brauner) 22
- Figure 2.** **A.** Adult female *Diadromus pulchellus* parasitizing a leek moth, *Acrolepiopsis assectella*, cocoon; **B.** Adult female *Conura albifrons* parasitizing a leek moth cocoon. (Photos: A.M. Brauner) 23
- Figure 3.** Greenhouse cage setup showing the buckwheat + vetch treatment. (Photo: A.M. Brauner) 46
- Figure 4.** Greenhouse setup with randomized arrangement of cage treatments on benches. (Photo: A.M. Brauner) 47
- Figure 5.** Leek moth, *Acrolepiopsis assectella*, pupae pinned to a young **A.** uncovered and **B.** covered (parasitoid exclusion) leek plant in preparation for a greenhouse exposure. (Photos: A.M. Brauner) 48
- Figure 6.** Newly-formed leek moth, *Acrolepiopsis assectella*, pupae with a pin placed between the pupal case and woven cocoon in preparation for greenhouse exposures. (Photo: A.M. Brauner) 49
- Figure 7.** Number of leek moth, *Acrolepiopsis assectella*, pupae killed by *Diadromus pulchellus* females by exposure number and cage treatment: water; vetch; sucrose; buckwheat; and buckwheat + vetch. Non-reproductive parasitoid-induced mortality was calculated using a correction derived from unexposed controls (see experimental procedure above) 50
- Figure 8.** Total (left) and daily (right) reproductive and non-reproductive parasitoid-induced mortality of leek moth, *Acrolepiopsis assectella*, pupae for each cage treatment; water (W), vetch (V), sucrose (S), buckwheat (B), buckwheat + vetch (B+V), by **A.** *Diadromus pulchellus* and **B.** *Conura albifrons*. (Different letters indicate significant differences [$p < 0.05$] between treatments as determined by Tukey contrasts: Upper case = parasitoid offspring emergence; Lower case = non-reproductive parasitoid-induced mortality) 51
- Figure 9.** Number of leek moth, *Acrolepiopsis assectella*, pupae killed by *Conura albifrons* females by exposure number and cage treatment: water; vetch; sucrose;

buckwheat; and buckwheat + vetch. Non-reproductive parasitoid-induced mortality was calculated using a correction derived from unexposed controls (see experimental procedure above).....	52
Figure 10. Proportional longevity of female <i>Diadromus pulchellus</i> and <i>Conura albifrons</i> when provided with various nutritional treatments: water; vetch; sucrose; buckwheat; and buckwheat + vetch. (Different letters indicate significant differences [$p < 0.05$] between treatments as determined by Tukey contrasts).....	53
Figure 11. Proportion of male offspring produced by <i>Diadromus pulchellus</i> and <i>Conura albifrons</i> when provided with various nutritional treatments: water (W); vetch (V); sucrose (S); buckwheat (B); and buckwheat + vetch (B+V). (Different letters indicate significant differences [$p < 0.05$] between treatments as determined by Tukey contrasts).	53
Figure 12. Wide-angle aerial photograph of 2016 life-table study site and surrounding habitat at the Central Experimental Farm, Ottawa, Ontario, Canada. A. actual field plot used for buckwheat and no-buckwheat leek blocks; B. adjacent tilled habitat with no available floral resources; C. field plot where original releases of <i>Diadromus pulchellus</i> occurred. Note: photograph was taken on September 5, 2016; approximately 2 months following completion of the study (Google Inc, 2017).....	81
Figure 13. Aerial photograph of 2016 life-table study site at the Central Experimental Farm, Ottawa, Ontario, Canada. A. leek blocks with no buckwheat; B. leek blocks intercropped with buckwheat. Note: photograph was taken on September 5, 2016; approximately 2 months following completion of the study (Google Inc, 2017).....	82
Figure 14. Life-table study site at the Central Experimental Farm, Ottawa, Ontario, Canada. A. 2016 no-buckwheat leek plot; B. 2016 buckwheat-enhanced leek plot. (Photos: A.M. Brauner).....	83
Figure 15. Sentinel plants containing newly-hatched leek moth, <i>Acrolepiopsis assectella</i> , larvae in a no-buckwheat leek plot. (Photo: A.M. Brauner).....	84
Figure 16. Natural enemy exclusion cages on sentinel leeks prior to field placement. (Photo: A.M. Brauner)	85
Figure 17. Potted leeks in realized fecundity cages, each containing a mating pair of leek moths, <i>Acrolepiopsis assectella</i> . (Photo: A.M. Brauner).....	86

Figure 18. Stage-specific mortality (\pm SE) by treatment, of leek moth, *Acrolepiopsis assectella*, in 2015 and 2016. Caged (C), uncaged (U) treatments in buckwheat (B) and no-buckwheat (NB) blocks. (Stars indicate significant differences [$p < 0.05$] between treatments as determined by Type II Wald chisquare tests: * = caged and uncaged treatments; ** = buckwheat and no-buckwheat treatments). 87

Figure 19. Proportion of leek moth, *Acrolepiopsis assectella*, larval and pupal parasitism (\pm SE) in caged (C), uncaged (U) treatments in buckwheat (B) and no-buckwheat (NB) blocks. (Stars indicate significant differences [$p < 0.05$] between treatments as determined by Type II Wald chisquare tests: * = caged and uncaged treatments; ** = buckwheat and no-buckwheat treatments). 88

Figure 20. Parasitoid species composition (%) from larval and pupal stages of leek moth, *Acrolepiopsis assectella*, in buckwheat and no-buckwheat leek plots in 2015 and 2016..... 89

Figure 21. Current food web as observed in the leek moth system from Chapter 3 field experiments. Solid lines represent known relationships between hosts (food resources) and consumers (herbivores and predators); hollow lines represent potential but currently unknown relationships between food resources and consumers. 100

List of Appendices

Appendix 1: Aerial images of 2015 and 2016 buckwheat and no-buckwheat life table study field site.

Appendix 2: Complete stage-specific life tables for second generation leek moth in 2015 and 2016 caged and uncaged, buckwheat and no-buckwheat treatments.

Acronyms

CBC	conservation biological control
BCA	biological control agent
IGP	intraguild predation
NACF	National Arthropod Containment Facility
AAFC	Agriculture and Agri-Food Canada
CEF	Central Experimental Farm (Ottawa, Ontario, Canada)
L:D	light:dark
RH	relative humidity
SE	standard error
NRPIM	non-reproductive parasitoid-induced mortality

Glossary

* Definitions for the following terms were described (whenever possible) in the context in which the term was used and/or intended in the body of this thesis. Unless otherwise indicated (e.g., JM), definitions were reproduced from established biological, ecological and/or entomological literature. Sources for each definition are indicated in brackets following the definition.

Eclosion: escape of the adult insect from the cuticle of the pupa (Torre-Bueno, 1989).

Ecological niche: the set of conditions under which species can maintain populations in the long term without an in-migration of individuals (Peterson, 2003).

Ectoparasitoid: a parasitoid that develops externally on a host, and feeds through an integumentary lesion (Torre-Bueno, 1989).

Endoparasitoid: a parasitoid that feeds internally within a host (Torre-Bueno, 1989).

Facultative hyperparasitoid: parasitoids of which their progeny can develop as either a primary or secondary parasitoid (Sullivan, 1987).

Feeding niche: the temporal and spatial habitat in the ecosystem that is filled by an organism as a result of its eating habits (JM).

Guild: a group of species that exploit the same class of environmental resources, or that exploit different resources in related way (Root, 1967)

Host: the organism in or on which a parasite or parasitoid lives (Torre-Bueno, 1989).

Hyperparasitoid: a secondary insect parasitoid that develops at the expense of a primary parasitoid (syn. 'secondary parasitoid') (Sullivan & Völkl, 1999).

Idiobiont: a parasitoid that prevents further development of the host by paralyzing or killing it (Torre-Bueno, 1989).

Indigenous community: organisms that are already residing in an area or system of study; not limited to native species (syn. 'resident community') (JM).

Koinobiont: parasitoid attacking exposed or weakly concealed active immature insects, allowing them to continue to develop for some time after oviposition by the parasite (Torre-Bueno, 1989).

Natural enemy: an organism that inflicts mortality on an individual of a species (e.g., parasitoids, pathogens, and predators) (JM).

Niche separation: the process by which different, competing species utilize the environment in a way that enables them to coexist (JM).

Non-reproductive parasitoid-induced mortality (NRPIM): effects caused by a parasitoid that result in host death but not in offspring emergence including but not limited to: host-feeding, oviposition without development, pseudoparasitism, superparasitism, multiparasitism, predation, competition, and venom injection. (P. Abram, personal communication).

Obligate hyperparasitoid: a parasitoid that always acts as a hyperparasitoid (Sullivan, 1987)

Oviposition: the act of depositing eggs (Torre-Bueno, 1989)

Ovigenesis: development of mature eggs (JM).

Parasite: an organism that lives in or on another (the host), from which it obtains food, shelter, or other beneficial requirements (Torre-Bueno, 1989).

Parasitoid: an internal or external parasite, e.g., many Hymenoptera and Diptera (Tachinidae), which slowly kills the host, this event generally occurring near the end of the parasite's larval development (Torre-Bueno, 1989).

Parasitoid-induced host mortality: any mortality that occurs to a host with the cause of the mortality being associated with an action performed by a parasitoid including but not limited to: host-feeding, oviposition with and without development, pseudoparasitism, superparasitism, multiparasitism, predation, competition, and venom injection (JM).

Predator: an organism that obtains energy (as food) by consuming and usually killing, 2 or more prey organisms during its lifetime (Torre-Bueno, 1989).

Primary parasitoid: the first-arriving parasitoid that forms a parasitic relationship with a host resulting in death of the host (JM).

Proovigenic: completing ovigenesis after emergence from the pupa (Torre-Bueno, 1989).

Synchronous development: referring to overlapping temporal relationship between a host and its parasitoid(s) in nature (JM).

Synovigenic: developing successive numbers of eggs to maturity throughout adult life (Torre-Bueno, 1989).

Chapter 1: General Introduction

Biological control of insect pests

In its broadest sense, biological control is the deliberate use of a living organism to suppress a pest (Eilenberg *et al.*, 2001; Hajek, 2004). More specifically, it involves the use of one or more natural enemy species – predators, parasites, pathogens, and competitors – to control a variety of pests including insects, nematodes, weeds, and plant diseases (Gurr *et al.*, 2000). Biological control of insect pests is most often applied to food production systems – greenhouse and field – as it can help to improve yield and crop quality, reduce the need for insecticides, and create a more sustainable and safer food supply (Pimentel, 2008). There are four main types of biological control used against insect pests: inundation, inoculation, classical, and conservation. All four of these involve either introducing a biological control agent (BCA) or agents, or taking measures to preserve or enhance natural enemies to suppress pests.

Inundation/augmentation biological control of insect pests involves releasing a BCA with the intent of achieving immediate pest suppression by the agent. The success of this strategy is measured by the impact the released individuals have on the pest, rather than by progeny produced as persistence and proliferation of the agent is not the desired outcome. This type of biological control is often employed to reduce pest pressure that exceeds economic thresholds, particularly in countries with strict regulations on insecticides (e.g., European Union members) (Eilenberg *et al.*, 2001; Razinger *et al.*, 2014). Examples of commonly used inundation BCAs are the lepidopteran egg parasitoids *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), which are used

against pests in field crops (Mansfield & Mills, 2002; Ulrichs & Mewis, 2004), in greenhouse operations (Cabello *et al.*, 2014; Tabone *et al.*, 2010) and as control agents against pests in food processing and storage operations (Steidle *et al.*, 2001).

Inoculation biological control is another technique that involves releasing a BCA against a pest; however, it differs from inundation biological control in that the agent is expected to multiply and provide pest suppression through future generations of progeny, without becoming permanently established (Eilenberg *et al.*, 2000; Eilenberg *et al.*, 2001). This strategy is most often used in greenhouses to prevent pests from reaching economically damaging levels. Following harvest, the greenhouse is cleaned, a new crop is planted, and new inoculative releases are made (Eilenberg *et al.*, 2000; van Lenteren, 2000; van Lenteren, 2012).

Classical biological control is the intentional introduction of an exotic species (BCA) into nature with the goal of establishing the agent to achieve long-term pest suppression (Greathead, 1994; Coombs & Hall, 1998; Eilenberg, 2001). Based on the observation that most introduced pest species are not problematic in their areas of origin due to the existing community of natural enemies, the release of a co-evolved (with the pest) BCA is often one of the most promising management strategies (Hajek, 2004). Evidence of successful classical biological releases against pest insects can be found as far back as 1877 when the vedalia beetle, *Rodalia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), and the parasitic cottony-cushion scale killer, *Cryptochaetum iceryae* (Williston) (Diptera: Criptochaetidae), were introduced to California from Australia, causing a drastic decrease in the cottony cushion scale, *Icerya purchasi* Maskell

(Hemiptera: Margarodidae), an introduced pest of citrus. Since then, more than 6158 introductions have been made worldwide using 2384 different insect BCAs against 588 pest insect species. However, of the 172 pests that have been monitored, establishment of the released agent has occurred only about 32% of the time, and acceptable levels of control have been attained only about 10% of the time (Cock *et al.*, 2016). Abiotic and biotic factors can influence why an introduced BCA may fail to establish, and include, but are not limited to, the following: failure to detect the pest, poor adaptation to climate in the area of release including inability to overwinter, poor life cycle synchronization with the host, and inadequate dispersal ability. Stiling (1993) examined reviews of 148 failed attempts to substantially reduce pest populations and found that more than 70% of the reasons for failure could be grouped into three major categories: climate (34.5%), predation or parasitism by native fauna (20.3%), and lack of alternative hosts or food (16.9%). The climate-related factors included conditions at the time of release, as well as direct (e.g., temperature, aridity) and indirect (e.g., lack of parasitoid-host synchronization) climatic effects – all problems that could, in principle, be addressed when evaluating a BCA for release. This suggests that the chances of an agent establishing could be increased by more than 50% simply by releasing individuals into a favourable environment. Ensuring access to non-host food resources upon release to increase the probability of successfully finding and parasitizing target hosts is another possibility. Classical biological control is often researched and considered separately from the other types of biological control (e.g., inundation, inoculation); but by combining the classical approach with another type, conservation biological control

(CBC), population establishment of the introduced BCA(s) and long-term management of the pest might result in higher levels of success.

Conservation biological control includes modification of the environment and farming practices to enhance or preserve natural enemies in order to reduce the negative effects of a pest (Eilenberg, 2001). There are numerous potential benefits offered to natural enemies through a CBC program that manipulates habitat to improve availability of resources required by natural enemies (see Barbosa, 1998; Landis *et al.*, 2000; Wilkinson & Landis, 2005; Tschardtke *et al.*, 2007). Habitat diversification near or within a cropping system can offer microclimate refuges for natural enemies as a buffer against extreme conditions (e.g., Dyer & Landis, 1996; Orr & Fox, 2012), as well as more suitable overwintering habitats (e.g., Pfiffner & Luka, 2000). It also offers the added benefit of additional prey or host availability for natural enemies, and non-host food resources such as honeydew, pollen, and nectar. Several studies have demonstrated that provisioning of sugar or nectar sources in generally sugar-deprived cropping systems has corresponded with increases in natural enemy abundance (particularly for parasitoids) and resulting pest-suppression (Cappuccino *et al.*, 1999; see also Landis *et al.*, 2000; Lee & Heimpel, 2008; Tschumi *et al.*, 2016; Hatt *et al.*, 2017).

Nectar-feeding requirements of hymenopteran parasitoids

Parasitoids use sugars for energy and to assist with ovigenesis, locomotion, and foraging activities (Wäckers, 1994; Lee *et al.*, 2004). Laboratory and field studies have demonstrated that feeding on sugar sources (i.e., sugar water, honey, hemipteran honeydew, nectar) can result in increased longevity and host-killing ability throughout

the lifespan of parasitoids (Lewis, 1961; Foster & Ruesink, 1984; Idris & Grafius, 1995, Lee *et al.*, 2004), but can also reduce the risk of starvation, especially in agricultural settings which are often relatively devoid of available non-host foods due to regular agricultural practices (Siekmann *et al.*, 2001).

Effects of nectar provisioning on biological control

By increasing non-crop plant diversity, the activities of natural enemies may be enhanced simply by providing them with favourable abiotic conditions such as a more moderate microclimate or refuge habitat when field conditions are extreme or with biotic resources such as alternative hosts or prey, and plant-based foods such as pollen, nectar and honeydew (Root, 1973; Landis *et al.*, 2000). When suitable floral and nectar resources are unavailable within an agricultural habitat, the biological control services provided by hymenopteran parasitoids may be limited or absent (Wratten *et al.*, 2002), but, through resources provisioning in the form of intercropping and wildflower plantings in a monoculture agricultural setting, an increase in biological control of some pests may result (Gurr & Wratten, 1999, Lewis (1961a,b).

To properly address the effect(s) that nectar-provisioning may have on the efficacy and success of a parasitoid BCA, several conditions and outcomes need to be explored in addition to the nutritional requirements of the parasitoid. Wratten *et al.* (2002) developed a “hierarchy of research outcomes” needed to demonstrate success of a parasitoid BCA through the provisioning of floral (nectar) resources. Similarly, Heimpel & Jervis (2005) developed a hypothesis that combined nectar-feeding as the underlying mechanism and improved biological control as the outcome in their “parasitoid nectar

provision hypothesis.” In both cases, several behavioural and ecological requirements need to be met in order to achieve the goal of reducing a pest population below an economic threshold. The components of the Wratten *et al* (2002) and Heimpel & Jervis (2005) hypotheses include several overlapping conditions; however, in both cases, each of the outcomes are progressively more difficult to obtain. The combined (merged) components of both hypotheses are: 1) sugar (nectar) is a limited resource; 2) parasitoids aggregate at or near flowers; 3) parasitoids feed on nectar in the field; 4) parasitoid fitness and fecundity is improved due to nectar consumption; 5) parasitism levels increase due to nectar feeding; 6) pest densities are reduced below an economic threshold in the presence of nectar

The combined Wratten (2002) and Heimpel & Jervis (2005) hypotheses are interesting for four reasons: a. monocultures, and particularly agricultural systems tend to be lacking in sugar resources, b. sugar-fed parasitoids have been shown to live longer and attack more hosts when compared to those that are sugar-starved, c. many parasitoids utilize sugar-sources in natural settings, and d. parasitoids use nectar-resources without sacrificing their host-utilization. By evaluating each of the components and required outcomes from the combined hypotheses individually, mechanisms that determine pest suppression by parasitoids, with the goal of improving crop yield, can be better assessed.

Improved crop yield in the presence of nectar

Although increased yield as a result of nectar-provisioning to natural enemies was not a key component of either of the previously mentioned hypotheses, it is nonetheless the desired outcome of a conservation biological control program. There are few examples of increased crop yield in the field as a result of increased parasitism due to nectar-provisioning; however, a review by Letourneau *et al.* (2011) reported that crop yield in diversified plant settings was about 14% lower than in those fields without added diversity, likely due to a decrease in actual crop plantings. Although it did not specifically assess the impact of added nectar on biological control of the pea aphid due to natural enemies, a study by Cardinale *et al.* (2003) demonstrated that multiple natural enemies in an aphid-parasitoid-predator system had a better impact on the pest than the additive effect of each natural enemy individually. This implies that an increase in natural enemy abundance (as is predicted to occur in a more diversified habitat), may actually have a synergistic effect on pest suppression, leading to increased crop yield. In another study by Jonsson *et al.* (2015), kale (*Brassica oleracea* L. [Brassicaceae]) fields that contained buckwheat strips had increased aphid and diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) parasitism levels compared to simple kale fields with no floral supplements. By measuring the impact of parasitism on the abundance of *P. xylostella*, the authors indirectly determined that the addition of floral resources to this moderately simple agricultural system positively impacted crop yield.

Natural enemy guilds and trophic interactions

Natural enemies of field crop pests exist as an assemblage of interacting species within and between trophic levels and guilds (Brodeur & Boivin, 2006). This community may be complex and include multiple species that compete with each other for resources through competition processes including acquisition of host and non-host foods, that interact through higher order predation and hyperparasitism, or that coexist without these antagonistic interactions by occupying different feeding and biological niches (niche separation) (Pedersen & Mills, 2004). Intraguild predation (IGP) occurs when two species attack the same host or prey (potential competition) and undergo a trophic interaction (e.g., parasitism or predation) (Rosenheim *et al.*, 1995; Rosenheim, 1998). Higher-order predators and omnivores are likely to influence the efficacy of a BCA by consuming both the host (i.e., competition) and the host's parasitoids (whether intentionally or unintentionally) (Meyhöfer & Klug, 2002; Müller & Brodeur, 2002). Obligate hyperparasitoids, those that develop at the expense of another parasitoid, may affect the establishment, abundance and distribution of their hosts, potentially releasing the pest herbivore from parasitoid pressure (Boivin & Brodeur, 2006; May & Hassell, 1981); whereas, facultative hyperparasitoids, those that can act as either higher-order natural enemies of another parasitoid or as an intraguild competitor, might have other effects on both the primary parasitoid and the pest (Rosenheim, *et al.*, 1995; Brodeur, 2000; Boivin & Brodeur, 2006). It is difficult to predict the dynamics of host use by facultative parasitoids, especially in the case of generalists, as host communities and densities (i.e., parasitoids and primary consumers) may differ between habitats, and when

different spatial and time scales are involved (Brodeur, 2000; Boivin & Brodeur, 2006). When interguild hosts are rare, a facultative hyperparasitoid is likely to act as both a primary parasitoid of the herbivore and as a secondary parasitoid of the pest's parasitoids; however, when herbivores are abundant, a facultative hyperparasitoid may demonstrate a tendency to exploit the primary consumer, supplementing the level of control already achieved by the primary parasitoid(s) (Rosenheim *et al.*, 1995; Pedersen & Mills, 2004; Boivin & Brodeur, 2006). This may also occur in situations where the facultative hyperparasitoid (acting as a primary consumer) obtains higher nutritional value from the herbivore than it would from the primary parasitoid. The life-history strategy of the facultative hyperparasitoid likely plays a large role in determining if it has a disruptive or additive effect on a host-parasitoid system (Godfray, 1994; Brodeur, 2000). Aside from direct competition and predation, this disruptive effect might present itself through decreased foraging by primary parasitoids (potential hosts to facultative hyperparasitoids), especially by generalists who might seek out alternative hosts in a system with a reduced community of hyperparasitoids (Nofemela, 2013). Likewise, a facultative hyperparasitoid may choose to accept additional or alternate hosts within the same trophic level which may reduce competition from other parasitoids; however, in both cases, this behaviour is likely constrained by searching efficiency, dispersal ability, patterns of aggregation and availability of hosts (Pedersen & Mills, 2004; Boivin & Brodeur, 2006).

Despite the fact that competition, predation and parasitism by indigenous fauna are reported as some of the main causes for hindered or failed establishment of BCAs

(Hokkanen, 1985; Stiling, 1993), the effect of nectar-provisioning on interactions within and between the third and fourth trophic levels has not yet been thoroughly addressed. It is critical to further develop our understanding of how applying this component of CBC will affect the introduced (primary) BCA agent and the interacting community members (i.e., competitors and predators) that could reduce the impact on the pest.

Effects of nectar provision on other trophic levels

Studies have shown that supplementary nectar-provisioning can result in increased pest suppression from natural enemies in the third trophic level (Landis *et al.*, 2000; Gurr *et al.*, 2000; Berndt & Wratten, 2005; Jonsson *et al.*, 2015, see review by Veras *et al.*, 2015). However, it is important to consider the potential positives and/or negatives that resource provisioning will have on all four trophic levels, including the pest, prior to implementing a CBC program (Baggen & Gurr, 1998; Norris and Kogan, 2005; Tscharrntke *et al.*, 2005; Banks *et al.*, 2008). Recent refinements in CBC have led to careful screening of supplemental plants to determine which species provide minimal benefit to the second trophic level (herbivore) and maximal benefit to the third trophic level (e.g., primary parasitoid) (Lavandero *et al.*, 2006). Only a few laboratory and field studies have demonstrated that nectar-provisioning can benefit the fourth trophic level (hyperparasitoids or parasitoids of predators), which in turn can have a detrimental effect on the members of the third trophic level (parasitoids) in a system (Araj *et al.*, 2006; Araj *et al.*, 2009; Jonsson *et al.*, 2009; Araj *et al.*, 2011). In one example, Jonsson *et al.* (2009) used field cage experiments to determine how the addition of flowering buckwheat to an alfalfa, *Medicago sativa* L. (Fabaceae), system impacted Tasmanian lacewing, *Micromus*

tasmaniae Rambur (Neuroptera: Hemerobiidae), predation of pea aphids, *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae) and parasitism by a primary parasitoid of *M. tasmaniae*, *Anachris zealandica* Ashmead (Hymenoptera: Figitidae). They determined that at higher aphid densities, the buckwheat had an adverse effect on the lacewing population, likely due to increased parasitism from *A. zealandica*, which was previously shown to benefit from nectar feeding. At lower aphid densities however, buckwheat had a positive effect on lacewing predation of *A. pisum*. In another cage study, Araj *et al.* (2009) measured the effects of buckwheat flowers on levels of parasitism by *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) on *A. pisum*, and hyperparasitism by *Dendrocerus aphidum* (Rondani) (Hymenoptera: Megaspilidae), in alfalfa. They determined that parasitism by the introduced BCA, *A. ervi*, was significantly higher in the presence of buckwheat, despite the increased levels of hyperparasitism in the same treatments. This suggests that the increased hyperparasitism did not cause enough of a trophic cascade to significantly impact the second or first trophic levels. Although these two examples did not clearly demonstrate that the addition of floral resources had a sustained detrimental effect on natural enemies in the third trophic level, the apparent lack of information on this subject highlights the need for more studies to examine the potential effects of CBC on IGP and competition.

Experimental approach and study system

Use of life tables to measure success of biological control agents, and natural enemy impacts

A life table is a summary of statistics that is used to estimate the number of deaths, the number of survivors, and the level of age-specific mortality of a particular cohort within a population (Deevey, 1947; Carey, 1993). Causes of mortality from each cohort are often assigned to a specific agent (e.g. predation, parasitism, various abiotic factors), a task that is made easier when examining each developmental stage independently (Bellows & Van Driesche, 1999). The development and use of life tables to quantify and summarize the distribution of generational mortality in insect populations was first proposed by Morris & Miller (1954) who used them to analyze stage-specific mortality of spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae). Since then, life-table studies have been used to determine stage-specific vulnerability and key mortality factors (including natural enemy impact) of a variety of pests in the field (Harcourt, 1969; Ghould *et al.*, 1992; Miranda *et al.*, 1998; Liu *et al.*, 2004; Gholizadeh *et al.*, 2010; Jenner *et al.*, 2010). An interesting and informative component of life-table analyses is their usefulness in quantifying the impact of a particular natural enemy species or guild (e.g., parasitism) on each host stage. Boettner *et al.* (2000) constructed life tables for all stages of the native cecropia moth, *Hyalophora cecropia* (L) (Lepidoptera: Saturniidae) and determined that the BCA, *Compsilura concinnata* (Meigen) (Diptera: Tachinidae), which was introduced to North America in the early 1900's to control gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae), was responsible for more than 80% of the larval mortality in the field. These results confirm earlier reporting that native saturniid moths were in heavy decline in the areas of *C. concinnata* introduction (Culver, 1919; Stamp & Bowers, 1990). Gould *et al.* (1992) used a life-table analysis to measure the

positive impact that an introduced BCA, *Encarsia partenopea* (Walker) (Hymenoptera: Aphelinidae) was having on the invasive *Siphoninus phillyreae* (Haliday) (Insecta: Hemiptera: Aleyrodidae), a major pest of fruit trees in California. They found that the net reproductive rate of the pest was stable or in decline compared to unity at sites where *E. partenopea* had been introduced, but indicated that populations were increasing in density at sites where the introduced BCA was not present. Finally, Jenner *et al.* (2010) constructed life tables to estimate the stage-specific mortality levels of leek moth in Switzerland. They employed an approach that introduced a fresh cohort of insects for each life stage, and compared their results to a similar study by Noyes (1974), who measured mortality from a single, continuous cohort in the UK. Both studies estimated the mortality levels of each life stage of leek moth: Jenner *et al.* (2010) found that the highest mortality occurred during the neonate stage which begins immediately after egg hatching; however, Noyes (1974) determined that the prepupal stage was the most susceptible to mortality. These differing results, likely based on the different methods used to place individuals onto plants in addition to the geographical distance between both studies, indicate that it is important to consider life-table data gathered using a variety of techniques across multiple generations and locations.

Study system

One example of a system where interactions between inter- and intraguild parasitoids may be limiting the establishment and impact of an introduced BCA is the case of *Diadromus pulchellus* (Wesmael) (Hymenoptera: Ichneumonidae), a solitary pupal parasitoid introduced into eastern Canada to control the invasive European leek moth,

Acrolepiopsis assectella (Zeller) (Lepidoptera: Acrolepiidae). The potential effect of resident parasitoids on the introduced agent, *Diadromus pulchellus*, was the driving force behind the experiments in this thesis.

Leek moth

The leek moth, *Acrolepiopsis assectella* (Zeller) (Lepidoptera: Acrolepiidae) (Figure 1 A, B), native to Europe, is a recently introduced pest of *Allium* spp. (Amaryllidaceae) in North America. It was first detected near Ottawa, Ontario, Canada in 1993 in organic garlic, *Allium sativum* L. (Amaryllidaceae) fields, and has since spread throughout much of eastern Canada (Québec, New Brunswick, Nova Scotia and Prince Edward Island) and into northern New York and Vermont, USA (Mason *et al.*, 2013a,b; Seto & Shelton, 2016). CLIMEX modelling of the known distribution of this pest in its native range predicts that leek moth has the potential to expand its area of invasion into much of eastern North America, with the potential to cause significant production losses to *Allium* spp. growers (Mason *et al.*, 2011). Preliminary surveys indicated that few indigenous parasitoids were present in the leek moth system following its North American introduction. As a result, Jenner *et al.* (2008) studied populations of leek moth in Switzerland in an attempt to find a suitable BCA to introduce in the areas of North American leek moth invasion. A number of larval and pupal parasitoids were reported to attack leek moth in its native range (see Table 1); however, most of those species were known to have a broad host range and were not suitable for introduction. Among these, *Diadromus pulchellus* (Wesmael) (Hymenoptera: Ichneumonidae) (Figure 2A), was found to be the only species that was host-specific and highly synchronous with the host.

Diadromus pulchellus

Diadromus pulchellus is a solitary pupal endoparasitoid that has been shown to have a narrow host range in the laboratory and in the field (Bekkaoui & Thibout, 1993; Gauthier *et al.*, 2004; Jenner *et al.*, 2014). This synovigenic species requires non-host food for energetic requirements and egg-production, and has been observed feeding on host haemolymph in the laboratory (Jenner *et al.*, 2012). Adults of another *Diadromus* species, *D. subtilicornis*, have demonstrated an increase in longevity, fecundity and host-parasitization when provided with a combination of honey and host haemolymph compared with those fed only honey, so it is likely that host-feeding by *D. pulchellus* plays at least a minor role in its oviposition activity (Tran & Takasu, 2000). Jenner *et al.* (2010) conducted laboratory studies examining the overwintering strategy and cold hardiness of *D. pulchellus* and determined that this parasitoid would be likely to survive the winters in the area of leek moth invasion in North America. Releases of *D. pulchellus* in eastern Ontario, Canada began in 2010 (Mason *et al.*, 2013a). Post-release monitoring between 2010–2017 has determined that *D. pulchellus* is capable of surviving the winters in the area of release; however, it is too soon to estimate the relative impact that *D. pulchellus* will have on leek moth as recovery of *D. pulchellus* in the field is still low (Mason *et al.*, 2013a; Miall *et al.*, 2014; PM, unpublished data).

Conura albifrons

Following its introduction, ongoing surveys to determine the establishment and spread of *D. pulchellus* determined that a suite of indigenous parasitoids had entered the leek moth-parasitoid system (Mason *et al.*, 2013a; Miall *et al.*, 2014; PGM, unpublished data). One

of the key members of this community is *Conura albifrons* (Walsh) (Hymenoptera: Chalcididae) (Figure 3B), a facultative pupal hyperparasitoid of numerous lepidopteran, hymenopteran, and coleopteran hosts, among others (Arthur 1958; Peck, 1963; Miall *et al.*, 2014). This long-lived, synovigenic species needs non-host food for energetic requirements and egg-production, and often requires multiple host encounters before oviposition begins. *Conura albifrons* has also been observed feeding on host haemolymph in the laboratory; however, it is not known what contribution this provides to longevity, fecundity or host-killing ability. A few studies have examined *Conura* spp. either in the context of its disruption of biological control or in consideration for introduction as a BCA (Gaines, 1997; Miall *et al.*, 2014; Bulgarella *et al.*, 2017). Gaines (1997) studied the potential impact of *C. torvina* on the disappearance of *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae), an introduced parasitoid of the cabbage butterfly, *Pieris rapae* (Lepidoptera: Pieridae) in Virginia, USA. Due to the poor reproductive and host-finding ability of *C. torvina*, he concluded that it was unlikely the cause of the disappearance of *C. rubecula*. After finding an increasing number of *C. albifrons* in the areas of *D. pulchellus* release, Miall *et al.* (2014) used laboratory choice tests to determine if *C. albifrons* was able to attack and develop on *D. pulchellus*-parasitized leek moth hosts. They determined that *C. albifrons* was able to act as a primary parasitoid of leek moth and a hyperparasitoid of *D. pulchellus*, and indicated that it has the potential to disrupt establishment and efficacy of *D. pulchellus* due to competition and intraguild predation. A third study explored the life cycle and host specificity of *Conura annulifera*, a potential BCA of *Philornis downsi* (Dodge and Aitken) (Diptera:

Muscidae), a parasitic fly that is negatively impacting populations of Darwin's finches (Thraupidae) in the Galapagos Islands. They found that *C. annulifera* is a promising candidate for release against *P. downsi* due to its host specificity and host-killing ability (Bulgarella *et al.*, 2017).

Post-release monitoring of the establishment and spread of *D. pulchellus* revealed that although establishment had occurred, the prevalence of indigenous competitors and hyperparasitoids in the leek moth system may be impacting the efficacy of *D. pulchellus*. I thus examined the possibility of introducing additional nectar sources to aid the establishment and impact of *D. pulchellus*, while also evaluating to what extent this added resource might affect both the introduced *D. pulchellus* and the indigenous parasitoids that had moved into the system.

I employed a variety of approaches to answer this question, beginning with large-cage greenhouse experiments, to assess the effect of nectar provision by two plants (buckwheat, *Fagopyrum esculentum* Moench [Polygonaceae], and common vetch, *Vicia sativa* L. [Fabaceae]) on *D. pulchellus* and the often-encountered, native facultative hyperparasitoid, *Conura albifrons* (Walsh) (Hymenoptera: Chalcididae). I then designed field experiments to determine, in part, if the addition of buckwheat to the sugar-deprived leek moth system would impact the mortality and parasitism levels of leek moth, and shift the parasitoid abundance and composition of indigenous parasitoids and the introduced *D. pulchellus* compared to standard (no-buckwheat) plots. I assessed population-level impacts of mortality caused by natural enemies by conducting a life-table study, which to my knowledge has not been applied to a CBC question before.

The general hypotheses for these two studies were:

- 1) All things being equal (i.e., equal parasitoid state, including ability to find and access the nectar source), nectar provisioning should improve longevity, fecundity, and the number of hosts killed both by the primary parasitoid and facultative hyperparasitoid;
- 2) Nectar provisioning by a given plant species is likely to differentially affect the primary parasitoid and its facultative hyperparasitoids, due to differences in physiology and life history of the parasitoids. In principle, this could mean that providing a given resource in a system could shift the balance of parasitism between primary parasitism and hyperparasitism.

Based in part on previous studies that have demonstrated that parasitoid fitness generally increases when provided with a nectar source to feed on, I predicted that:

- 1) Both *D. pulchellus* and *C. albifrons* will experience greater longevity, increased fecundity, and a higher impact on the target host when a sugar source is provided to them under simplified (greenhouse) settings.
- 2) The response of the parasitoids will vary among buckwheat and common vetch, and a combination of the differing nectar sources (multiple different nectar resources) will benefit parasitoid fitness and performance to a greater extent than a single nectar source.
- 3) Field experiments will show different relative abundances of primary versus facultative hyperparasitoids attacking the target pest depending on floral

treatment, in a way that reflects the relative benefits of floral resource addition to *D. pulchellus* and *C. albifrons* observed in the greenhouse experiment.

- 4) Host mortality levels in the field will be increased by the addition of buckwheat compared to no-buckwheat plots, due to increased parasitism by both the primary parasitoid and the facultative hyperparasitoid, unless the two show evidence of interference.

Figures



Figure 1. Leek moth, *Acrolepiopsis assectella*, **A.** Adult, **B.** Late-instar larvae causing extensive feeding damage to a garlic, *Allium sativum*, plant. (Photos: A.M. Brauner)



Figure 2. **A.** Adult female *Diadromus pulchellus* parasitizing a leek moth, *Acrolepiopsis assectella*, cocoon; **B.** Adult female *Conura albifrons* parasitizing a leek moth cocoon. (Photos: A.M. Brauner)

Chapter 2: Conservation meets classical: testing the effect of floral enrichment on the efficacy of an introduced parasitoid of leek moth and their facultative hyperparasitoid.

Introduction

The enemies hypothesis states that lower pest densities in plant polycultures versus monocultures occur because the higher plant diversity of polycultures enhances the activity of natural enemies (Root, 1973). Accordingly, one of the main principles behind conservation biological control (CBC) – *i.e.*, the manipulation of habitat to enhance the survival, behaviour and/or efficacy of a natural enemy – is that adding plant diversity provides resources that benefit natural enemies (Barbosa, 1998). Indeed, many studies have shown that adding plant diversity to a system can increase the lifespan of natural enemies, and increase their parasitism levels in the laboratory or greenhouse (Araj *et al.*, 2006; Jamont *et al.*, 2013; Irvin *et al.*, 2014; Irvin *et al.*, 2015) and in the field (see review by Andow, 1991; Leius, 1967; Idris and Grafius, 1995; Tylianakis *et al.*, 2004; Lee and Heimpel, 2005; Berndt *et al.*, 2006; Lee & Heimpel, 2008; Tajmiri *et al.*, 2017). However, improved pest suppression has not always been achieved (Berndt *et al.*, 2002), and in some cases, increases in pest densities (Baggen & Gurr, 1998) or decreases in target natural enemy populations (Stephens *et al.*, 1998) have been observed. Targeted habitat management which involves the use of appropriate plant species (reviewed in Landis *et al.*, 2000) could help to provide alternative or additional hosts or prey, as well as plant-derived food sources (e.g., pollen, floral and extrafloral nectar, honeydew) and shelters for natural enemies.

Carbohydrates, lipids and proteins are important components of the diets of many insect species that require them for metabolic processes such as flight, foraging, and reproduction (Wolcott, 1942; Jervis & Kidd, 1986; Jervis *et al.*, 1993; Jervis *et al.*, 1996). This is particularly the case for synovigenic hymenopteran parasitoids, which require resources to continually develop eggs throughout their lives (Jervis *et al.*, 2001). For example, sugar feeding has been shown to improve parasitoid fecundity by increasing the rate of egg maturation in synovigenic species, and/or by increasing reproductive lifespan (Leius, 1961a,b; Heimpel *et al.*, 1997; Schmale *et al.*, 2001; Tylianakis *et al.*, 2004; Zhang *et al.*, 2011; Segoli & Rosenheim, 2013). In addition, Van Emden (1962) demonstrated that parasitoids showed enhanced activity levels, including host-searching, in habitats with flowers present compared to those without flowers, likely due to sugar feeding in the flowering plants. Wäckers (1994) showed reduced host searching efficiency in parasitoids that were sugar deprived, likely due to the parasitoid reducing its host searching activity in favour of food provisioning. This combination of enhanced host-searching ability and improved fecundity in sugar-fed parasitoids suggests that sugar-dependent biological control agents (BCAs) could be more effective at pest targeting when nectar sources are provided (see also Wratten *et al.*, 2002; Heimpel & Jervis 2005).

Conservation biological control techniques that incorporate nectar provisions, while they may be targeted towards benefiting a particular natural enemy species, are likely to affect multiple members of an arthropod community – including the pest (Baggen & Gurr, 1998; Williams & Hendrix, 2008), as well as members of higher trophic

levels such as competitors (Sholes, 1984; Jervis *et al.*, 1996), intraguild predators (predators that attack the pest as well as the target beneficial) (Jervis, 1990; Patt *et al.*, 1997a; Lee *et al.*, 2001), hyperparasitoids (parasitoids that attack the target beneficial) (Araj *et al.*, 2011), and facultative hyperparasitoids (parasitoids that attack the pest as well as the target beneficial) (Miall *et al.*, 2014; see review by Wäckers *et al.*, 2007). Adding flowering resources for CBC thus has the potential to have positive, neutral, or disruptive effects on biological control of the target pest, depending on (i) the nature of trophic interactions and (ii) the relative benefit of adding the flowering resource to each community member (e.g., Rijk *et al.*, 2016). Appropriate plant selection is thus a critical aspect of CBC if the goal is to enhance the efficacy of a specific natural enemy (Landis *et al.*, 2000) without also benefiting disruptive competitors, intraguild predators, or hyperparasitoids.

Targeted plant selection is based on the principle that different flowers or nectar sources may be differentially beneficial to pests and natural enemies (Landis *et al.*, 2000). Selecting plants for CBC may require the consideration of a number of factors, including various components of flower morphology (i.e., nectar accessibility), quality or quantity of nectar production, timing of flower presentation in the field, morphological relatedness to the host plant of a pest, and its value as a source of protection from biotic and abiotic factors that may otherwise impact a pest or natural enemy (Jervis *et al.*, 1993; Landis *et al.*, 2000). An ideal scenario would be to provide a ‘permanent’ nectar source that strictly benefits natural enemies that have the largest suppressive impact on a target pest, and not the pest itself or disruptive intraguild predators or hyperparasitoids. While it can be

difficult to establish perennial flowering plants in frequently-disturbed agricultural settings, the presence of annual nectar-producing plants throughout the complete growing season of a crop, might be a more feasible solution.

Diadromus pulchellus Wesmael (Hymenoptera: Ichneumonidae) was introduced from Europe to Canada beginning in 2010 to control the invasive European leek moth, *Acrolepiopsis assectella* (Zeller) (Lepidoptera: Acrolepiidae) (Mason *et al.*, 2013a,b). Post-release monitoring in 2011 and 2012 indicated that overwintering populations of *D. pulchellus* had established. However, the establishment of this BCA coincided with an increasing number of *Conura albifrons* (Walsh) (Hymenoptera: Chalcididae) reared from field-collected and sentinel leek moth pupae (Mason *et al.*, 2013a,b). Miall *et al.* (2014) demonstrated that *C. albifrons* can develop in both leek moth pupae as a primary parasitoid and in *D. pulchellus*-parasitized leek moth pupae (from the primary parasitoid's egg stage to fully-developed pupae) as a facultative hyperparasitoid. The occurrence of this indigenous, facultative hyperparasitoid within the leek moth system has the potential to impact the establishment of *D. pulchellus* due to both competition and intraguild predation. As *D. pulchellus* has been shown to be the most promising BCA to manage leek moth in eastern North America, it is important to consider what cultural and agricultural practices could be implemented to help establish this classical biocontrol agent at new sites, increase its efficacy once established, and minimize the negative impacts on the BCA from competitors and predators. I explored whether CBC, involving addition of nectar-producing plants to generally sugar-deprived leek fields, could be

combined with classical biological control to help accomplish these goals, while also considering its effect on potentially disruptive members of the arthropod community.

Because it is difficult to predict how a particular nectar source or habitat manipulation technique will affect a complete food web, I first investigated the effect of adding different plants (alone or in combination) and sugar resources on the fitness parameters and biological control efficacy of individual natural enemy species in this system: *D. pulchellus* (the primary parasitoid) and *C. albifrons* (a facultative hyperparasitoid also acting as a primary parasitoid). Since neither species targets hosts that produce honeydew, they would likely be seeking additional resources in order to fulfill metabolic and life history needs. Several laboratory and field studies have examined the effects of nectar on pests and natural enemy communities, but only one laboratory study has tested the effect of nectar provisioning on the efficacy of a primary parasitoid and its obligate hyperparasitoid (Araj *et al.*, 2008). To my knowledge, there have been no studies that have explored the potential impact of sugar provisioning on a facultative hyperparasitoid. Based on the knowledge that both *D. pulchellus* (Jenner *et al.*, 2012) and *C. albifrons* (Arthur, 1958; Miall *et al.*, 2014) require non-host food sources to maximize their ability as primary (i.e., *D. pulchellus* & *C. albifrons*) and secondary (i.e., *C. albifrons*) parasitoids, I predicted that parasitoids provided with sucrose, floral nectar and/or extrafloral nectar resources, would live longer, produce more offspring, and kill more hosts than those provided only with water. Differences in life-history traits of the two parasitoids (Noyes, 1974; Jenner *et al.*, 2014; Miall *et al.*, 2014), including the effect of prior host-handling experience and duration of preoviposition

period, indicated that there could be different temporal effects of sugar-provisioning on *D. pulchellus* and *C. albifrons*. Determining how sugar or nectar resources impact these life-history traits and overall host-killing ability of parasitoids could help to provide important insight into how different habitat management strategies might shift the balance between parasitism and hyperparasitism in the context of enhanced biological control.

Materials & Methods

Insect colonies

Acrolepiopsis assectella

All leek moth eggs, larvae, pupae, and adults were obtained from a continuous rearing culture established from adult leek moths collected at sites in the Ottawa (Ontario, Canada) area beginning in 2003. This culture, housed at the National Arthropod Containment Facility (NACF) at AAFC, Ottawa, Canada, was supplemented annually with wild material from various additional sites in eastern Ontario. The leek moth rearing methods for this study were adapted from those described by Mason *et al.* (2010).

Newly-emerged adult moths were housed in a wooden-framed, screen-sided oviposition cage (25 x 25 x 45 cm) and provided with a 10% sucrose solution, replenished weekly. Sections of fresh leek, *Allium ampeloprasum* var. *porrum* L. (Amaryllidaceae), leaves (approximately 5 x 10 cm) were placed in the oviposition cage and replaced daily to stimulate oviposition. These leek leaves were divided into smaller sections, each containing approximately 150 eggs, and placed in the leaf whorls of

individually-potted, mature leek plants purchased from local produce distributors. Sets of five of these infested plants were placed into wooden-framed, screen-sided rearing cages (30 x 30 x 80 cm), and the larvae were allowed to feed for approximately two weeks. Prior to leek moth pre-pupation, a 104 x 94 mesh bag (48 x 71 cm, DC3148 MegaView Science Co., Taichung, Taiwan) was placed over the group of five leeks within each cage, to provide an easy-to-access medium on which pupation could occur. Bags were removed from cages daily, and all pre-pupae and pupae were picked from the bags and placed in Petri dishes (91 x 10 mm, Semadeni AG, Ostermundigen, Switzerland) for experimentation or perpetuation of the colony. Adult moths from pupae designated for the rearing colony were transferred into the oviposition cage following emergence in Petri dishes. The culture was kept in a rearing room at $25 \pm 1^\circ\text{C}$ on a 16L: 8D light:dark cycle and $50 \pm 10\%$ relative humidity (RH).

Diadromus pulchellus

All experimental *D. pulchellus* material was obtained from a culture housed in the NACF, and reared on hosts from the aforementioned leek moth culture. This colony was originally established from *D. pulchellus* individuals collected near Bern, Switzerland from 2005–2009. Beginning in 2012, the culture was supplemented annually with new individuals from various sites in western Switzerland. Adults were held in 150 x 150 mesh-walled BugDorm-4TM cages (47.5 x 47.5 x 47.5 cm, BD4F4545 MegaView Science Co., Ltd., Taiwan) and provided with a 10% sucrose solution. Groups of approximately 50 newly-formed leek moth pupae were placed in open Petri dish lids (91 x 10 mm, Semadeni AG, Ostermundigen, Switzerland), and added to each cage for approximately

24 hours before being removed. Emerged adult wasps were used in experiments or to perpetuate the colony. The culture was kept in a rearing room at $22 \pm 1^\circ\text{C}$ on a 16L: 8D light cycle and $50 \pm 10\%$ RH.

Conura albifrons

A culture of *C. albifrons* was established from individuals emerging from sentinel leek moth pupae placed in a variety of locations in the Ottawa Valley between 2012 and 2015. Newly-emerged *C. albifrons* adults were placed in 150 x 150 mesh-walled BugDorm-4™ cages (32.5 x 32.5 x 32.5 cm, BD4F3030 MegaView Science Co., Ltd., Taiwan), with a 10% sucrose solution, and allowed to mate for approximately two weeks. Following mating, open Petri dish lids (91 x 10 mm, Semadeni AG, Ostermundigen, Switzerland), each containing approximately 50 newly-formed leek moth pupae, were placed in the cages for 48–72 hours before being removed. Emerged and mated adult wasps were used in experiments or to perpetuate the colony. The culture was kept in a rearing room at $22 \pm 1^\circ\text{C}$ on a 16L: 8D light cycle and $50 \pm 10\%$ RH.

Plants used as nectar sources

Buckwheat, *Fagopyrum esculentum* Moench (Polygonaceae), is a fast-growing, annual plant that produces hexose-dominant nectar, with fructose making up more than 50% of the sugar at peak flowering (Bowie *et al.*, 1995; Lee & Heimpel, 2004; Cawoy *et al.*, 2006; Cawoy *et al.*, 2009). Common vetch, *Vicia sativa* L. (Fabaceae), is a leguminose annual plant often grown for seed, pasture, hay, and as green manure due to its strong nitrogen-fixing capacity (Acikgoz & Rum-Baugh, 1979). These plants produce and

secrete nectar through extrafloral nectaries located on their stipules from early leaf development until full leaf maturity (Koptur, 1979). These extrafloral nectaries precede flower development, offering access to nectar for a wide variety of insect groups (Koptur, 2005; Heneidak, 2007). Several laboratory studies have previously used buckwheat and/or common vetch in experiments to determine the effects of nectar-provisioning on parasitoids (Araj & Wratten, 2015; Irvin & Hoddle, 2015). These plants were chosen for this experiment because of their differing nectar accessibility features (i.e., floral and extrafloral) and the likeliness that both plant species could be incorporated into future CBC field studies.

Plant maintenance

Leek, *Allium ampeloprasum* var. *porrum* L. “Hannibal” (Alliaceae), seeds were sown into 48-cell flats (25 x 45 cm) and placed on benches in a greenhouse (23 ± 10°C on a 15L: 9D light cycle and 30 ± 10% RH). After about 90 days, approximately 400 well-established leek seedlings were transplanted from these flats into 12 cm fibre pots for use in greenhouse cage experiments with *D. pulchellus*. A second cohort of leek flats was sown approximately 45 days after the first one to provide host plant material for a second set of experiments using *C. albifrons*.

Buckwheat (AAFC Ottawa, Ontario, Canada) and common vetch (Homestead Organics; Berwick, Ontario, Canada) seeds were sown directly (four seeds and three seeds per pot, respectively) into 12 cm fibre pots and placed on benches in a greenhouse (23 ± 10°C on a 15L: 9D light cycle and 30 ± 10% RH) until flowering began. These bi-

weekly plantings continued for the duration of the greenhouse cage experiments to provide a continuous supply of fresh nectar.

Plants were watered as needed and provided with a weekly feeding of quick release, water soluble fertilizer (20% Nitrogen, 20% Phosphorous, 20% Potassium) (Master Plant-Prod Inc. Brampton, Ontario, Canada). Prior to experimental use, a 1 cm layer of 1:1 Plaster of Paris (Rona Certaineed 20 kg) and water mix was poured into each pot and allowed to fully harden in order to coat exposed soil and facilitate location of parasitoids after their death.

Experimental procedure

D. pulchellus

Same-cohort (emerged within the same 12-hour period), newly-emerged *D. pulchellus* females were placed in a 150 x 150 mesh-walled BugDorm-4™ cages (32.5 x 32.5 x 32.5 cm, BD4F3030 MegaView Science Co., Ltd., Taiwan) with newly-emerged males for a period of 24 hours, to allow mating (ratio of 2:1 male to female ratio to optimize likelihood of successful mating – JM, unpublished data). Wasps were provided with access to water, and cages were misted lightly as needed throughout the mating period. Following this mating period, female wasps were removed from mating cages and placed in 60 x 60 x 120 cm observation cages (1466CV, BioQuip, Rancho Dominguez, California, USA) in a greenhouse (Figure 3). Each cage contained one of the following five experimental treatments:

- 1) Water treatment (two water-soaked yellow sponges held in clear plastic trays.)

- 2) Sucrose treatment (two 10% sucrose solution-soaked yellow sponges held in clear plastic trays.)
- 3) Buckwheat treatment (two flowering buckwheat plants).
- 4) Vetch treatment (two mature common vetch plants with extrafloral nectaries present.)
- 5) Buckwheat + Vetch treatment (two flowering buckwheat plants and two mature common vetch plants with extrafloral nectaries present.)

Each cage also contained a white Styrofoam tray (40 x 30 cm) on which the treatment plants or sponges were placed. This provided a level surface for pots and sponges and assisted with locating the parasitoids each day. Each cage had a green microfiber cloth (35 x 35 cm) placed on top of the cage which was soaked in water multiple times daily. These cloths served to provide shade, and to help regulate humidity. In addition, all cages were misted multiple times daily to provide parasitoids in all treatments with continuous access to water. Cages were randomly assigned to positions on the greenhouse benches, each bench containing a cage from each treatment (Figure 4). All greenhouse experiments took place under $23 \pm 10^{\circ}\text{C}$ on a 15L: 9D light cycle and $30 \pm 10\%$ RH.

After two days in a cage with its assigned treatment, each three-day-old *D. pulchellus* female was provided with two young leek plants each containing ten newly-formed leek moth pupae (Figure 5A). Pupae were carefully pinned (Black Enameled #0, BioQuip, Rancho Dominguez, California, USA) through the centre of their loose cocoon to avoid damage to the pupa and to avoid obstructing oviposition (Figure 6). To measure baseline leek moth pupal mortality when they were not exposed to parasitoids (e.g., due

to natural non-viability or abiotic conditions during the experiment), one of the two leek plants in each treatment was covered with a 104 x 94 mesh rearing sleeve (30 x 10 cm, DC3210 MegaView Science Co., Ltd., Taiwan) with the drawstring at the bag's base tightened on the leek stem to prevent access by *D. pulchellus* females (Figure 6B). After 24 hours, each leek was removed from the cage, and the pupae were placed in a Petri dish (50 x 15 mm, Semadeni AG, Ostermundigen, Switzerland) in a rearing room until resulting emergence (leek moth or *D. pulchellus*) was complete. Numbers of emerged leek moth and *D. pulchellus* adults from each treatment were recorded daily. All pupal dishes were kept in a rearing room at $22 \pm 1^\circ\text{C}$ on a 16L: 8D light cycle and $50 \pm 10\%$ RH.

After each 24-hour pupal exposure, the *D. pulchellus* females were provided with a two-day rest period without leek moth pupae to renew their egg load (determined through preliminary experiments; JM, unpublished data) with continuous access to their assigned treatment. All cages were checked daily for parasitoid survival, and the date was recorded in the event of death. When females were deemed to be missing, the cage and treatment were left intact for 24 hours to ensure that the female was no longer in the cage and not simply well hidden. In the event that a female was not recovered, she was considered dead, the date of her absence was recorded, and data from that female was included in the analysis.

Exposures continued for the duration of the lifespan of the wasp (between 1 and 21 exposures; i.e., 4–75 days), and plant and non-plant food resources were replaced or replenished as needed, to ensure a continuous supply of healthy food throughout the

experiments. Female longevity, offspring emergence, offspring sex ratio, leek moth emergence, and number of dead hosts were recorded for each exposure of each female wasp. A total of 22 replicates (110 *D. pulchellus* females) were tested in two temporal blocks (12 replicates in the first block, 10 replicates in the second block).

C. albifrons

The *C. albifrons* experiments were performed under the same mating, rearing, greenhouse and treatment conditions as the previously described *D. pulchellus* experiments. The only difference was the number of replicates performed (19), again in two blocks (10 and 9), and the duration of the experiments. The experiment was stopped after 36 days (11 exposures) due, in part, to the long-lived nature of *C. albifrons* (Miall *et al.*, 2014).

Estimation of non-reproductive parasitoid-induced mortality

Parasitoids often kill hosts via mechanisms that do not result in offspring production (e.g., due to ovipositor probing, venom injection, or host feeding; see Abram *et al.*, 2016), which can collectively be grouped under the term “non-reproductive effects” (hereafter NRPIM). To estimate NRPIM for pupae exposed to parasitoids (i.e., the number of hosts killed by parasitoids, taking into account natural mortality), I used Abbott’s formula as described in Abram *et al.* (2016):

$$\text{Corrected NRPIM} = M_E - \left(\frac{M_C * Tot_E}{Tot_C} \right)$$

where M_E and M_C are, respectively, the number of dead pupae observed on the uncaged and caged plants Tot_E and Tot_C , the total number of pupae on the uncaged and caged plants.

Statistical analyses

I used different mixed-model analyses, tailored to model error distributions, to test the effect of floral treatment (fixed factor) on the total number of offspring produced, the number of offspring produced per day of life (i.e., adjusted for longevity), total NRPIM, NRPIM per day of life, female parasitoid longevity, and offspring sex ratio. Temporal block (January 4, 2016 and January 27, 2016 – *D. pulchellus*; February 16, 2016 and March 3, 2016 – *C. albifrons*) was included in each model as a random effect. For each response variable, separate models were run for *D. pulchellus* and *C. albifrons*. The effects of floral treatment on the number of hosts killed and the number of offspring produced were tested with generalized linear mixed models (GLMMs) with a Poisson error distribution and a log-link function. Linear mixed models (Gaussian error distribution) were used to assess the effect of floral treatment on the number of offspring per day and NRPIM per day, as these response variables were normally distributed and homoscedastic. Floral treatment effects on female longevity, which was not normally distributed, as is typical of time-to-event data, were assessed with Cox proportional hazards mixed-effects models, after verifying the proportional hazards assumption. These models can also be fit to censored data, such as the longevity data for *C. albifrons*, where some individuals were still alive at the end of the experiment. Finally, the effect of floral treatment on offspring sex ratio was modeled with a GLMM with a binomial error

distribution and a logit link function. In all of the above mixed models, temporal block was included as a random factor, and the significance of the fixed factor (floral treatment) was assessed with Wald Chi-squared tests (linear mixed models, generalized linear mixed models in the lme4 package of R software (Bates, 2015) or likelihood ratio tests (Cox proportional hazards models) (Therneau, 2015). Post-hoc differences among the levels of floral treatment were tested with Tukey multiple comparisons tests (glht function in the multcomp package of R software) (Hothorn *et al.*, 2008). All analyses were performed in R software version 3.2.2 (R Core Team, 2016).

Results

Offspring production and host killing

D. pulchellus

Parasitism and parasitoid-induced host mortality showed a similar temporal pattern across all treatments (Figure 7). Host mortality resulting in parasitoid emergence was typically highest in the early and middle exposures. Host-mortality not resulting in parasitoid emergence tended to be more prevalent in later exposures in all treatments.

Total offspring production by *D. pulchellus* was significantly affected by treatment ($\chi^2 = 362.71$; $df = 4$; $P < 0.001$) and differed among all treatments except for sucrose and buckwheat (Figure 8A – left). *Diadromus pulchellus* in the water-only treatment produced the fewest offspring, while those in the buckwheat + vetch treatment produced the most. Parasitoids in the buckwheat + vetch treatment produced 5.5 and 1.6

times more offspring than those in the vetch treatment and buckwheat treatment alone, respectively. Total parasitoid-induced host mortality also varied significantly among treatments ($\chi^2 = 14.59$; $df = 4$; $P < 0.01$), being lowest in the water treatment and similar across the four remaining treatments (Figure 8A – Left).

Daily offspring production was higher in the buckwheat + vetch treatment than all other treatments ($\chi^2 = 34.37$; $df = 4$, $P < 0.005$), but did not differ significantly among the vetch, sucrose and buckwheat treatments (Figure 8A – Right). Female *D. pulchellus* in the buckwheat + vetch treatment produced an average of 0.3 more offspring per day than those in the vetch treatment, and 0.12 more offspring per day than females given the buckwheat treatment. Daily levels of parasitoid-induced host mortality did not differ among treatments ($\chi^2 = 8.62$; $df = 4$; $P = 0.071$) (Figure 8B – Right).

C. albifrons

Similar to the parasitism and parasitoid-induced host mortality levels demonstrated in the *D. pulchellus* exposures, *C. albifrons* exhibited a consistent temporal pattern among the different treatments (Figure 9). However, this trend differed from that of *D. pulchellus* in that host mortality resulting in parasitoid emergence was highest in the middle and later exposures in all treatments. There was a slightly increasing trend in NRPIM throughout the exposure progressions.

Total offspring production differed depending on what treatment female *C. albifrons* received ($\chi^2 = 208.27$; $df = 4$; $P < 0.001$) (Figure 8B – Left). Females in the buckwheat + vetch treatment produced the most offspring, while those receiving the water only treatment produced the fewest. The number of offspring produced in the vetch

and sucrose treatments was similar, and was higher in the buckwheat treatment. *Conura albifrons* females in the buckwheat + vetch treatment produced 3.4 and 2.2 times more offspring than when provided with vetch or buckwheat alone, respectively. Total parasitoid-induced host mortality also differed among treatments ($\chi^2 = 18.06$; $df = 4$; $P < 0.001$) (Figure 8B – Left). As was the case for offspring production, those receiving the buckwheat + vetch combination killed the most hosts; however, the number of hosts killed by females provided with the water-only treatment did not differ from those killed in the vetch, sucrose, or buckwheat treatments. Females provided with the buckwheat + vetch treatment killed 8.7 times more offspring than those provided with water only.

Daily offspring production did not differ among the water, vetch, sucrose and buckwheat treatments, but was higher in the buckwheat + vetch treatment ($\chi^2 = 24.58$; $df = 4$; $P < 0.001$) (Figure 8B – Right). Daily parasitoid-induced host mortality did not differ among treatments ($\chi^2 = 5.69$; $df = 4$; $P = 0.224$).

Female longevity

D. pulchellus

Female *D. pulchellus* longevity was lowest in the water and vetch treatments, and highest in the sucrose, buckwheat and buckwheat + vetch treatments ($\chi^2 = 40.48$; $df = 4$; $P < 0.001$) (Figure 10 – Left). More than half of the females receiving the sucrose, buckwheat and buckwheat + vetch treatments survived for more than 31 days, while half of the females who received the water and vetch treatments were dead after ten days.

Diadromus pulchellus in the buckwheat + vetch treatment (35.5 ± 4.5 days) survived for an average of 21 days longer than those in the vetch only treatment (14.5 ± 2.4 days).

C. albifrons

Female *C. albifrons* longevity differed among treatments ($\chi^2 = 53.50$; $df = 4$; $P < 0.001$) (Figure 10 – Right). Females receiving the buckwheat treatment and the buckwheat + vetch treatment had the greatest survivorship for the duration of the experiments, with more than half surviving for the full length of the experiment (36 days). Females in the water-only treatment survived for the shortest amount of time; none survived past day 17 of the experiment. *Conura albifrons* females in the buckwheat + vetch treatment (31.3 ± 1.7 days) survived an average of 23 days longer than those in the water only treatment (8.3 ± 0.8 days).

Offspring sex ratio

D. pulchellus

The proportion of male offspring produced was higher in the vetch treatment than in any of the other four treatments ($\chi^2 = 36.82$; $df = 4$; $P < 0.001$) (Figure 11 – Left). Female *D. pulchellus* in the vetch treatment produced an average of 63% male offspring, while females provided with the sucrose treatment produced 39% males.

C. albifrons

Male offspring production differed among treatments ($\chi^2 = 24.11$; $df = 4$; $P < 0.001$), with the water, sucrose and buckwheat treatments producing the greatest proportion of male

offspring, and the vetch and buckwheat + vetch treatments producing the least (Figure 11 – Right). Female *C. albifrons* in the sucrose treatment produced an average of 86% male offspring, while females provided with the vetch treatment produced 60% males.

Discussion

I tested the effect that supplemental sugar resources had on the host-killing ability, longevity and offspring sex ratio of *D. pulchellus* and *C. albifrons*, which typically co-exist in *Allium* fields, an agricultural system likely to be poor in sugar resources for parasitoids. In line with my general predictions, I found that both parasitoid species responded to treatments with added sugar resources (artificial and plant-produced) by producing more offspring, killing more hosts, and living longer. While these general responses of the two species were similar among treatments, there were also some important differences indicating that habitat manipulation could differentially affect them.

In line with previous studies showing that parasitoids benefitted from supplemental sugar sources such as sucrose and plant nectar (Idris & Grafius, 1995; Baggen & Gurr, 1998; Wäckers, 2001; Vattala *et al.*, 2006; Tompkins *et al.*, 2010), *D. pulchellus* and *C. albifrons* females that were provided with added sugar resources generally produced more offspring daily and overall than those provided with water-only, with the exception of offspring produced daily by *C. albifrons*. This suggests that the parasitoids were able to exploit artificial and plant-derived sugar sources leading to behavioural and metabolic advantages that led to improved reproductive success. In addition, both species appeared to benefit from multiple plant-derived sugar sources to

find and kill the maximum number of hosts, as those provided with buckwheat + vetch produced the most offspring and killed the most hosts. For both parasitoids, daily NRPIM remained consistent among all treatments suggesting that host-killing was still occurring from those parasitoids provided with the water-only treatments. Parasitoids provided with only water seemed to lack the necessary carbohydrates needed for both egg production and locomotion, the latter of which was apparent by the number of females from those treatments that remained relatively stationary during much of this experiment (JM, personal observation). Similar to my predictions, *D. pulchellus* and *C. albifrons* longevity was positively influenced by the addition of buckwheat and buckwheat + vetch treatments, but was surprisingly low in the vetch treatment. This could indicate that the parasitoids did not recognize the vetch extrafloral nectaries as a potential sugar source, or were unable to exploit the resource to increase longevity (e.g., Irvin *et al.*, 2007; but see Patt *et al.*, 1997b; Jervis, 1998; Lewis *et al.*, 1998; Winkler *et al.*, 2009a). These females may have been able to use the plant as a microclimate refuge from abiotic factors (temperature extremes) during the experiment, but were unable to fully benefit from it in the absence of an additional sugar source. Although at least one study has examined the synergistic effect of combining multiple food resources on a predator (see Pekas & Wäckers, 2017), to my knowledge, no studies have evaluated the potential additive effects of multiple floral resources on parasitoid efficacy and longevity in the lab, greenhouse, or field. As such, it is difficult to predict how abiotic and biotic protection gained from the refuge provided by multiple nectar resources and/or increased plant density from these resources, will translate to the field as these experiments were

performed in the absence of the complete natural enemy community associated with the leek moth system. From an ecological point of view, it would be interesting to explore this further by evaluating if multiple nectar resources provided additional benefits to parasitoids than the sum of each resource provided individually.

In experiments with *D. pulchellus*, host mortality resulting in parasitoid emergence was highest during the early and middle exposures, consistent with previous findings involving this parasitoid's oviposition behaviour (PM, unpublished data). Females generally have a full complement of mature eggs ready for oviposition after about three days but peak around ten days following eclosion, before fecundity declines and resorption begins to occur (Labeyrie, 1960). Early sugar provisioning may mean that they produce viable eggs more quickly; however, egg production slows down with increasing parasitoid age (Jenner *et al.*, 2012). An observed increase in NRPIM in later exposures may indicate that parasitoids are seeking a protein source through host feeding, for survival or to supplement sugar reserves for egg production and locomotion (Balzan & Wäckers, 2013; Liu *et al.*, 2015). This corresponds with the observation that these parasitoids need to oviposit into young hosts otherwise their offspring's ability to develop becomes unreliable (Majerus *et al.*, 2000; Vinson, 2010). For *C. albifrons*, parasitoid emergence was highest in the middle and later exposures in all treatments, consistent with findings from previous studies (Arthur, 1958; Miall *et al.*, 2014). This species generally takes about seven days to develop mature eggs following eclosion, and often requires multiple host experiences before initiating oviposition (Arthur, 1958; Miall *et al.*, 2014). Also, being a generalist facultative hyperparasitoid, it may not immediately recognize the

host as being suitable for development (JM, unpublished data). Alternatively, it may be “holding out” for a more suitable host, but once it recognizes that a better host is not available, it parasitizes the less desirable host (see Turlings *et al.*, 1990; Brododjojo & Walter, 2006). Once it has gained oviposition experience with that host, it is more likely to continue to oviposit (Miall *et al.*, 2014). Several of the females in this experiment demonstrated this behaviour as was evident by continual offspring production in later exposures. Additionally, most of the earlier emerging offspring were males, again indicative that naïve *C. albifrons* viewed leek moth as an inferior host (e.g., Godfray, 1990; Heinz & Parrella, 1990; Ueno, 2015).

The present study demonstrated that both the primary pupal parasitoid and a facultative hyperparasitoid of leek moth can benefit from supplementation of sugar sources in a sugar-deprived environment. The combination of two nectar-producing plants, buckwheat (floral) and common vetch (extrafloral) led to increased longevity and host-killing ability for both *D. pulchellus* and *C. albifrons*, showing promise that this system can be manipulated to improve biological control of leek moth. To further explore how nectar will impact the full interaction between *D. pulchellus* and *C. albifrons* as intraguild competitors, and the resulting effect on leek moth parasitism, it would be useful to perform more interactive experiments in the laboratory and in the field with these and additional nectar-producing plants.

Figures



Figure 3. Greenhouse cage setup showing the buckwheat + vetch treatment. (Photo: A.M. Brauner)



Figure 4. Greenhouse setup with randomized arrangement of cage treatments on benches. (Photo: A.M. Brauner)

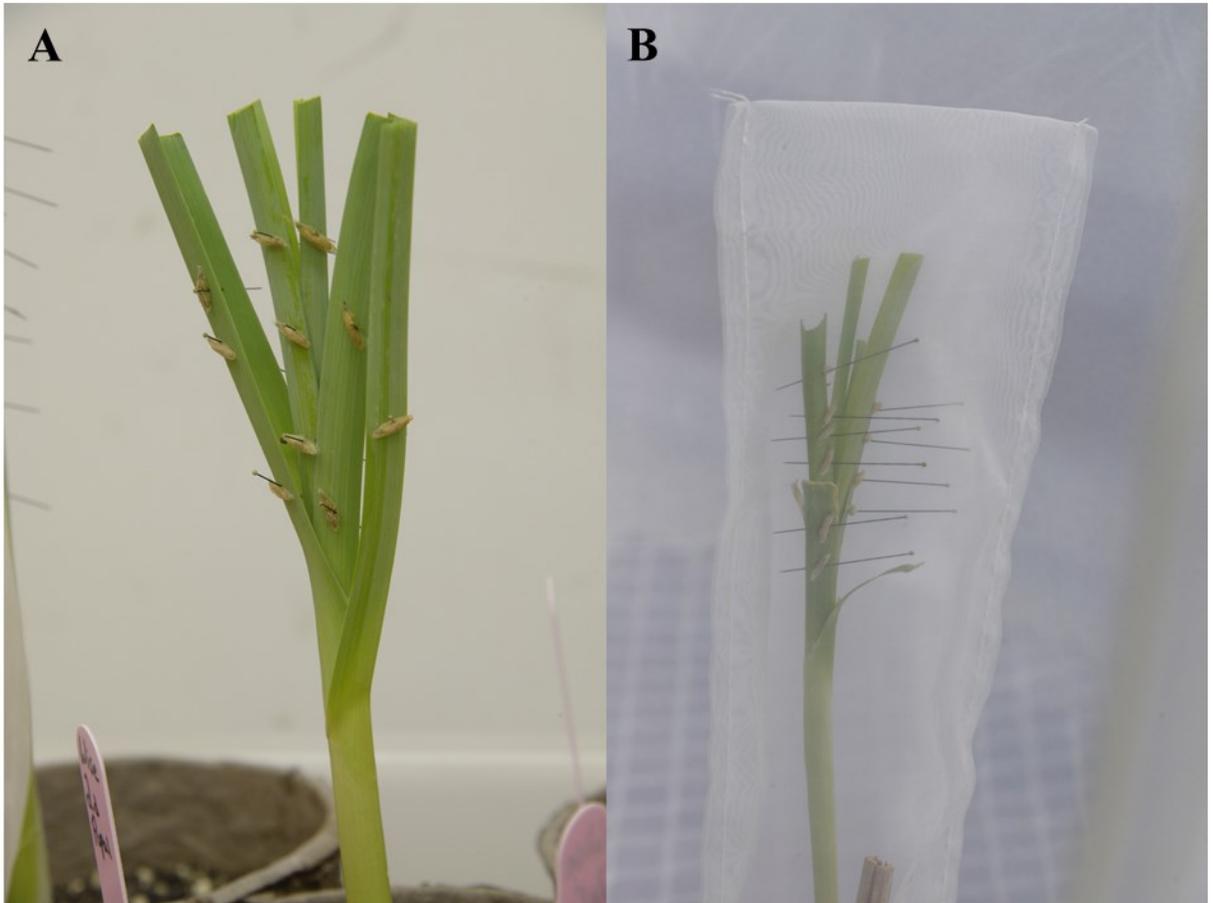


Figure 5. Leek moth, *Acrolepiopsis assectella*, pupae pinned to a young **A.** uncovered and **B.** covered (parasitoid exclusion) leek plant in preparation for a greenhouse exposure. (Photos: A.M. Brauner)

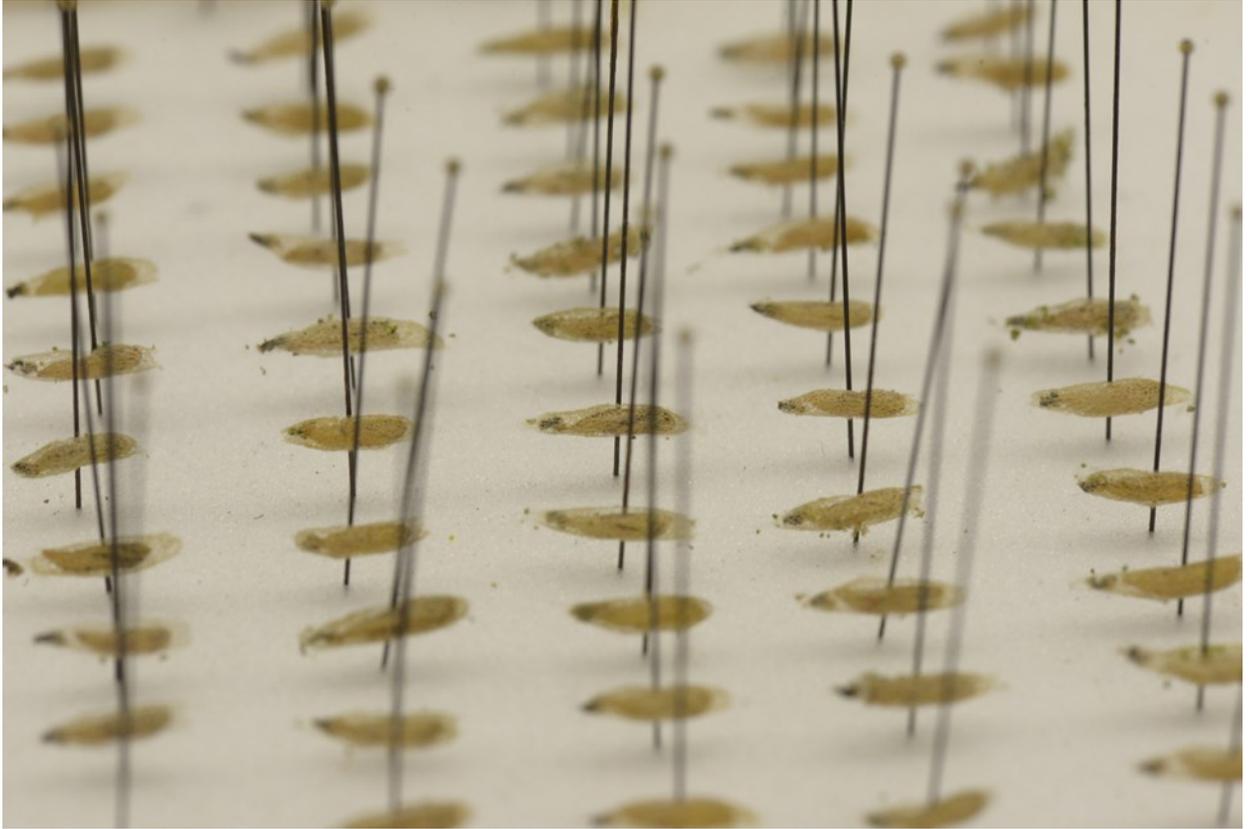


Figure 6. Newly-formed leek moth, *Acrolepiopsis assectella*, pupae with a pin placed between the pupal case and woven cocoon in preparation for greenhouse exposures. (Photo: A.M. Brauner)

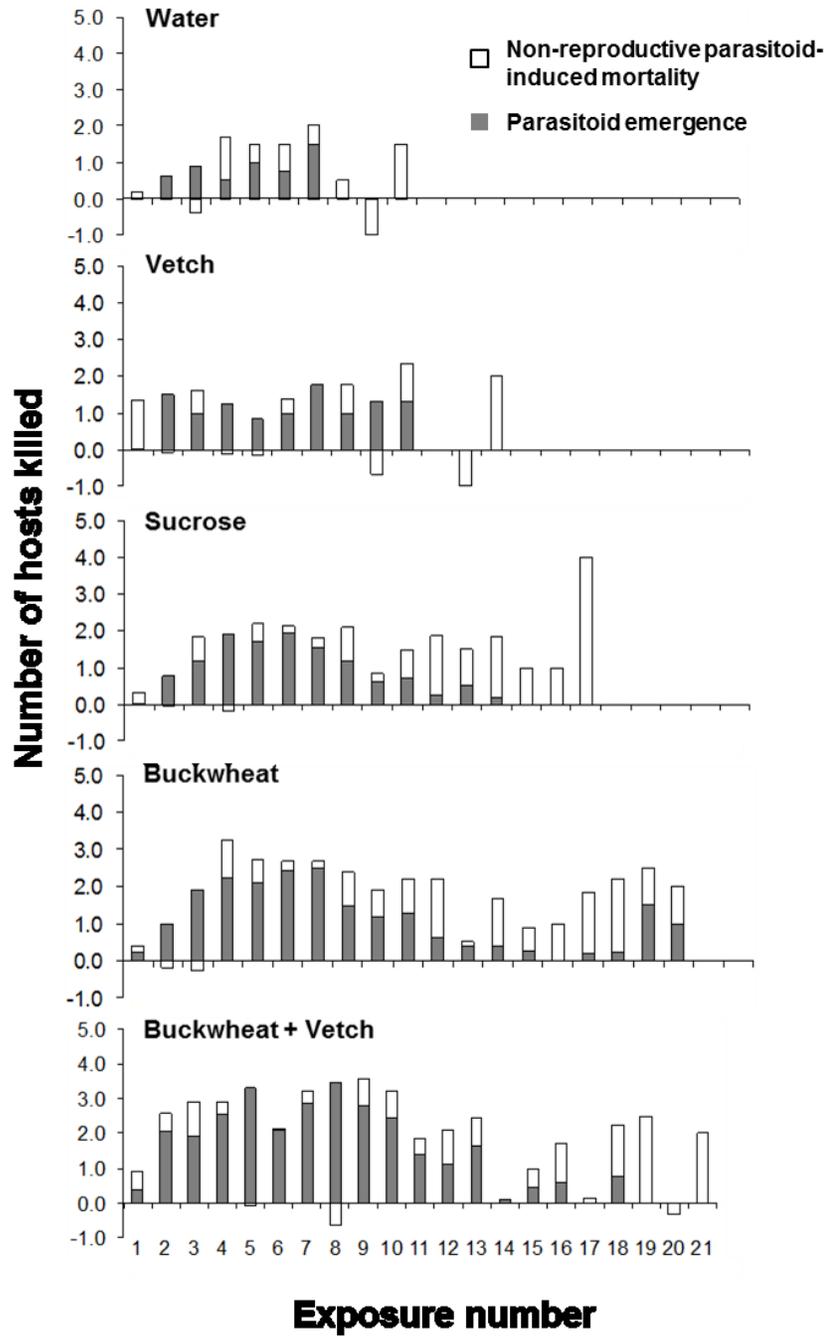


Figure 7. Number of leek moth, *Acrolepiopsis assectella*, pupae killed by *Diadromus pulchellus* females by exposure number and cage treatment: water; vetch; sucrose; buckwheat; and buckwheat + vetch. Non-reproductive parasitoid-induced mortality was calculated using a correction derived from unexposed controls (see experimental procedure above).

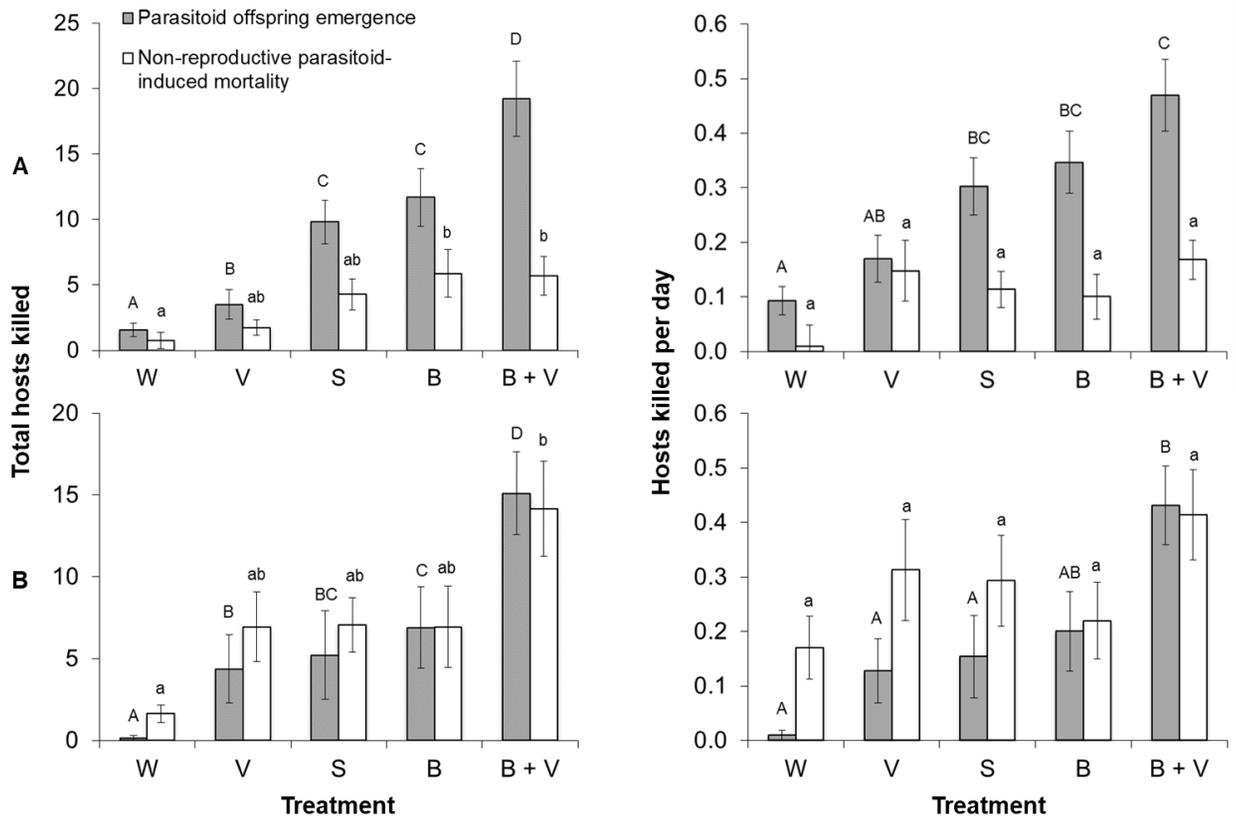


Figure 8. Total (left) and daily (right) reproductive and non-reproductive parasitoid-induced mortality of leek moth, *Acrolepiopsis assectella*, pupae for each cage treatment; water (W), vetch (V), sucrose (S), buckwheat (B), buckwheat + vetch (B+V), by **A.** *Diadromus pulchellus* and **B.** *Conura albifrons*. (Different letters indicate significant differences [$p < 0.05$] between treatments as determined by Tukey contrasts: Upper case = parasitoid offspring emergence; Lower case = non-reproductive parasitoid-induced mortality).

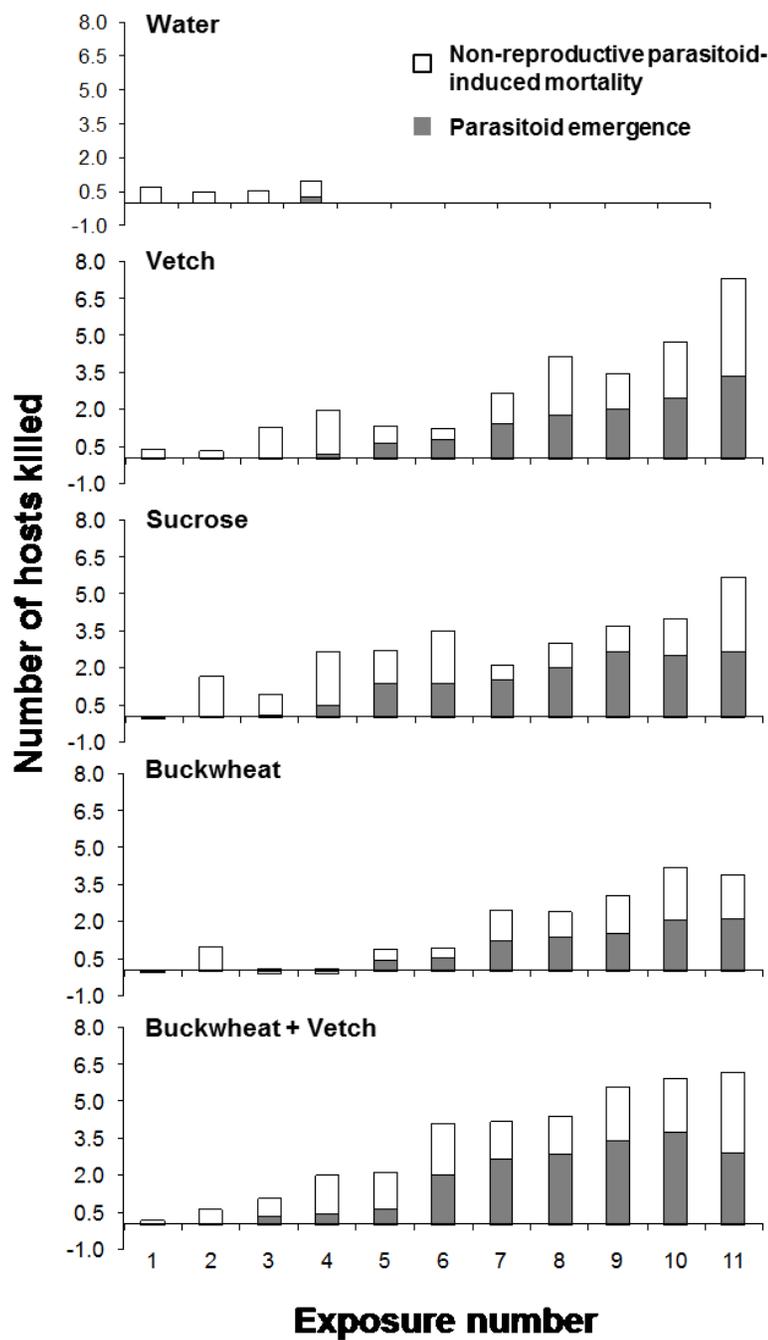


Figure 9. Number of leek moth, *Acrolepiopsis assectella*, pupae killed by *Conura albifrons* females by exposure number and cage treatment: water; vetch; sucrose; buckwheat; and buckwheat + vetch. Non-reproductive parasitoid-induced mortality was calculated using a correction derived from unexposed controls (see experimental procedure above).

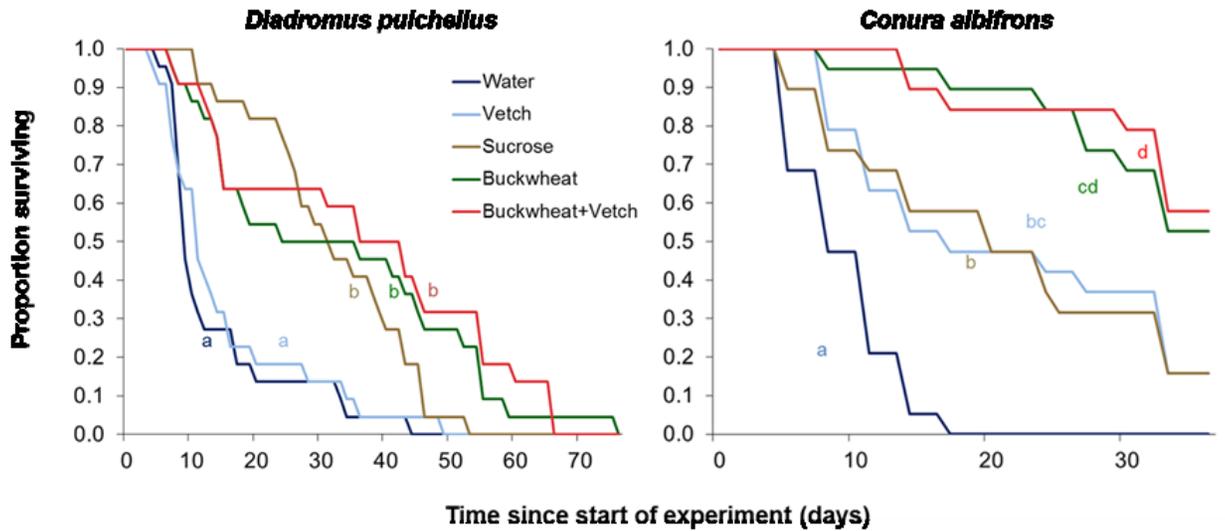


Figure 10. Proportional longevity of female *Diadromus pulchellus* and *Conura albifrons* when provided with various nutritional treatments: water; vetch; sucrose; buckwheat; and buckwheat + vetch. (Different letters indicate significant differences [$p < 0.05$] between treatments as determined by Tukey contrasts).

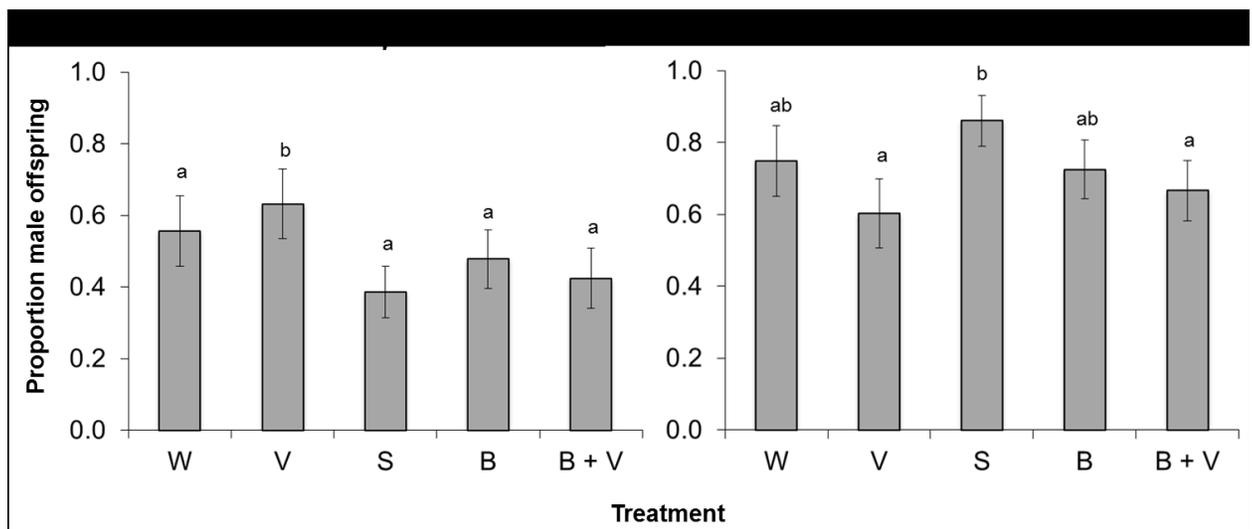


Figure 11. Proportion of male offspring produced by *Diadromus pulchellus* and *Conura albifrons* when provided with various nutritional treatments: water (W); vetch (V); sucrose (S); buckwheat (B); and buckwheat + vetch (B+V). (Different letters indicate significant differences [$p < 0.05$] between treatments as determined by Tukey contrasts).

Chapter 3: Evaluating the effect of floral enrichment on generational mortality and the parasitoid community of leek moth in the field.

Introduction

Several studies have demonstrated that increased plant diversity and density often increase the species richness and abundance of beneficial insects, including natural enemies, in the field (see review by Poveda *et al.*, 2008; Langellotto & Denno, 2004; Berndt *et al.*, 2006). Conservation biological control (CBC) strategies, including the addition of cover crops and nectar resources to agricultural systems, can provide refuges and supplementary sugar sources for both pests and natural enemies; however, these strategies do not directly translate to reduced crop damage or improved pest suppression (Wratten *et al.*, 2000; Poveda *et al.*, 2008; Tscharrntke *et al.*, 2016).

Addition of floral resources may improve biological control by specific agents through niche separation by providing potential competitors with a variety of habitats and food sources within an agricultural system (Pedersen & Mills, 2004), but might also drastically shift the parasitoid and natural enemy complex found in the field (Baggen & Gurr, 1998; Tscharrntke *et al.*, 2007). This type of manipulation may disrupt the natural enemy-pest “balance” that is already in place in some systems, or lead to higher incidences of indigenous or generalist pests, parasitoids and predators (Messing & Garipey, 2012). It is important to consider this full range of consequences before implementing a CBA strategy, as added effort to support redundant natural enemies may reduce overall crop yield in areas where arable land is used for intercropping or companion planting in place of crop production (Tscharrntke *et al.*, 2005; Letourneau *et al.*, 2011).

Along with determining which companion plant species will provide the most benefit to a natural enemy assemblage in a given system, it is important to measure the impact of these conservation strategies in terms of pest reduction. Several theoretical hypotheses (i.e., resource concentration hypothesis, enemies hypothesis: Root, 1973; trap crop hypothesis: Vandermeer, 1989; nectar-provision hypothesis: Heimpel & Jervis, 2005) have been introduced to explain how increased plant diversity will lead to improved pest control through trap-cropping, natural enemy recruitment, resource subsidies, and masking the attractiveness of the host plant to the pest. However, there is little evidence to support the theory that increased natural enemy abundance in resource-subsidized systems leads to population-level pest suppression that would be expected to improve crop yield (Cardinale *et al.*, 2003; Ostman *et al.*, 2003).

Evaluating the population-level impact of natural enemies in a cropping system is an important step towards implementing an effective CBA strategy. By manipulating experimental variables in the laboratory or field, effects of these strategies can be looked at in isolation (e.g., exclusion or containment cages, hedgerows, trap crops, etc.) or together (natural enemy abundance, trophic interactions, intra- and inter-guild competition, etc.). One popular method of measuring the impact of natural enemies on a system – but that has not previously been applied to CBA evaluations – is a life-table approach, which estimates stage-specific mortality levels and generational reproductive output of a pest (Bellows *et al.*, 1992). Life tables can assist with the development of pest management programs by providing insight into which factors are the most important in reducing pest population growth, what life stages they impact, and, by extension, which

life stages should be targeted when evaluating a candidate biological control agent (BCA) to supplement a natural enemy community. In some cases, the goal of the life-table analysis may be to evaluate the impact of a single natural enemy or a specific group of natural enemies such as parasitoids (Harcourt, 1969; Bellows *et al.*, 1992; Jenner *et al.*, 2010; Yu *et al.*, 2013;). The information gained from this type of life-table analysis could help to determine the impact of CBC strategies on the life stage-specific impact of a particular parasitoid species or guild, further refining our understanding of how to manipulate habitat to maximize pest suppression in an agricultural system.

First discovered in North America near Ottawa, Ontario, Canada, the leek moth, *Acrolepiopsis assectella* (Zeller) (Lepidoptera: Acrolepiidae), has established and spread throughout much of eastern Canada and parts of New York, and Vermont in the USA (Landry, 2007; Mason *et al.*, 2010; Mason *et al.*, 2013a,b; Seto & Shelton, 2015). Native to much of the eastern hemisphere, this pest of *Allium* spp. plants can cause heavy economic losses and has induced trade restrictions on commercial *Allium* crops (Jenner *et al.*, 2010). *Diadromus pulchellus* (Wesmael) (Hymenoptera: Ichneumonidae) is a solitary pupal endoparasitoid, native to Europe, that has been continually released against leek moth in Canada since 2010 as part of an integrated pest management strategy that also includes the use of biopesticides and physical barriers to establish long-term control of leek moth populations (Mason *et al.*, 2013a,b). *Conura albifrons* (Walsh) (Hymenoptera: Chalcididae) is a facultative hyperparasitoid known to exploit numerous insect hosts across several different orders (Peck, 1963; Hansen, 1980). It has recently become a subject of interest due to its potential threat to the establishment of *D. pulchellus* after

post-release monitoring determined that it was prevalent in field-collected leek moth pupae (Mason *et al.*, 2013a,b). *Conura albifrons* can act as both a facultative hyperparasitoid of *D. pulchellus* and a primary parasitoid of *A. assectella* (Miall *et al.* 2014); however, it is not yet clear if it demonstrates a distinct behavioural preference for one host or the other. A CBA approach could potentially help to recruit and enhance the impact of introduced (e.g., *D. pulchellus*) and indigenous (e.g., *C. albifrons*) natural enemies in the leek moth system (see Chapter 2), increasing leek moth mortality rates and decreasing population growth. However, it is important to consider that an increase in leek moth mortality due to improved *D. pulchellus* parasitism could be offset if the food web is affected in such a way that *C. albifrons* acts principally as a hyperparasitoid of the biocontrol agent rather than as a primary parasitoid of the pest.

In this study, I tested how the addition of flowering buckwheat, *Fagopyrum esculentum* Moench (Polygonaceae), to standard leek, *Allium ampeloprasum* var. *porrum* L. “Hannibal” (Amaryllidaceae), plots affected leek moth mortality and estimated generational reproductive output compared to standard (no-buckwheat) plots. I hypothesized that adding floral resources (buckwheat) to leek plots would affect biological control efficacy and parasitoid species composition by providing beneficial nectar resources and shelter to a normally sugar-deprived system. I predicted that all else being equal (i.e., the addition of buckwheat will not disproportionately benefit hyperparasitoids), total leek moth mortality caused by natural enemies (including parasitoids) should be higher when buckwheat is added to standard leek plots. I also predicted that parasitoid species composition would differ between plots with buckwheat

versus no-buckwheat plots, and that the buckwheat plots will have increased parasitoid species diversity.

Materials & Methods

Leek moth culture

All leek moth eggs, larvae, pupae, and adults were obtained from a continuous rearing culture established from adult leek moths collected at sites in the Ottawa (Ontario, Canada) area beginning in 2003. This culture, housed at the National Arthropod Containment Facility (NACF) at AAFC, Ottawa, Canada, was supplemented annually with wild material from various additional sites in eastern Ontario. The leek moth rearing methods for this study were adapted from those described by Mason *et al.* (2010).

Newly-emerged adult moths were housed in a wooden-framed, screen-sided oviposition cage (25 x 25 x 45 cm) and provided with a 10% sucrose solution, replenished weekly. Sections of fresh leek, *Allium ampeloprasum* var. *porrum* L. (Amaryllidaceae), leaves (approximately 5 x 10 cm) were placed in the oviposition cage and replaced daily to stimulate oviposition. These leek leaves were divided into smaller sections, each containing approximately 150 eggs, and placed in the leaf whorls of individually-potted, mature leek plants purchased from local produce distributors. Sets of five of these infested plants were placed into wooden-framed, screen-sided rearing cages (30 x 30 x 80 cm), and the larvae were allowed to feed for approximately two weeks. Prior to leek moth pre-pupation, a 104 x 94 mesh bag (48 x 71 cm, DC3148 MegaView

Science Co., Taichung, Taiwan) was placed over the group of five leeks within each cage, to provide an easy-to-access medium on which pupation could occur. Bags were removed from cages daily, and all pre-pupae and pupae were picked from the bags and placed in Petri dishes (91 x 10 mm, Semadeni AG, Ostermundigen, Switzerland) for experimentation or perpetuation of the colony. Adult moths from pupae designated for the rearing colony were transferred into the oviposition cage following emergence in Petri dishes. The culture was kept in a rearing room at $25 \pm 1^\circ\text{C}$ on a 16L: 8D light cycle and $50 \pm 10\%$ relative humidity (RH).

Host plant maintenance

Leek seeds were sown into 48-cell flats (25 x 45 cm) and placed on benches in a greenhouse ($23 \pm 10^\circ\text{C}$ on a 16L: 8D light cycle and $30 \pm 10\%$ RH). After about 90 days, approximately 400 well-established leek seedlings were transplanted from these flats individually into 12 cm peat pots for use in the stage-specific mortality component of the life-table field experiments. The remaining seedlings were kept in their flats for approximately 45 more days until they were transplanted into the field plots.

Plant selection

Buckwheat was selected as a companion crop for this study as it has been shown to promote female bias in parasitoid offspring and increase relative abundance and levels of parasitism (Berndt *et al.*, 2006; Lee & Heimpel., 2008). Due to its relatively low cost, long flowering season, erosion protection, and adaptability to poor growing conditions, buckwheat is often used as a source of green manure, a crop border or as a companion

plant in intercropping systems (Angus *et al.*, 1982; Bowie *et al.*, 1995). I thus considered it to be a good candidate to provide a consistent, long-lasting resource for natural enemies both as a nectar source and as a refuge. It was also seen as having low potential for improving conditions for the study pest as leek moth larvae are specific to *Allium* spp. plants.

Field plot

The two-year life-table study was conducted in a cultivated field (40 x 70 m) at the Central Experimental Farm in Ottawa, Ontario Canada (45.3899°N, 75.7141°W) (see Figures 12 and Appendix 1 for a detailed visual description of the study site and surrounding habitat). Four rectangular blocks were set up on the corners of the field, which was tilled continually throughout the growing season to prevent establishment and maturation of unwanted flowering plants (Figure 13). Each 12 x 25 m block was prepared with ten furrowed rows, 1 m apart, into which greenhouse-grown leeks were planted every 50 cm (the length of each east-west row) (Figure 14A). Two of the blocks, designated as “buckwheat,” were machine sown with buckwheat seed (AAFC, Ottawa, Ontario, Canada) (Figure 14B) approximately one week prior to the leek plantings beginning in the middle of May of both study years. The other two blocks, designated as “no-buckwheat,” consisted of leek plantings only. Any ‘volunteer’ flowering plants were removed from the no-buckwheat blocks on a weekly basis. In both 2015 and 2016, buckwheat blocks were placed in opposite corners from each other, as were the no-buckwheat blocks, to maximize the spacing between treatments (Figure 13).

Experimental procedure

To control age and density in all stages of *A. assectella*, a sentinel-host exposure approach was used to evaluate field mortality using a lab-reared colony. For each leek moth life stage, two infested leeks were placed in each row within each of the four blocks (= 20 infested leeks per block per life stage). In order to provide access to soil-dwelling predators, potted leek plants were dug into the ground so the base of the potted plant was level with the base of the field-grown leeks (Figure 15). The life-table study began when adult leek moth pheromone trap counts indicated the beginning of the second generation. This generation was selected for the life-table study due to its relatively high percentage of leek moth parasitism compared to the first and third generations (PM, unpublished data). In addition, the flowering period of the buckwheat in the buckwheat blocks corresponded with the timing of the second generation.

Stage-specific mortality

All sentinel leek moth eggs, larvae, pupae and adults were held in a rearing room under the rearing conditions described above, then transferred to leek plants and placed in the field once they had reached the desired developmental stage. Sentinel setups of each life stage were placed sequentially, and synchronized with the phenology of the naturally-occurring leek moth population. Sentinel eggs, larvae, and pupae were placed in the field only for the duration of that life stage (with the exception of the egg-neonate stage which was left out in the field for the duration of egg hatching and first instar development), after which they were retrieved and evaluated in the laboratory. For each block, two potted leeks (one “caged” and one “uncaged”) were placed in a random location in each

leek row, for each life stage (a total of 20 plants per block). The placement within the rows was marked with a flag, and utilized for each life stage throughout the experiment. Simultaneously with the experiments, a subset of infested leek plants containing sentinel eggs, larvae and pupae, were placed adjacent to the field site and monitored closely to accurately monitor developmental progression.

Leek moth eggs were obtained by hanging strips of young leek leaves (approximately 2 x 15 cm) from the ceiling of the leek moth oviposition cage overnight (approximately 15 hours). The next morning, the leaves were removed from the oviposition cages and divided into approximately 2 cm sections containing 20–40 healthy eggs. Infested leaf sections were then attached to potted leeks from the greenhouse, and transferred to the field (one section per plant). Each leaf section was pinned to the ventral side of a strong leaf using insect pins (Black Enameled #2, BioQuip, Rancho Dominguez, California, USA). Following collection from the field, eggs were placed in Petri dishes (50 x 15 mm, Semadeni AG, Ostermundigen, Switzerland) and kept in a rearing room at $22 \pm 1^\circ\text{C}$ on a 16L: 8D light cycle and $50 \pm 10\%$ RH to record survival.

Following the same preparation methods as above, a second cohort of 20–40 eggs was pinned to leek plants and placed in the field at the same time as the first cohort. Instead of retrieving eggs prior to hatching, this second cohort was given additional time (three days) to hatch, and neonates the opportunity to mine into leek leaves. Following retrieval from the field, infested plants were brought back to the laboratory for dissection to determine the number of first instar larvae that had successfully entered the leek plant tissues.

To measure mortality of the larval stages of leek moth, 20 newly-hatched (< 2 hours old) first instar leek moth larvae were transferred to freshly cut tips of each potted leek plant, using a fine paint brush. Infested plants were placed in a rearing room ($15 \pm 1^\circ\text{C}$ on a 16L: 8D light cycle and $50 \pm 10\%$ RH) overnight, to allow neonates to enter the plant. The following morning, infested plants were transported to the field and placed into their assigned row and block. Care was taken to ensure that any adjacent leek leaves from nearby plants were trimmed to deter larvae from walking off the experimental plants. Larvae were left in the field for the duration of their development and retrieved just prior to pre-pupal formation on the plant. All retrieved plants were brought back to the laboratory for dissection and evaluation of larval survivorship, mortality and parasitism. Recovered larvae were placed in rearing cylinders (100 x 53 mm, Semadeni AG, Ostermundigen, Switzerland) with leek sections in order to record any emergence of parasitoids. Any dissected larvae that were visibly parasitized (i.e., had ectoparasitoids present on or near paralyzed leek moth larvae) were placed in capped and labelled 96-well plates (Nunc MaxiSorp flat-bottom, LS44240421 Invitrogen ThermoFisher Scientific, Waltham, Massachusetts, USA) and kept in a rearing room at $22 \pm 1^\circ\text{C}$ on a 16L: 8D light cycle and $50 \pm 10\%$ RH for the duration of parasitoid development.

Mortality in the pupal stage was measured by attaching ten newly-formed (< 12 hours old) leek moth pupae to the ventral side of potted leek plants. Pupae were obtained by collecting pre-pupae from the leek moth rearing culture and pinning them to a foam pinning block, carefully placing the pin (Black Enameled #0, BioQuip, Rancho Dominguez, California, USA) between the middle of their pupal casing and silk cocoon

(so as not to cause damage to the pupa) into the foam. Pinned pre-pupae were placed in a rearing room ($25 \pm 1^\circ\text{C}$ on a 16L: 8D light cycle and $50 \pm 10\%$ RH) overnight to allow for development into pupae. The following morning, newly-formed pinned pupae were attached to the plants, which were dug into their assigned row and block in the field. All pupae were recovered and placed into labelled Petri dishes (50 x 15 mm, Semadeni AG, Ostermundigen, Switzerland) just prior to adult emergence, and brought back to the laboratory to monitor mortality and adult leek moth or parasitoid emergence. All pupae were kept in a rearing room at $22 \pm 1^\circ\text{C}$ on a 16L: 8D light cycle and $50 \pm 10\%$ RH.

Natural enemy impact

An exclusion technique was used to evaluate the effect that predators and parasitoids had on leek moth survivorship. For each life stage, half of the infested plants were covered with a fine-mesh bag (30 x 10 cm, DC3210 MegaView Science Co., Ltd., Taiwan) prior to being placed in the field (Figure 16). The bag was placed over the whole plant and cinched at the base of the plant just above the soil surface. These “caged” plants were used to exclude biotic mortality factors that the fully “uncaged” plants were susceptible to (i.e., parasitoids, predators). The natural enemy impact was estimated by comparing stage-specific survivorship between the caged and uncaged leeks.

Potential and realized fecundity

To construct complete life tables for the field experiments, it was necessary to estimate the potential and realized female leek moth fecundity. To determine potential fecundity, a pair of newly-emerged male and female leek moths were placed in a Petri dish (91 x 20

mm, Semadeni AG, Ostermundigen, Switzerland) with a piece of filter paper lining the bottom, and a 1 cm long cotton bud soaked with a 10% sucrose solution. Each pair was also provided with a freshly cut piece of leek leaf (approximately 3 x 2 cm). Oviposition arenas were kept in a rearing room at $22 \pm 1^\circ\text{C}$ on a 16L: 8D light cycle and $50 \pm 10\%$ RH. Every 2–3 days, the adult moths were transferred to a clean Petri dish containing a new piece of filter paper, cotton sucrose bud, and leek leaf, and eggs laid in the vacated arenas and leek leaves were counted and recorded. This process continued for the lifetime of each female leek moth. A total of 19 and 21 leek moth pairs were observed in the lab in 2015 and 2016, respectively. Females that did not lay eggs were presumed to be unmated and were excluded from these experiments.

To determine the realized fecundity of leek moths in the field, a pair of newly-emerged male and female leek moths were placed on a young, potted leek plant (approximately 20 cm tall), encased by a windowed, fine-mesh bag (40 x 20 cm, DC3000W-S MegaView Science Co., Ltd., Taiwan), cinched around the rim of the pot (Figure 17). To prevent the mesh bag from collapsing into the leek plant, curved wire hoops were placed inside the pot and shaped to keep the mesh bag cylindrical. These oviposition plants were placed in the field margins of the life-table study plot at the beginning of the second field generation. Every 3–5 days, the adult moths were transferred to a new potted leek plant, and all eggs laid on the previously exposed plants were counted. This process continued for the lifetime of each female leek moth. A total of 22 and 18 leek moth pairs were observed in the field in 2015 and 2016, respectively.

Females that did not lay eggs were presumed to be unmated and were excluded from these experiments.

Construction of life tables

Following Jenner *et al.* (2010) and Haye *et al.* (2014), and using techniques described by Bellows & Van Driesche (1999), field mortality data was used to construct horizontal, stage-specific life tables for each treatment during the second leek moth generation in 2015 and 2016. Mortality probability was calculated using a known number of leek moth individuals for each life stage; however, to simplify and standardize the presentation of accumulated mortality, a starting number of 1000 eggs was used. Apparent mortality (q_x) caused by known (e.g., parasitism) and unknown abiotic and biotic factors, was determined as the proportion of individuals dying in a stage (d_x) to the number entering the stage (l_x). Real mortality (r_x) was calculated by determining the ratio of individuals dying in a particular stage to the initial starting number at the beginning of the study. The number of individuals entering each life stage was determined by subtracting the number dying in each stage from the number entering the previous stage. Marginal death rate (m_x) was used to estimate the number of individuals entering a stage that would be attacked by an agent (e.g., parasitoids) if it were acting in the absence of other mortality factors, or for contemporaneous factors (Bellows *et al.*, 1992). When no overlap between mortality factors occurs, or a specific factor was distinguishable, the marginal death rate was equal to the apparent mortality; however, when overlap did occur in the absence of discernable interaction factors, the marginal death rate was calculated by using the formula $m_x = 1 - (1 - q)^{q_i/q}$, where q_i is the apparent mortality caused by the i th factor and q is the stage

mortality rate caused by all interacting factors (Elkinton *et al.*, 1992). Once marginal death rates for each stage were calculated, k -values (intensity of mortality in a given stage) were determined as $-\log(1-m_x)$. Each k -value represented the impact of mortality in each stage on the total generational mortality (K_g), which was the sum of all of these k -values. Finally, the net reproductive rate of increase (R_o) was determined by dividing the number of estimated surviving progeny by the initial number of individuals used in the study. This value represents the factor by which a population increases (or decreases) in successive generations (Van Driesche *et al.*, 2009).

Statistical analyses

To assess the effects of floral treatment (buckwheat, no-buckwheat), cage treatment (caged, uncaged), and their interaction on proportion of leek moth mortality and parasitism, I used generalized linear mixed models (GLMMs) with a binomial error distribution and a logit link function, floral treatment and cage treatment as fixed factors, and field block as a random factor. For both mortality and parasitism response variables, I ran separate GLMMs for each leek moth life stage and year of data. The statistical significance of each model's fixed factors and their interaction were determined with Type II Wald chi-squared tests (Anova function in the "car" package of R statistical software) (Fox & Weisberg, 2011). To interpret significant floral treatment/cage treatment interaction effects, I examined the effect of floral treatment separately for each cage treatment using the same general GLMM and significance testing procedure as described above. The effects of floral treatment, year, and their interaction on parasitoid species composition (number of parasitoid species for plants from which at least one

parasitoid was reared) were modeled with permutational multivariate analysis of variance (a non-parametric analogue to a traditional MANOVA) with a Euclidian distance matrix (Anderson, 2001). Because there were too few ($n = 2$) true replicates of each floral treatment to run a robust analysis of parasitoid species composition among blocks, blocks of each treatment were pooled and plants were treated as independent replicates. Thus, I considered this analysis on partially pseudoreplicated data exploratory rather than definitive. In addition, as there was too little parasitism data from caged plants to include cage treatment as a factor in models, data from both cage treatments were pooled for these analyses. Models were run with 1000 permutations, and the assumption of equal multivariate spread was verified in all cases. A separate model was run for each attacked leek moth life stage (i.e., larvae, pupae). All analyses were conducted with R software version 3.3.2 (R Core Team, 2016).

Results

Stage-specific mortality

There were varying effects of floral treatment (i.e., buckwheat, no-buckwheat) and cage treatment (i.e., caged, uncaged) on leek moth mortality across life stages and years (Figure 18). In 2015, floral treatment did not affect leek moth mortality during the egg ($\chi^2 = 0.002$, $df = 1$, $P = 0.96$) or larval ($\chi^2 = 0.001$, $df = 1$, $P = 0.98$) stages. For neonates, the effect of floral treatment depended on cage treatment ($\chi^2 = 4.09$, $df = 1$, $P = 0.043$): in the uncaged treatment, neonate mortality tended to be lower in plots with buckwheat ($\chi^2 =$

23.88, $df = 1$, $P < 0.0001$); whereas there was no effect of buckwheat on neonate mortality in the caged treatment ($\chi^2 = 0.31$, $df = 1$, $P = 0.58$) (Figure 18). In the uncaged treatment, there was 100% pupal mortality in both the buckwheat and the no-buckwheat treatments. In the caged treatment, pupal mortality was similar in plots with and without buckwheat ($\chi^2 = 0.17$, $df = 1$, $P = 0.68$) (Figure 18). Mortality of eggs ($\chi^2 = 42.54$, $df = 1$, $P < 0.0001$), larvae ($\chi^2 = 103.53$, $df = 1$, $P < 0.0001$), and pupae (complete mortality in uncaged treatment) was lower on caged plants than on uncaged plants (Figure 18). Neonate mortality was higher in the caged treatments than in the uncaged treatments ($\chi^2 = 37.06$, $df = 1$, $P < 0.0001$). In 2016, the buckwheat treatment did not affect leek moth mortality during the egg ($\chi^2 = 1.308$, $df = 1$, $P = 0.25$) and pupal stage ($\chi^2 = 0.188$, $df = 1$, $P = 0.66$). Neonate mortality was lower in the buckwheat plots ($\chi^2 = 5.056$, $df = 1$, $P < 0.05$) than in the no-buckwheat plots, whereas larval mortality was higher in the buckwheat plots ($\chi^2 = 6.294$, $df = 1$, $P < 0.05$). In plots with and without buckwheat, neonate mortality was higher in the caged treatment than uncaged treatment ($\chi^2 = 4.541$, $df = 1$, $P < 0.05$). Mortality of eggs ($\chi^2 = 11.18$, $df = 1$, $P < 0.001$), larvae ($\chi^2 = 79.63$, $df = 1$, $P < 0.0001$), and pupae ($\chi^2 = 120.96$, $df = 1$, $P < 0.0001$), was lower on caged plants than on uncaged plants in both the buckwheat and no-buckwheat plots (Figure 18).

Generational mortality

The contribution of each life stage of leek moth to its generational mortality and the estimated net reproductive rate for each treatment in 2015 and 2016 is summarized in Table 1 (see Appendix 2 for complete life tables for both years). In all treatments in both years, generational mortality was highest in the egg stage, except for in the 2015 – caged,

buckwheat plots, and the 2016 – caged, no-buckwheat plots; both of which had slightly higher mortality in the neonate stage. The second highest mortality was seen in the neonate stage in all treatments in both years, except for the 2015 uncaged buckwheat plot, in-which the larval stage showed a slightly higher contribution to generational mortality. Larval mortality was the third highest contributor to generational mortality in all but the above cases, and pupal mortality contributed the least to generational mortality. The highest net reproductive rate was in the caged treatments in both years.

Parasitism levels and effects on leek moth population growth

Mortality due to parasitism was only observed in the larval and pupal stages of *A. assectella* (Figure 19). There was no effect of floral treatment on larval parasitism in 2015 ($\chi^2 = 0.18$, $df = 1$, $P = 0.67$), but on the 2016 uncaged plants it was almost twice as high in the buckwheat plots than in the no-buckwheat plots ($\chi^2 = 8.41$, $df = 1$, $P = 0.0037$) (Figure 19). Larval parasitism was higher in the uncaged treatment than the caged treatment in both 2015 ($\chi^2 = 10.85$, $df = 1$, $P < 0.001$) and 2016 ($\chi^2 = 41.24$, $df = 1$, $P < 0.0001$) (Figure 19). In 2015, the effect of floral treatment on pupal parasitism was affected by an interaction with cage treatment ($\chi^2 = 12.81$, $df = 1$, $P < 0.001$); pupae were parasitized at a lower level in buckwheat plots when they were on caged leeks ($\chi^2 = 9.55$, $df = 1$, $P = 0.0020$), but there was no effect of floral treatment on pupal parasitism for uncaged leeks ($\chi^2 = 0.12$, $df = 1$, $P = 0.72$). In 2016 however, there was no effect of floral treatment on pupal parasitism ($\chi^2 = 0.15$, $df = 1$, $P = 0.70$); there was greater pupal parasitism on uncaged leeks ($\chi^2 = 26.54$, $df = 1$, $P < 0.0001$). In both years, some pupal

and larval parasitism was observed in caged leeks, indicating that parasitoids were, to some degree, able to oviposit through the mesh bags (Figure 19).

To evaluate parasitoid impact on leek moth population growth in each year and treatment, I calculated hypothetical differences in net reproductive rate (R_0) when removing leek moth mortality due to parasitism (Table 2). The net reproductive rate in both the caged and uncaged treatments demonstrated increasing population growth in all cases except the 2015 uncaged buckwheat and no-buckwheat plots. In 2015, R_0 increased by 23.3% in the caged buckwheat plots and 54.8% in the caged no-buckwheat plots. The R_0 in the uncaged buckwheat plots ranged from 0 (no reproduction) to 1.09 (slightly increasing growth), and from 0 to 0.58 (declining growth) in the uncaged no-buckwheat plots. In 2016, R_0 increased by 4.2% and 5.6% in the caged buckwheat and no-buckwheat plots respectively. The highest percentage increase in R_0 in the absence of parasitism was found in the 2016 buckwheat and no-buckwheat plots, which saw increases of 256.6% and 246.2%, respectively. In the 2015 uncaged buckwheat treatment, and the 2016 uncaged buckwheat and no-buckwheat treatments, the R_0 increased from that of a declining population to that of a growing population when parasitism was removed.

Parasitoid composition

The species composition of parasitoids attacking leek moth larvae in buckwheat and no-buckwheat plots differed between years ($F_{1,59} = 10.53$, $R^2 = 0.15$, $P < 0.001$), but was not affected by floral treatment ($F_{1,59} = 3.21$, $R^2 = 0.052$, $P = 0.059$) or an interaction between year and floral treatment ($F_{1,59} = 2.92$, $R^2 = 0.039$, $P = 0.084$). In 2015, the community of larval parasitoids consisted of 15–25% *Scambus* sp., 5% *Bracon* sp., and the remaining

70–80% *Habrobracon* sp. (Figure 20 – Top Left). In 2016, there were no *Scambus* sp. present, and only about 2% *Bracon* sp. *Habrobracon* sp. dominated the remaining larval parasitoid species (Figure 20 – Top Right). The number of parasitoid species (three) was the same in buckwheat and no-buckwheat plots in both years.

The pupal parasitoid complex was made up of *Itoplectis* sp., *D. pulchellus*, and *C. albifrons* in buckwheat and no-buckwheat plots. However, the relative prevalence of different parasitoid species differed between buckwheat and no-buckwheat plots ($F_{1,82} = 12.19$, $R^2 = 0.13$, $P < 0.001$) but was not affected by year ($F_{1,81} = 4.57$, $R^2 = 0.02$, $P = 0.19$) or an interaction of year and floral treatment ($F_{1,80} = 1.14$, $R^2 = 0.01$, $P = 0.32$) (Figure 20). Although the same parasitoid species were present in all year/floral treatment combinations, *C. albifrons* made up more than 60% of the parasitoids in the buckwheat treatments, whereas *Itoplectis* sp. made up more than 60% of the parasitoids in the no-buckwheat plots. In both 2015 and 2016, the prevalence of *D. pulchellus* in the caged and uncaged treatments was lower than 10% (Figure 20).

Discussion

Adding flowering plants to a leek field – an environment otherwise mostly devoid of sugar sources – had limited effects on generational mortality or parasitism levels of leek moth. In both years generational mortality was highest in the egg stage with the exception of the caged, buckwheat plot in 2015, and the caged, no-buckwheat plot in 2016, which both had slightly higher mortality in the neonate stage. Larval and pupal parasitism contributed to generational mortality in both years; however, the impact was not generally increased by the addition of buckwheat. Overall parasitoid species composition

was the same for both the buckwheat and no-buckwheat plots; however, floral treatment did heavily influence the relative abundance of the various pupal, but not larval, parasitoid species. The results of this study suggest that resource manipulation as a component of CBC in this system, can drastically change the natural enemy community while having basically neutral effects on leek moth control. In addition, the nature of these changes may depend on the life stage of the pest.

I predicted that adding floral resources (buckwheat) to standard leek field plots would cause aggregation of nectar-feeding parasitoids and predators, resulting in an increase in total leek moth mortality, and a reduction in population growth, due to improved longevity, fecundity, and activity levels of natural enemies (i.e., parasitoids) (Suarez *et al.*, 2005; Desouhant, 2010). This was generally not true as stage-specific and total generational mortality in buckwheat and no-buckwheat plots was similar in both years. In fact, neonate mortality was generally lower in buckwheat plots than no-buckwheat plots. This might be attributed to a reduction in the severity of detrimental abiotic factors (e.g., wind) within the buckwheat plots, which would lessen disturbances on the leek plants compared to more exposed, no-buckwheat plots. After hatching and prior to entering the plant, neonates are vulnerable to abiotic factors and can easily be knocked off their plant (Jenner *et al.*, 2010). Accordingly, life-table generation mortality estimates were also similar between the two floral treatments. This again suggests that these floral treatments did not dramatically alter the effective natural enemy abundance between plots. Comparison of caged versus uncaged plants in both plots suggests that predation is likely an important contributor to leek moth mortality. In the absence of

exposure to natural enemies, caged plants experienced a greater than ten-fold increase in estimated net reproductive output resulting in much lower generational reproductive growth than that of uncaged plants in the same treatments. Generational mortality was generally highest in the egg stage for both years, followed by neonates, larvae and pupae. The effect of cage treatment on mortality was most prominent in the egg stage as the eggs in the caged plants were seemingly better protected than the uncaged plants; however, neonates experienced higher mortality levels in the caged treatments compared to the uncaged treatments. Both larval and pupal stage mortality levels were lower in caged treatments compared to uncaged treatments, with the exception of pupae in the caged floral treatments in 2015. However, again, life-stage-specific mortality was generally not affected by floral treatment. Results from these life tables differ from those of Jenner *et al.*, (2010) in Europe, who found that neonate mortality was the primary factor influencing population increase or decrease followed by larvae, pupae and finally, eggs.

I predicted that parasitism would be higher in the buckwheat plots than in the no-buckwheat plots because the increased nectar resources provided by the buckwheat plants would recruit more parasitoids and improve their fitness (see Berndt and Wratten, 2006; Lee & Heimpel, 2008; Chapter 2 of this thesis). Although there were sometimes differences in parasitism between buckwheat and non-buckwheat plots – higher larval parasitism on uncaged leeks in 2016, for example – these effects were not consistent across leek moth life stages or years. This may be due in part to any beneficial effect of nectar feeding being diluted by immigration of nectar-fed parasitoids from buckwheat plots into no-buckwheat plots, although the dispersal abilities of leek moth parasitoids are

not known. Parasitoids that are naturally likely to travel greater distances for food searching and host-finding may have had an easier time migrating between the buckwheat and no-buckwheat plots. This may be the case for the majority of those recovered in this study as they are almost all known to be generalists (Mason *et al.*, 2010; Daane, 2013). Larval and pupal primary parasitoids, as well as facultative hyperparasitoids, were observed and recovered in both years of this study. Interestingly, the presence of *C. albifrons* was much greater in the buckwheat plots than in the no-buckwheat plots in both study years, indicating that *C. albifrons* may have benefited greatly from the constant supply of nectar-producing buckwheat (see below). While I saw minimal effects of this pilot CBC strategy, results from the life-table analysis, indicate that increasing overall parasitism within this system is a worthwhile goal, as it greatly reduced estimated leek moth population growth rates, particularly when affecting the pupal stage.

Based in part on results from the previous sugar and nectar resource-provisioning experiments in the greenhouse, I predicted that the addition of buckwheat to leek plots in the field could differentially affect the two main parasitoids known from this system, *D. pulchellus* and *C. albifrons*. However, I was surprised to find that several other parasitoid species played significant, even dominant roles in overall leek moth parasitism in both floral treatments. Although all of these species had been recovered previously from leek moth, the high prevalence of several of them in this study was not anticipated. The parasitoid assemblage of both buckwheat and no-buckwheat treatments was essentially the same in both years; however, the differing relative abundance of the pupal parasitoids

between the two treatments demonstrated that some of them were most likely benefitting from the buckwheat more than others, or that the presence of buckwheat modified the outcome of competitive interactions. For example, *C. albifrons* was recovered more often in the buckwheat plots, whereas *Itopectis* sp. was more common in the no-buckwheat plots. This might be attributed to differing life-history strategies of these two facultative hyperparasitoids (Leius, 1961a; Hansen, 1980). As was reported by Arthur (1958) and discussed by Miall *et al.* (2014), *C. albifrons* requires up to nine days following eclosion to mate and develop mature eggs. This parasitoid has been observed to feed on both sugar and protein sources during this period of time; however, the infrequency of observed host-feeding and low level of foraging once a sugar source is found likely means that the protein source acquired from the host is not a preoviposition requirement. Alternatively, Leius (1961) determined that fecundity and longevity were both positively affected by sugar and host-feeding by *Itopectis conquisitor* (Say) (Hymenoptera: Ichneumonidae) in the laboratory. In fact, this previous study showed that sugar-feeding soon after eclosion (<2 days) and continual host-feeding, were both required to initiate egg development and oviposition, and to prevent egg resorption. *Itopectis* spp. are relatively short-lived in the laboratory (upwards of 60 days when provided with carbohydrate and protein source) compared to *C. albifrons* (often more than five months when provided with carbohydrates only); however, while *C. albifrons* ecloses with undeveloped eggs and requires a long preoviposition period (up to nine days), *Itopectis* spp. are able to begin oviposition as early as three days after eclosion. Both parasitoids are known to attack a wide-variety of hosts (*Itopectis* spp: Leius, 1961; House, 1980; Kuhlmann *et al.*, 1998;

C. albifrons: Burks, 1940; Arthur, 1958; Peck, 1963; Hansen, 1980; Gaines, 1997; Gaines, 1999; Miall *et al.*, 2014) but it is unknown if either generalist prefers to act as a primary parasitoid of a particular host species or as a facultative hyperparasitoid of a preferred primary parasitoid. *Itopectis* sp. may have utilized nectar from buckwheat before moving around more to seek out a particular host to feed on – or a potential oviposition host. It is important to consider that a mobile species like *Itopectis* sp. could be demonstrating selective host-feeding by utilizing a different host to obtain additional nutrition as higher levels of immature parasitoid mortality have been seen in parasitized pupae that have been fed on (Ueno, 1998). It is also possible that *Itopectis* sp. was able to recognize *C. albifrons* as a potential intra-guild competitor or predator, and therefore moved from areas of higher parasitoid density (buckwheat plots) to reduced density plots (no-buckwheat). It has also been noted by Leius (1961) that *I. conquisitor* has been observed feeding on aphid honeydew, which also might explain why it was found outside of the buckwheat plots more often than *C. albifrons*.

In this study, I examined how intercropping leek plots with buckwheat affected leek moth mortality, generational population growth, parasitism levels, and overall parasitoid species composition and relative abundance compared to conventional leek plots. Based on these results, future studies should focus on manipulating plot designs (i.e., larger field plots, isolating plots with hedgerows, etc.) and test different or combined nectar sources, to better understand how to manipulate the parasitoid species composition to provide more pest suppression. These studies could be enhanced by developing methods to exclude particular parasitoid species. For example, *D. pulchellus* requires

complete access to the head end of the leek moth pupa to oviposit, whereas *Habrobracon* sp. and *C. albifrons* can both penetrate through the bags used in the caged treatments (see Costamagna *et al.*, 2007). This information would also be useful to help shape parasitoid assemblages across all three leek moth generations in eastern Canada with the goal of maximal host suppression. While efforts have been made to increase leek moth suppression by establishing *D. pulchellus* in the field (Mason *et al.*, 2013a), it is obvious from this study that there are likely additional indigenous parasitoids that play important roles in this system. Understanding the life histories of these other players is an important step in designing field plots that effectively cater to their needs. In more general terms, CBC strategies need to address how whole food webs, and not just a few selected species, may be affected by habitat manipulation techniques.

Tables

Table 1. Contribution of each leek moth, *Acrolepiopsis assectella*, life stage to generational mortality for caged and uncaged, buckwheat and no-buckwheat leek plots in 2015 and 2016. Resulting net reproductive rate (R_0) is tabulated for each treatment in 2015 and 2016.

% contribution to generational mortality								
Treatment		Egg	Neonate	Larval	Pupal	Total generational mortality (%)	Net reproductive rate (R_0)	
2015	Caged	Buckwheat	35.6	36.9	12.5	9.4	94.4	2.92
		No-buckwheat	40.0	38.2	9.9	7.7	95.7	2.21
	Uncaged	Buckwheat	55.8	16.4	19.6	8.1	100.0	0.00
		No-buckwheat	51.3	26.1	15.8	8.0	100.0	0.00
2016	Caged	Buckwheat	35.9	35.7	13.9	3.8	89.3	5.95
		No-buckwheat	36.4	37.6	11.6	4.1	89.7	5.73
	Uncaged	Buckwheat	47.1	25.7	20.3	6.0	99.0	0.52
		No-buckwheat	41.1	32.9	16.6	8.5	99.1	0.52

Table 2. Influence of parasitism on leek moth, *Acrolepiopsis assectella*, reproductive output (R_o) for each larval, pupal and combined stages for cage and uncaged, buckwheat and no-buckwheat leek plots in 2015 and 2016.

Year	Stage	Buckwheat			No-buckwheat		
		Actual R_o	Parasitism absent R_o	% decrease in R_o due to parasitism	Actual R_o	Parasitism absent R_o	% decrease in R_o due to parasitism
Caged	Larval		2.94	0.68		2.21	0
	2015 Pupal	2.92	3.58	22.6	2.21	3.42	54.75
	Combined		3.6	23.29		3.42	54.75
	Larval		6.04	1.51		5.8	1.22
	2016 Pupal	5.95	6.11	2.69	5.73	5.97	4.19
	Combined		6.2	4.2		6.05	5.58
Uncaged	Larval		0	-		0	-
	2015 Pupal	0	0.88	-	0	0.48	-
	Combined		1.09	-		0.58	-
	Larval		0.83	56.6		0.63	21.15
	2016 Pupal	0.53	1.21	128.3	0.52	1.49	186.54
	Combined		1.89	256.6		1.8	246.15

Figures



Figure 12. Wide-angle aerial photograph of 2016 life-table study site and surrounding habitat at the Central Experimental Farm, Ottawa, Ontario, Canada. **A.** actual field plot used for buckwheat and no-buckwheat leek blocks; **B.** adjacent tilled habitat with no available floral resources; **C.** field plot where original releases of *Diadromus pulchellus* occurred. Note: photograph was taken on September 5, 2016; approximately 2 months following completion of the study (Google Inc, 2017).

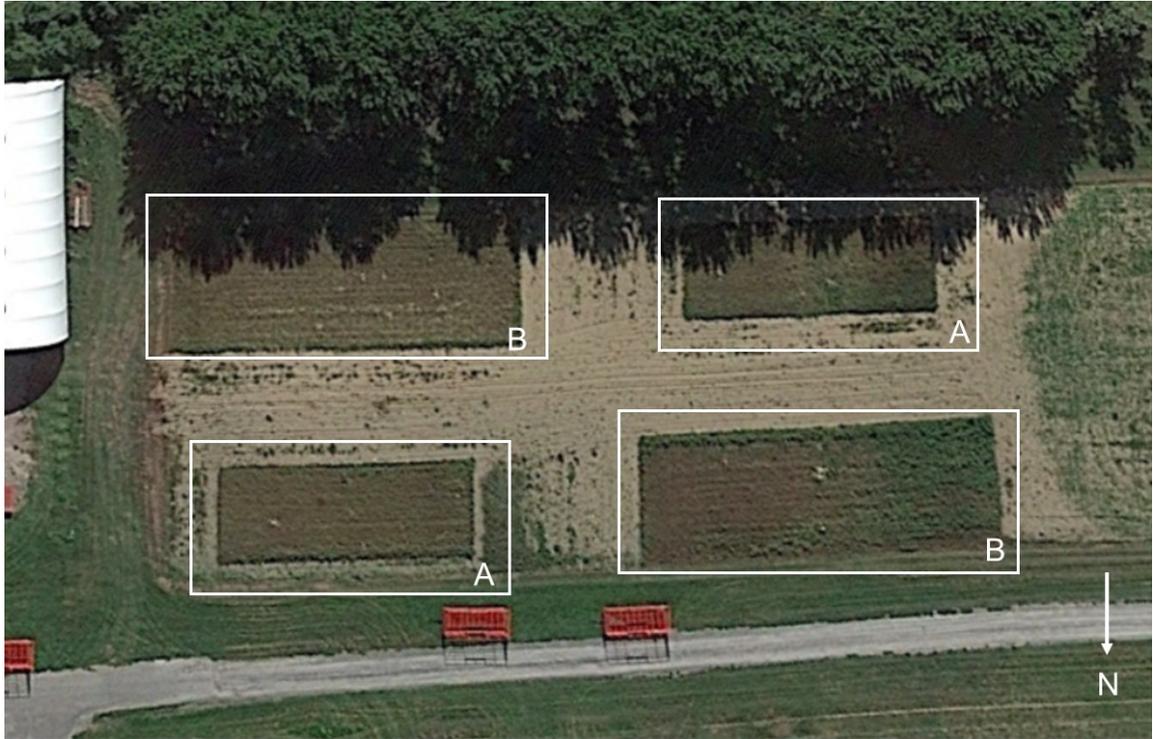


Figure 13. Aerial photograph of 2016 life-table study site at the Central Experimental Farm, Ottawa, Ontario, Canada. **A.** leek blocks with no buckwheat; **B.** leek blocks intercropped with buckwheat. Note: photograph was taken on September 5, 2016; approximately 2 months following completion of the study (Google Inc, 2017).



Figure 14. Life-table study site at the Central Experimental Farm, Ottawa, Ontario, Canada. **A.** 2016 no-buckwheat leek plot; **B.** 2016 buckwheat-enhanced leek plot. (Photos: A.M. Brauner)



Figure 15. Sentinel plants containing newly-hatched leek moth, *Acrolepiopsis assectella*, larvae in a no-buckwheat leek plot. (Photo: A.M. Brauner)



Figure 16. Natural enemy exclusion cages on sentinel leeks prior to field placement.
(Photo: A.M. Brauner)



Figure 17. Potted leeks in realized fecundity cages, each containing a mating pair of leek moths, *Acrolepiopsis assectella*. (Photo: A.M. Brauner)

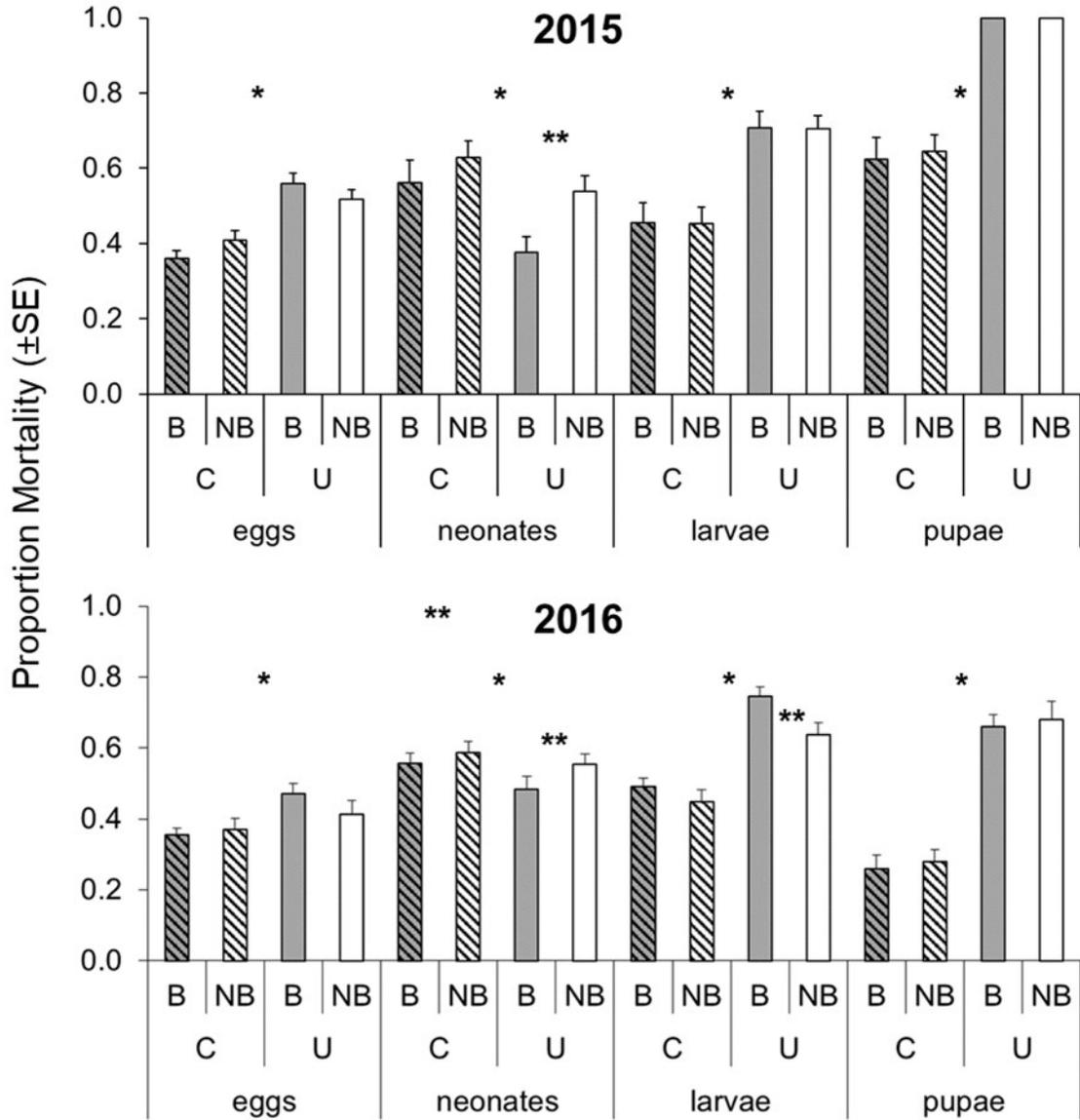


Figure 18. Stage-specific mortality (\pm SE) by treatment, of leek moth, *Acrolepiopsis assectella*, in 2015 and 2016. Caged (C), uncaged (U) treatments in buckwheat (B) and no-buckwheat (NB) blocks. (Stars indicate significant differences [$p < 0.05$] between treatments as determined by Type II Wald chi-square tests: * = caged and uncaged treatments; ** = buckwheat and no-buckwheat treatments).

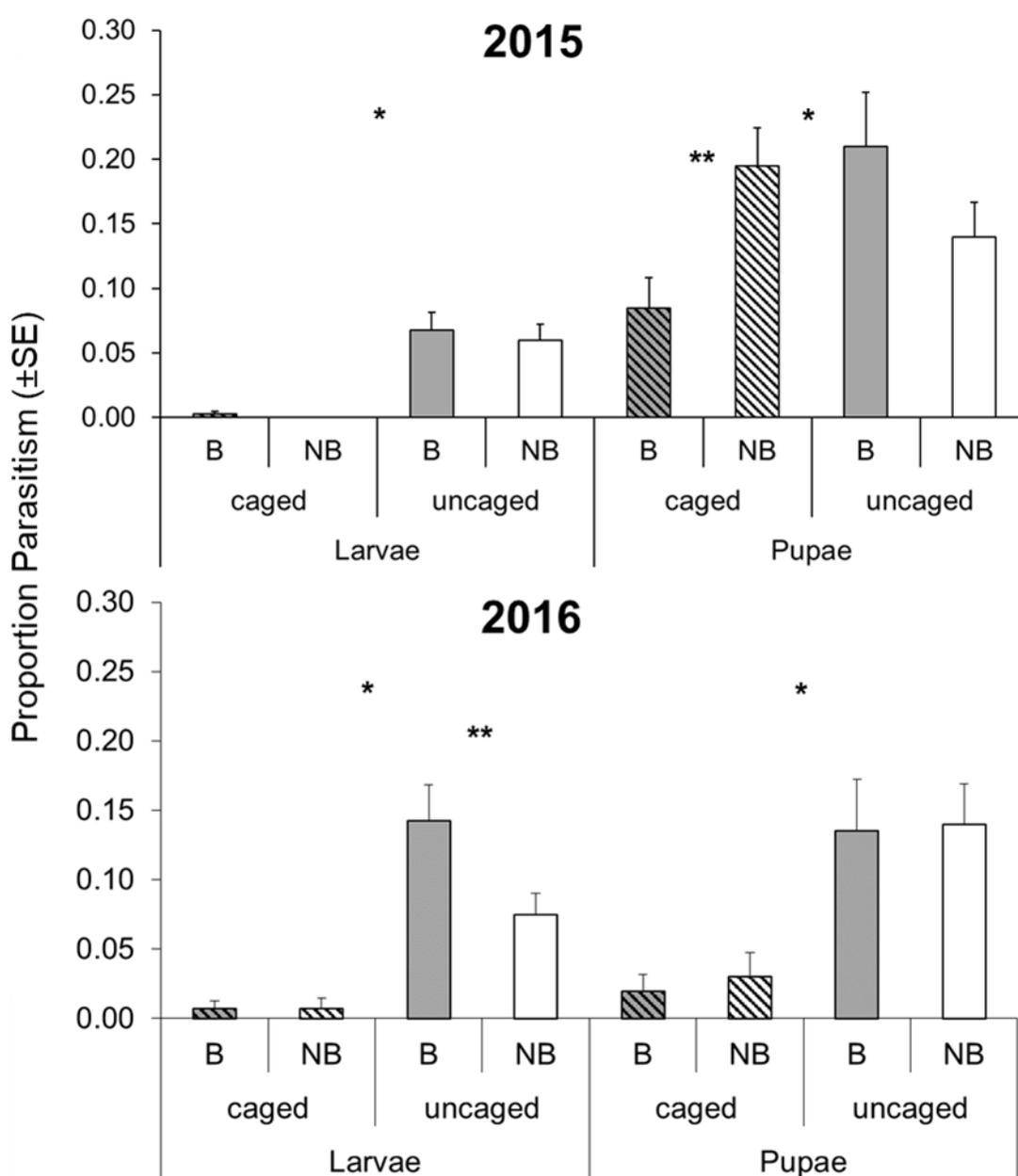


Figure 19. Proportion of leek moth, *Acrolepiopsis assectella*, larval and pupal parasitism (\pm SE) in caged (C), uncaged (U) treatments in buckwheat (B) and no-buckwheat (NB) blocks. (Stars indicate significant differences [$p < 0.05$] between treatments as determined by Type II Wald chisquare tests: * = caged and uncaged treatments; ** = buckwheat and no-buckwheat treatments).

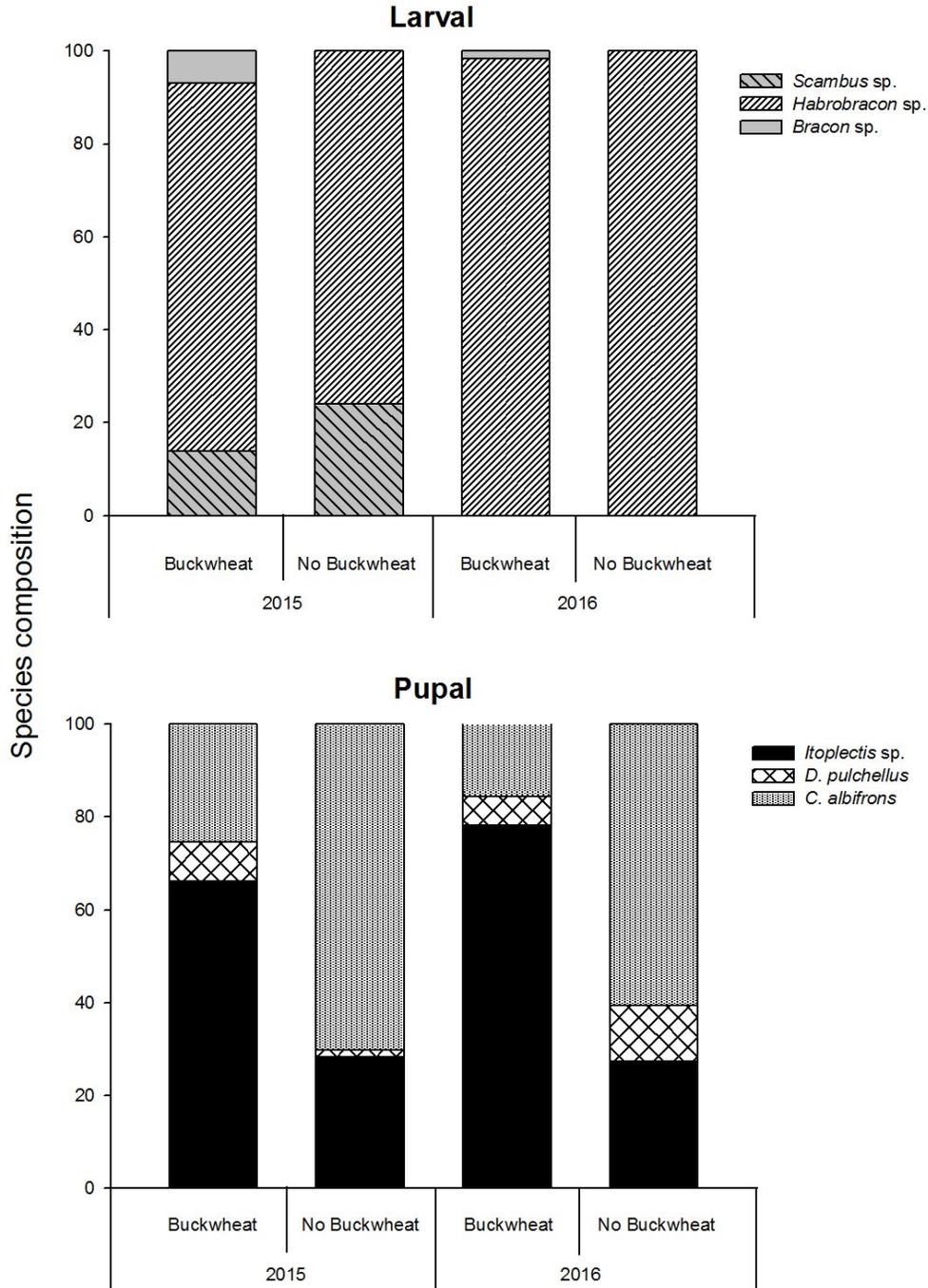


Figure 20. Parasitoid species composition (%) from larval and pupal stages of leek moth, *Acrolepiopsis assectella*, in buckwheat and no-buckwheat leek plots in 2015 and 2016.

Chapter 4: General Discussion

Summary of findings

Agricultural systems are often dominated by monocultures, resulting in an absence of sugar availability for natural enemies (Landis, 2000, Wratten *et al.*, 2002). Crops in these systems are vulnerable to pest outbreaks, and the resulting damage can cause significant economic losses (Dalin *et al.*, 2009). Improving ecosystem services to address this sugar void could help indigenous and introduced natural enemies to suppress pest populations. Floral resource addition is often employed as a strategy to increase natural enemy impact on pests (Landis *et al.*, 2000; Gurr *et al.*, 2000), as many lab experiments show clear benefits of nectar feeding on important natural enemies, often resulting in increased longevity, fecundity and host suppression (See Chapter 2; Winkler *et al.*, 2006; Wade and Wratten, 2007. Many predators and most hymenopteran parasitoids require carbohydrates (sugar) to help them perform basic ecological functions such as foraging and prey/host acquisition (Jervis *et al.*, 1993; Jervis *et al.*, 2001), which helps to explain why agricultural pests are seemingly better managed in polycultures with readily available and accessible sugar resources (e.g., honeydew and nectar) (Rusch *et al.*, 2016). The establishment of the classical biological control agent (BCA) *Diadromus pulchellus* in eastern Canada is a positive first step in managing the introduced leek moth, *Acolepiopsis assectella* in *Allium* spp. crops. There are however, several possible “next steps” that could improve the overall impact *D. pulchellus* has on leek moth populations and hasten its establishment in new areas, including a conservation biological control (CBC) approach to increase sugar availability to female *D. pulchellus* to improve fitness

(longevity, sex ratio and fecundity) and parasitism levels in *Allium* fields, which often lack sugar sources. In addition, identifying which nectar-producing plants have attributes beneficial to the natural enemies of leek moth could be used to develop and implement better cropping strategies. As it was shown previously that *C. albifrons* can act as both a primary parasitoid of leek moth and a facultative hyperparasitoid of *D. pulchellus* (Miall *et al.*, 2014), it was pertinent to explore how the addition of sugar resources affected both of these species under the same conditions. This would not only serve to specifically address the impact on an important parasitoid in this system, but also the so-far poorly understood effect of supplementary sugar resources on a generalist facultative hyperparasitoid. In Chapter 2, the benefits of sugar sources (sucrose, floral and extra-floral nectar) to the primary pupal parasitoid, *D. pulchellus*, of leek moth, and their facultative hyperparasitoid, *C. albifrons*, were discussed in the context of longevity, fecundity, and host-killing. In a greenhouse setting, I demonstrated significant effects of nectar provisioning for both *D. pulchellus* and *C. albifrons*, by testing different artificial (sucrose) and plant-derived (buckwheat and common vetch) sugar sources alone and in combination. Knowing how these resources affected this system under simplified (caged) conditions, I designed a field study (Chapter 3) using a life-table approach to quantify the effects of providing an additional nectar source to the leek moth system, in terms of population-level generational mortality of the pest. Although the addition of floral resources had no major impact on pest mortality, I demonstrated that it could significantly shift the relative abundance of indigenous and exotic parasitoids, within the community. This effect was repeatable, as it was observed in both years of the study.

Below, I discuss the results of this study in the context of combining classical and CBC to leek moth management, as well as to biological control programs in general.

Opportunities and limits for applying CBC techniques to the leek moth system in Canada

The relatively recent invasion of leek moth and establishment of *D. pulchellus* allowed us to explore the potential opportunities and limitations of a combined classical and CBC strategy, in the absence of a “stabilized” natural enemy community. While I am aware that some indigenous parasitoids are already having an impact on the leek moth system, it remains difficult to predict the long-term consequences of the presence of these other species for *D. pulchellus* establishment, spread, and impact on leek moth, as changes in the natural enemy community appears to be ongoing. Field surveys beginning ten years after leek moth’s discovery in Ontario, Canada, revealed that few indigenous parasitoid species were targeting leek moth, and those few that were discovered were having almost no effect on pest suppression (PM, unpublished data). Prior to expanding its range beyond the Ottawa area, leek moth was having a detrimental effect on small-scale organic garlic producers. In these settings, conventional methods of achieving pest management through pesticide applications were replaced with techniques such as placing row cover over crops, crop rotation, removal or destruction of infested material, and promotion of natural enemies through habitat manipulation (e.g., reduction of tilling, hand weeding rather than machine or chemical weed removal, establishment of flowering perennial plants). It is in these settings where the effectiveness of a CBC approach might be most

easily measured. In larger-scale operations (e.g., mass leek production in southern Ontario and eastern Quebec) where more conventional pest management techniques predominate (regular and preventative pesticide applications, low incidence of crop rotation, low non-crop plant diversity), a classical and/or conservation biological control program might be less effective. Aside from the efforts made to minimize the non-buckwheat weeds in each of the experimental leek blocks, the field conditions of the resource-provisioning study in Chapter 3 more closely-resembled those found in small organic plots as opposed to a conventional system. Furthermore, it is possible that the resources provided in the buckwheat blocks were close enough to the no-buckwheat blocks that they were providing a refuge and a nectar source to those parasitoids more likely to migrate (see Wilkinson & Landis, 2005).

Surprisingly, I found that *D. pulchellus* is currently only a small part of the host-parasitoid system (Chapter 3) despite the location of this experimental field site having been within 200m of the original 2010 *D. pulchellus* releases. Some causes of the seemingly low levels of establishment and/or impact could be related to competitive exclusion or hyperparasitism by the increasing number of indigenous parasitoids moving into the leek moth system since the initial 2010 releases (Mason *et al.*, 2013a,b; PGM unpublished data). Perhaps in this case, enough time had passed between leek moth establishment, initial natural enemy surveys, and *D. pulchellus* releases for the indigenous natural enemies to move into the system, making it much harder for *D. pulchellus* to find an unoccupied niche. As was observed in the greenhouse cage experiments (Chapter 2), buckwheat seems to have a similar overall positive effect on

fitness and host-killing ability of *D. pulchellus* as on the facultative hyperparasitoid, *C. albifrons*. It is tempting to speculate that the beneficial effects of buckwheat on these two players, will likely extend to the full suite of parasitoids now found in the leek moth field system, all things being equal. But all things are not necessarily equal. The life history, phenology and behaviour of parasitoids differs immensely between families, genera, and in some cases strains of the same species (Jervis & Kidd, 1986; Jervis *et al.*, 2008; Mayhew, 2016; Harvey *et al.*, 2017). Factors as basic as different mouth structures may enable access to flowers by some species but not others (Jervis *et al.*, 1996; Jervis, 1998; Wäckers, 2005; Vattala *et al.*, 2006). Host-specific parasitoids may be far less likely to migrate in and out of fields that have available hosts, as the trade-off between feeding and host-searching may not be worthwhile (Bernstein & Jervis, 1996; Jamont, *et al.*, 2014). Importantly, in the field study (Chapter 3), many of the parasitoids recovered from leek moth larvae and pupae were generalists which are known to attack a wide range of lepidopteran hosts as well as several other non-lepidopteran orders as is the case with *C. albifrons*, and *Scambus* sp. (*C. albifrons*: Peck, 1963; Miall *et al.*, 2014; *Scambus* spp.: Gauld, 1991). The different host-searching behaviour of these generalists may mean that they are more affected (either positively or negatively) by a plentiful and nearby source of nectar. These normally otherwise “wanderer” species may decide to remain in or near the resource-enriched fields, and seek out leek moth (if present and suitable) rather than taking on the risk of finding additional hosts somewhere less suitable. Lastly, all three pupal ‘generalists’ found in the field study were known to be facultative hyperparasitoids (*C. albifrons*: Arthur, 1958; Peck, 1963; Miall *et al.*, 2014; *Scambus* sp.: Gauld, 1991;

Gomez & Yabar-Landa, 2015; *Itopectis* spp.: Ueno, 1997). Under the assumption that these species are capable of developing in both unparasitized (*C. albifrons*: confirmed by Miall *et al.*, 2014; *Scambus* sp. & *Itopectis* sp.: confirmed by PM, unpublished data) and *D. pulchellus*-parasitized hosts (*C. albifrons*: confirmed by Miall *et al.*, 2014), the window of opportunity for these parasitoids to feed, host-search, and attack is significantly longer than that of *D. pulchellus* (JM, unpublished data), as observed by Cusumano *et al.*, (2013) in a different facultative hyperparasitoid/primary parasitoid system. In this leek moth parasitoid community dominated by facultative hyperparasitoids, the “primary parasitoid-only” life history of *D. pulchellus* thus puts it at a clear disadvantage in terms of competitive ability and the time window of host availability.

General potential and limits for combining classical and conservation biological control strategies

As presented in Chapter 1, a variety of reasons can be given for why a classical BCA does not establish or provide an acceptable level of pest suppression. Some of these include easily-addressed issues such as unfavourable weather conditions at the time of release, poor timing with susceptible host stage in the field, and poor choice of release location (e.g. low or undetectable pest levels) (Stiling, 1993). Others such as inability to survive or thrive in the area of introduction (e.g., failure to find a suitable overwintering refuge), poor host-BCA synchronicity, or failure of the BCA to detect the host, may be hurdles that are insurmountable to climb in order for an agent to succeed (Stiling, 1993

and references therein). Practising due diligence to get past these roadblocks and provide the conditions favourable and necessary for a BCA to establish and achieve desired pest suppression (e.g., applying CBC strategies), is more likely to lead to a success. However, a series of unknowns will always exist when it comes to making concrete predictions about the outcomes of a biological control program, especially when the indigenous natural enemy community is in a constant state of ecological and evolutionary flux. Understanding the basic biology of the pest, and the natural enemy community either already present or likely to migrate into a system, is essential if the addition of nectar resource with the goal of enhancing biological control of a pest is being considered (Winkler *et al.*, 2004; see Wäckers *et al.*, 2007). This is especially true in the case of parasitoid wasps, as differing life-history traits between species may result in drastically different responses to supplementary food and refuges (Jervis & Kidd, 1995). Parasitoids within the same guild or feeding niche might demonstrate similar across-guild responses to the resource, thus making targeted enhancement of a particular guild easier to accomplish once the best nectar resource has been determined. Sugar-deprived agricultural systems may benefit the most from the addition of nectar resources and refuges, if the goal is to increase the abundance and maximize the pest-suppression capabilities of all natural enemies in that system. However, if the sole initial goal is to improve the chances of pest suppression by a host-specific BCA, then minimizing the effort to recruit competing natural enemies into the system might be the best approach. Beyond looking at the life-history strategies of an individual or group of natural enemies, further consideration should be given to the potential benefits provided to the pest by

supplementary resources. These could be as simple as the pest using the selected resource as an alternative or additional food source (Baggen & Gurr, 1998; Baggen *et al.*, 1999; Winkler *et al.*, 2009b), or as complex as the host-finding ability of a parasitoid being hindered or prevented by the additional resource in or in proximity to the field (Sheehan, 1986; Andow, 1991). Other conventional and/or cultural practices may hinder the establishment or efficacy of an introduced BCA, regardless of additional resources being present in the system. For example, farm operations that use row cover as a means of protecting the crop from pests would drastically alter the ability of a BCA to access the host, even if the host was present in the crop. Other practices such as poor timing of tilling (e.g., when parasitoid cocoons are still present in the soil), and poorly-planned or misguided pesticide applications, could significantly impact BCA survivorship, and hinder establishment. It is important to consider these factors and their interactions when evaluating the success or failure of a classical or CBC project. Lastly, there are likely yield-related trade-offs occurring when floral subsidies are introduced into a field, occupying arable land that could otherwise be used for crop production. As a result, measures of crop yield in a CBC-implemented system as an indicator of pest suppression, may not actually be representative of the level of control that is occurring in the field (Sastawa *et al.*, 2004; Fisher *et al.*, 2008; see Poveda *et al.*, 2008).

Future research

Chapters 2 and 3 provided some insight into how some parasitoids are affected by the presence (or absence) of sugar sources in the leek moth system. Chapter 2 discussed how

the provisioning of sugars impacted the lifespan and parasitism levels of an introduced primary parasitoid of the leek moth, and a native facultative hyperparasitoid acting as a primary parasitoid of the leek moth. Chapter 3 explored how the addition of buckwheat to the leek moth system influenced the associated parasitoid community and overall mortality levels of leek moth with and without the addition of buckwheat. Based on the consistency of the field study results across two years and comparisons with previously collected data (Mason *et al.*, 2013a; PGM unpublished data, 2003-2016), it is clear that the parasitoid community in this system is still changing, potentially due in part to resource availability and landscape complexity as is shown in Figure 21, which depicts the dynamic food web associated with the leek moth and its parasitoid community as was determined in Chapter 4. It remains unknown to what extent these parasitoids will impact leek moth populations if at some point the community becomes more static. However, the presence of these indigenous parasitoids does not necessarily mean that there is no hope for *D. pulchellus* establishing and impacting the system as part of the community. Although present, its proportional representation in terms of leek moth parasitism is seemingly very low. This situation presents a good opportunity to continue to seek out what CBC and conventional strategies and techniques can be implemented to make conditions more favourable for *D. pulchellus* in a dynamic system (see Keane *et al.*, 2003). When leek moth was first detected, initial assessment of the impact of indigenous natural enemies on the pest was likely not indicative of the overall level of suppression that might be achieved once the parasitoid community had time to adapt to the novel host. In this case, earlier introductions of *D. pulchellus* might have favoured its

establishment as it would likely have been more entrenched in the system prior to the adaptation of indigenous parasitoids, potentially offering it a competitive advantage. It is important to consider that, when assessing a BCAs potential for suppressing a pest, the level of complexity that already exists in the indigenous parasitoid community, might actually correspond with the likelihood of failure of the agent. Releasing a BCA into well-established systems (e.g., diamondback moth, *Plutella xylostella* [L.] [Lepidoptera: Plutellidae] and its parasitoids) may lessen the likelihood of its establishment as the availability of an unoccupied niche may simply not exist. This study is another reminder that biological control introductions, and management techniques to enhance them, do not happen in an ecological vacuum: they exist in the context of complex and dynamic communities that change over time.

Figures

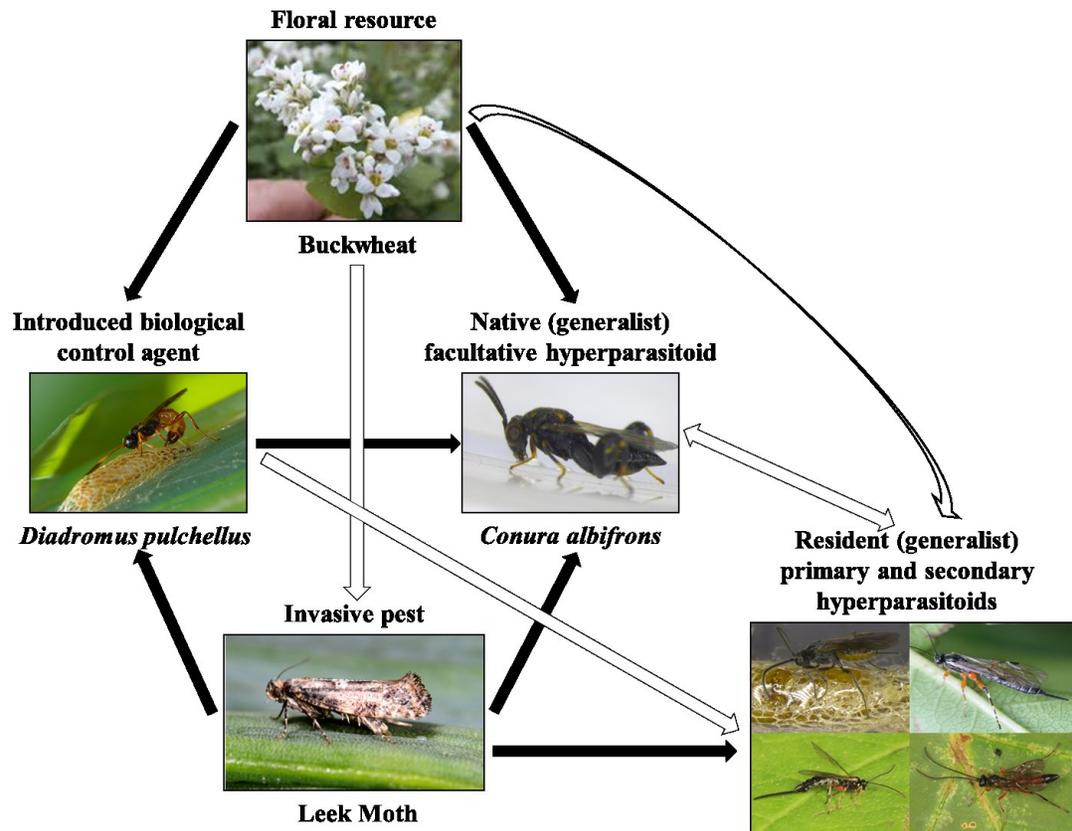


Figure 21. Current food web as observed in the leek moth system from Chapter 3 field experiments. Solid lines represent known relationships between hosts (food resources) and consumers (herbivores and predators); hollow lines represent potential but currently unknown relationships between food resources and consumers.

References

- Abram, P. K., Brodeur, J., Burte, V., & Boivin, G. (2016). Parasitoid-induced host egg abortion: an underappreciated component of biological control services provided by egg parasitoids. *Biological Control*, **98**, 52–60.
- Acikgoz, E., & Rum-Baugh, M. D. (1979). Variation in seed and seedling traits of common vetch (*Vicia sativa* L.). *Canadian Journal of Plant Science*, **59**, 511–513.
- Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, **26**, 32–46.
- Andow, D. A. (1991). Vegetational diversity and arthropod population response. *Annual Review of Entomology*, **36**, 561–586.
- Araj, S. A., Wratten, S. D., Lister, A. J., & Buckley, H. L. (2006). Floral nectar affects longevity of the aphid parasitoid *Aphidius ervi* and its hyperparasitoid *Dendrocerus aphidum*. *New Zealand Plant Protection*, **59**, 178–183.
- Araj, S. E., Wratten, S., Lister, A., & Buckley, H. (2008). Floral diversity, parasitoids and hyperparasitoids—A laboratory approach. *Basic and Applied Ecology*, **9**, 588–597.
- Araj, S. E., Wratten, S., Lister, A., & Buckley, H. (2009). Adding floral nectar resources to improve biological control: Potential pitfalls of the fourth trophic level. *Basic and Applied Ecology*, **10**, 554–562.
- Araj, S. E., Wratten, S., Lister, A., Buckley, H., & Ghabeish, I. (2011). Searching behavior of an aphid parasitoid and its hyperparasitoid with and without floral nectar. *Biological Control*, **57**, 79–84.
- Arthur, A. P. (1958). Development, behaviour, and descriptions of immature stages of *Spilochalcis* side (Walk.) (Hymenoptera: Chalcididae). *The Canadian Entomologist*, **90**, 590–595.
- Baggen, L. R., & Gurr, G. M. (1998). The influence of food on *Copidosoma koehleri* (Hymenoptera: Encyrtidae), and the use of flowering plants as a habitat management tool to enhance biological control of potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Biological Control*, **11**, 9–17.
- Baggen, L.R., Gurr, G.M., & Meats, A. (1999). Flowers in tri-trophic systems: mechanisms allowing selective exploitation by insect natural enemies for conservation biological control. *Entomologia Experimentalis et Applicata*. **91**, 155–161.

- Balzan, M. V., & Wäckers, F. L. (2013). Flowers to selectively enhance the fitness of a host-feeding parasitoid: adult feeding by *Tuta absoluta* and its parasitoid *Necremnus artynes*. *Biological Control*, **67**, 21–31.
- Banks, J. E., Bommarco, R., & Ekbom, B. (2008). Population response to resource separation in conservation biological control. *Biological Control*, **47**, 141–146.
- Barbosa, P., & Benrey, B. (1998). The influence of plants on insect parasitoids: implications for conservation biological control. Pp. 55–82 In: Barbosa, P. A. (Ed.) *Conservation biological control*. Academic Press, Cambridge, Massachusetts, United States.
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1–48. doi:10.18637/jss.v067.i01.
- Bekkaoui, A., & Thibout, E. (1993). Role of the cocoon of *Acrolepiopsis assectella* (Lep., Hyponomeutoidae) in host recognition by the parasitoid *Diadromus pulchellus* (Hym., Ichneumonidae). *BioControl*, **38**, 101–113.
- Bellows Jr, T. S., Van Driesche, R. G., & Elkinton, J. S. (1992). Life-table construction and analysis in the evaluation of natural enemies. *Annual Review of Entomology*, **37**, 587–612.
- Bellows Jr, T.S. & Van Driesche, R.G. (1999). Life table construction and analysis for evaluating biological control agents. Pp. 199–223 In: Fisher, T. W., Bellows Jr., T.S., Caltagirone, L. E., Dahlsten, D. L., Huffaker, C. B., & Gordh, G. (Eds.) *Handbook of biological control: principles and applications of biological control*. Academic Press, New York, New York.
- Berndt, L. A., Wratten, S. D., & Hassan, P. G. (2002). Effects of buckwheat flowers on leafroller (Lepidoptera: Tortricidae) parasitoids in a New Zealand vineyard. *Agricultural and Forest Entomology*, **4**, 39–45.
- Berndt, L. A., & Wratten, S. D. (2005). Effects of alyssum flowers on the longevity, fecundity, and sex ratio of the leafroller parasitoid *Dolichogenidea tasmanica*. *Biological Control*, **32**, 65–69.
- Berndt, L. A., Wratten, S. D., & Scarratt, S. L. (2006). The influence of floral resource subsidies on parasitism rates of leafrollers (Lepidoptera: Tortricidae) in New Zealand vineyards. *Biological Control*, **37**, 50–55.
- Bernstein, C., & Jervis, M. (2008). Food-searching in parasitoids: the dilemma of choosing between ‘immediate’ or future fitness gains. Pp. 129–171 In: Wajnberg,

- E., Bernstein, C., & Van Alphen, J. (Eds.) *Behavioral ecology of insect parasitoids: from theoretical approaches to field applications*. Blackwell Publishing, Malden, Massachusetts, United States.
- Boettner, G. H., Elkinton, J. S., & Boettner, C. J. (2000). Effects of a biological control introduction on three nontarget native species of saturniid moths. *Conservation Biology*, **14**, 1798–1806.
- Boivin, G., & Brodeur, J. (2006). Intra-and interspecific interactions among parasitoids: mechanisms, outcomes and biological control. Pp. 123-144 In: Brodeur, J., & Boivin, G. (Eds.) *Trophic and guild interactions in biological control*. Springer, New York, New York, United States.
- Bowie, M. H., Wratten, S. D., & White, A. J. (1995). Agronomy and phenology of “companion plants” of potential for enhancement of insect biological control. *New Zealand Journal of Crop and Horticultural Science*, **23**, 423–427.
- Brodeur, J. (2000). Host specificity and trophic relationships of hyperparasitoids. Pp. 163-183 In: Hochberg, M. E., & Ives, A. R. (Eds.) *Parasitoid population biology*. Princeton University Press, Princeton, New Jersey, United States.
- Brodeur, J., & Boivin, G. (2006). Preface. Pp. ix–x In: Brodeur, J., & Boivin, G. (Eds.) *Trophic and guild interactions in biological control*. Springer, New York, New York, United States.
- Bulgarella, M., Quiroga, M. A., Boulton, R. A., Ramírez, I. E., Moon, R. D., Causton, C. E., & Heimpel, G. E. (2017). Life cycle and host specificity of the parasitoid *Conura annulifera* (Hymenoptera: Chalcididae), a potential biological control agent of *Philornis downsi* (Diptera: Muscidae) in the Galápagos Islands. *Annals of the Entomological Society of America*, **110**, 317–328.
- Burks, B. D. (1940). Revision of the Chalcid-Flies of the Tribe Chalcidini in America north of Mexico. *Proceedings of the United States National Museum*, **88**, 237–354.
- Brotodjojo, R. R., & Walter, G. H. (2006). Oviposition and reproductive performance of a generalist parasitoid (*Trichogramma pretiosum*) exposed to host species that differ in their physical characteristics. *Biological Control*, **39**, 300–312.
- Cabello, T., Gallego, J. R., Fernandez, F. J., Gamez, M., Vila, E., Del Pino, M., & Hernandez-Suarez, E. (2012). Biological control strategies for the South American tomato moth (Lepidoptera: Gelechiidae) in greenhouse tomatoes. *Journal of Economic Entomology*, **105**, 2085–2096.

- Cappuccino, N., Houle, M. J., & Stein, J. (1999). The influence of understory nectar resources on parasitism of the spruce budworm *Choristoneura fumiferana* in the field. *Agricultural and Forest Entomology*, **1**, 33–36.
- Cardinale, B. J., Harvey, C. T., Gross, K., & Ives, A. R. (2003). Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecology Letters*, **6**, 857–865.
- Cardona, E. V. (1997). *Integrated pest management of the diamondback moth (Plutella xylostella L.) in cabbage in the Philippines: biological control with the use of the parasitoid Diadegma semiclausum Hellen*, Doctoral dissertation, Ghent University, Ghent, Belgium.
- Carey, J. R. (1993). *Applied demography for biologists: with special emphasis on insects*. Oxford University Press, New York, New York, United States.
- Cawoy, V., Deblauwe, V., Halbrechq, B., Ledent, J. F., Kinet, J. M., & Jacquemart, A. L. (2006). Morph differences and honeybee morph preference in the distylous species *Fagopyrum esculentum* Moench. *International Journal of Plant Sciences*, **167**, 853–861.
- Cawoy, V., Ledent, J. F., Kinet, J. M., & Jacquemart, A. L. (2009). Floral biology of common buckwheat (*Fagopyrum esculentum* Moench). *The European Journal of Plant Science and Biotechnology*, **3**, 1–9.
- Cock, M. J., Murphy, S. T., Kairo, M. T., Thompson, E., Murphy, R. J., & Francis, A. W. (2016). Trends in the classical biological control of insect pests by insects: an update of the BIOCAT database. *BioControl*, **61**, 349–363.
- Coombs, I., & Hall, K.E. (1998). *Dictionary of biological control and integrated pest management*. 2nd ed. CPL Press, Newbury, United Kingdom.
- Costamagna, A. C., Landis, D. A., & Difonzo, C. D. (2007). Suppression of soybean aphid by generalist predators results in a trophic cascade in soybeans. *Ecological Applications*, **17**, 441–451.
- Culver, J. J. (1919). *A study of Compsilura concinnata, an imported tachinid parasite of the gipsy moth and the brown-tail moth* (No. 766). US Department of Agriculture.
- Cusumano, A., Peri, E., Amodeo, V., McNeil, J. N., & Colazza, S. (2013). Intraguild interactions between egg parasitoids: window of opportunity and fitness costs for a facultative hyperparasitoid. *PloS One*, **8**, e64768.

- Daane, K. M., Wang, X., Duerr, S. S., Kuhn, E. J., Son, Y., & Yokota, G. Y. (2013). Biology of *Habrobracon gelechiae* (Hymenoptera: Braconidae), as a parasitoid of the obliquebanded leafroller (Lepidoptera: Tortricidae). *Environmental Entomology*, **42**, 107–115.
- Dalin, P., Kindvall, O., & Björkman, C. (2009). Reduced population control of an insect pest in managed willow monocultures. *PLoS One*, **4**, e5487.
- Deevey Jr, E. S. (1947). Life tables for natural populations of animals. *The Quarterly Review of Biology*, **22**, 283–314.
- Desouhant, E., Lucchetta, P., Giron, D., & Bernstein, C. (2010). Feeding activity pattern in a parasitic wasp when foraging in the field. *Ecological Research*, **25**, 419–428.
- Dyer, L. E., & Landis, D. A. (1996). Effects of habitat, temperature, and sugar availability on longevity of *Eriborus terebrans* (Hymenoptera: Ichneumonidae). *Environmental Entomology*, **25**, 1192–1201.
- Eilenberg, J., Enkegaard, A., Vestergaard, S., & Jensen, B. (2000). Biocontrol of pests on plant crops in Denmark: present status and future potential. *Biocontrol Science and Technology*, **10**, 703–716.
- Eilenberg, J., Hajek, A., & Lomer, C. (2001). Suggestions for unifying the terminology in biological control. *BioControl*, **46**, 387–400.
- Elkinton, J. S., Buonaccorsi, J. P., Bellows, T. S., & Van Driesche, R. G. (1992). Marginal attack rate, k-values and density dependence in the analysis of contemporaneous mortality factors. *Researches on Population Ecology*, **34**, 29–44.
- Fischer, J., Brosi, B., Daily, G. C., Ehrlich, P. R., Goldman, R., Goldstein, J., Lindenmayer, D.B., Manning, A.D., Mooney, H.A., Pejchar, L., Ranganathan, J., & Tallis, H. (2008). Should agricultural policies encourage land sparing or wildlife-friendly farming? *Frontiers in Ecology and the Environment*, **6**, 380–385.
- Foster, M. A., & Ruesink, W. G. (1984). Influence of flowering weeds associated with reduced tillage in corn on a black cutworm (Lepidoptera: Noctuidae) parasitoid, *Meteorus rubens* (Nees von Esenbeck). *Environmental Entomology*, **13**, 664–668.
- Fox, J. & Weisberg, S. (2011). *An {R} Companion to Applied Regression, Second Edition*. Sage Publications, Thousand Oaks, California, United States.

- Gaines, D. N. (1997). *Studies on Conura torvina (Hymenoptera: Chalcididae) Reproduction and Biology in Relation to Hosts in Brassica Crops*, Doctoral dissertation, Virginia Tech, Blacksburg, Virginia, United States.
- Gaines, D. N., & Kok, L. T. (1999). Impact of Hyperparasitoids on *Cotesia glomerata* in Southwestern Virginia. *Biological Control*, **14**, 19–28.
- Gardiner, M. M., Landis, D. A., Gratton, C., DiFonzo, C. D., O'neal, M., Chacon, J. M., Wayo, M.T., Schmidt, N.P., Mueller, E.E., & Heimpel, G. E. (2009). Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. *Ecological Applications*, **19**, 143–154.
- Gauld, I. D. (1991). The Ichneumonidae of Costa Rica, 1. *Memoirs of the Entomological Institute*, **47**, 1–589.
- Gauthier, N., Mandon, N., Renault, S., & Bénédet, F. (2004). The *Acrolepiopsis assectella* silk cocoon: kairomonal function and chemical characterisation. *Journal of Insect Physiology*, **50**, 1065–1074.
- Géneau, C. E., Wäckers, F. L., Luka, H., Daniel, C., & Balmer, O. (2012). Selective flowers to enhance biological control of cabbage pests by parasitoids. *Basic and Applied Ecology*, **13**, 85–93.
- Gholizadeh, A., Kamali, K., Fathipour, Y., & Abbasipour, H. (2010). Life table of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on five cultivated brassicaceous host plants. *Journal of Agricultural Science and Technology*, **11**, 115–124.
- Gillespie, M. A., Gurr, G. M., & Wratten, S. D. (2016). Beyond nectar provision: the other resource requirements of parasitoid biological control agents. *Entomologia Experimentalis et Applicata*, **159**, 207–221.
- Godfray, H. C. J. (1990). The causes and consequences of constrained sex allocation in haplodiploid animals. *Journal of Evolutionary Biology*, **3**, 3–17.
- Godfray, H. C. J. (1994). *Parasitoids: behavioral and evolutionary ecology*. Princeton University Press, Princeton, New Jersey, United States.
- Gomez, I. C., & Yabar-Landa, E. (2015). Description of the first species of *Scambus* Hartig (Hymenoptera, Ichneumonidae) from Peru, with a key to the Neotropical species. *Zootaxa*, **3956**, 437–443.
- Gontijo, L. M., Beers, E. H., & Snyder, W. E. (2013). Flowers promote aphid suppression in apple orchards. *Biological Control*, **66**, 8–15.

- Google Inc (2017). Google Earth Pro V7.3.0.3832. Downloaded January, 2018. Available from www.google.com/earth/download/gep/agree.html.
- Google^{CA} (2018). Google Earth for Chrome. Accessed January, 2018. Available from <https://earth.google.com/web>.
- Gould, J. R., Bellows, T. S., & Paine, T. D. (1992). Evaluation of biological control of *Siphoninus phillyreae* (Haliday) by the parasitoid *Encarsia partenopea* (Walker), using life-table analysis. *Biological Control*, **2**, 257–265.
- Greathead, D. J. (1994). History of biological control. *Antenna*, **18**, 187–199.
- Gurr, G. M., & Wratten, S. D. (1999). 'Integrated biological control': A proposal for enhancing success in biological control. *International Journal of Pest Management*, **45**, 81–84.
- Gurr, G. M., Barlow, N. D., Memmott, J., Wratten, S. D., & Greathead, D. J. (2000). A history of methodological, theoretical and empirical approaches to biological control. Pp. 3–37 In: Gurr, G.M. & Wratten, S.D (Eds.) *Biological control: measures of success*. Kluwer Academic Publishers. Dordrecht, Netherlands.
- Gurr, G. M., Wratten, S. D., & Barbosa, P. (2000). Success in conservation biological control of arthropods. Pp. 105–132 In: Gurr, G.M. & Wratten, S.D (Eds.) *Biological control: measures of success*. Kluwer Academic Publishers. Dordrecht, Netherlands.
- Gurr, G. M., Wratten, S. D., & Luna, J. M. (2003). Multi-function agricultural biodiversity: pest management and other benefits. *Basic and Applied Ecology*, **4**, 107–116.
- Gurr, G. M., Lu, Z., Zheng, X., Xu, H., Zhu, P., Chen, G., Yao, X., Cheng, J., Zhu, Z., Catindig, J.L., Villareal, S., Chien, H.V., Cuong, L.Q., Channoo, C., Chengwattana, N., Lan, L.P., Hai, L.H., Chaiwaong, J., Nicol, H.I., Perovic, D.J., Wratten, S.D., & Heong, K.L. (2016). Multi-country evidence that crop diversification promotes ecological intensification of agriculture. *Nature Plants*, **2**, 16014.
- Hajek, A. E. (2004). *Natural enemies: an introduction to biological control*. Cambridge University Press, Cambridge, United Kingdom.
- Hansen, J. D. (1980). The life history and behavior of *Spilochalcis albifrons* (Hymenoptera: Chalcididae), a parasite of the larch casebearer, *Coleophora*

- laricella* (Lepidoptera: Coleophoridae). *Journal of the Kansas Entomological Society*, **53**, 553–566.
- Harcourt, D. G. (1969). The development and use of life tables in the study of natural insect populations. *Annual Review of Entomology*, **14**, 175–196.
- Hatt, S., Mouchon, P., Lopes, T., & Francis, F. (2017). Effects of wildflower strips and an adjacent forest on aphids and their natural enemies in a pea field. *Insects*, **8**, 99.
- Heimpel, G. E., Rosenheim, J. A., & Kattari, D. (1997). Adult feeding and lifetime reproductive success in the parasitoid *Aphytis melinus*. *Entomologia Experimentalis et Applicata*, **83**, 305–315.
- Heimpel, G. E., & Jervis, M. A. (2005). Does floral nectar improve biological control by parasitoids. Pp. 267–304 In: Wäckers, F. L. van Rijn, C. J. & Bruin, J. (Eds.) *Plant-provided food for carnivorous insects: a protective mutualism and its applications*. Cambridge University Press, Cambridge, United Kingdom.
- Heneidak, S., & Hassan, A. E. (2007). Morphological and anatomical studies of floral and extrafloral nectaries in some *Vicia* taxa (Fabaceae). *International Journal of Botany*, **3**, 329–341.
- Heinz, K. M., & Parrella, M. P. (1990). The influence of host size on sex ratios in the parasitoid *Diglyphus begini* (Hymenoptera: Eulophidae). *Ecological Entomology*, **15**, 391–399.
- Hokkanen, H. M. (1985). Success in classical biological control. *Critical Reviews in Plant Sciences*, **3**, 35–72.
- Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.
- Idris, A. B., & Grafius, E. (1995). Wildflowers as nectar sources for *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera: Yponomeutidae). *Environmental Entomology*, **24**, 1726–1735.
- Irvin, N. A., Hoddle, M.S. & Castle, S. J. (2007). The effect of resource provisioning and sugar composition of foods on longevity of three *Gonatocerus* spp., egg parasitoids of *Homalodisca vitripennis*. *Biological Control*, **40**, 69–79.
- Irvin, N. A., Pinckard, T. R., Perring, T. M., & Hoddle, M. S. (2014). Evaluating the potential of buckwheat and cahaba vetch as nectar producing cover crops for enhancing biological control of *Homalodisca vitripennis* in California vineyards. *Biological Control*, **76**, 10–8.

- Irvin, N. A., and Hoddle, M.S.. (2015). The effect of buckwheat flowers and cahaba vetch extrafloral nectaries on fitness of the vine mealybug parasitoid *Anagyrus pseudococci* (Hymenoptera: Encyrtidae). *Florida Entomologist*, **98**, 237–242.
- Jamont, Marie, Solène Crépellière, and Bruno Jaloux. (2013). Effect of extrafloral nectar provisioning on the performance of the adult parasitoid *Diaeretiella rapae*. *Biological Control*, **65**, 271–277.
- Jamont, M., Dubois-Pot, C., & Jaloux, B. (2014). Nectar provisioning close to host patches increases parasitoid recruitment, retention and host parasitism. *Basic and Applied Ecology*, **15**, 151–160.
- Jenner, W. H., Kuhlmann, U., Mason, P. G., & Cappuccino, N. (2010). Comparative life tables of leek moth, *Acrolepiopsis assectella* (Zeller) (Lepidoptera: Acrolepiidae), in its native range. *Bulletin of Entomological Research*, **100**, 87–97.
- Jenner, W.H., Cappuccino, N., Kuhlmann, U. and Mason, P.G. (2012). Manipulation of parasitoid state influences host exploitation by *Diadromus pulchellus* Wesmael (Hymenoptera: Ichneumonidae). *Biological Control*, **63**, 264–269.
- Jenner, W. H., Kuhlmann, U., Miall, J. H., Cappuccino, N., & Mason, P. G. (2014). Does parasitoid state affect host range expression? *Biological Control*, **78**, 15–22.
- Jervis, M. A., and N. A. C. Kidd. (1986). Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews*, **61**, 395–434.
- Jervis, M. A. (1990). Predation of *Lissonota coracinus* (Gmelin) (Hymenoptera: Ichneumonidae) by *Dolichonabis limbatus* (Dahlbom) (Hemiptera: Nabidae). *Entomologist's Gazette*, **41**, 231–233.
- Jervis, M. A., Kidd, N. A. C., Fitton, M. G., Huddleston, T., & Dawah, H. A. (1993). Flower-visiting by hymenopteran parasitoids. *Journal of Natural History*, **27**, 67–105.
- Jervis, M., & Kidd, N. (1995). Incorporating physiological realism into models of parasitoid feeding behaviour. *Trends in Ecology & Evolution*, **10**, 434–436.
- Jervis, M. A., N. A. C. Kidd, and G. E. Heimpel. (1996). Parasitoid adult feeding behaviour and biological control—a review. *Biocontrol News and Information*, **17**, 11N–26N.
- Jervis, M. (1998). Functional and evolutionary aspects of mouthpart structure in parasitoid wasps. *Biological Journal of the Linnean Society*, **63**, 461–493.

- Jervis, M. A., Heimpel, G. E., Ferns, P. N., Harvey, J. A., & Kidd, N. A. (2001). Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. *Journal of Animal Ecology*, **70**, 442–458.
- Jonsson, M., Wratten, S. D., Robinson, K. A., & Sam, S. A. (2009). The impact of floral resources and omnivory on a four trophic level food web. *Bulletin of Entomological Research*, **99**, 275–285.
- Jonsson, M., Straub, C. S., Didham, R. K., Buckley, H. L., Case, B. S., Hale, R. J., Gratton, C., & Wratten, S. D. (2015). Experimental evidence that the effectiveness of conservation biological control depends on landscape complexity. *Journal of Applied Ecology*, **52**, 1274–1282.
- Kuhlmann, U., Carl, K. P., & Mills, N. J. (1998). Quantifying the impact of insect predators and parasitoids on populations of the apple ermine moth, *Yponomeuta malinellus* (Lepidoptera: Yponomeutidae), in Europe. *Bulletin of Entomological Research*, **88**, 165–175.
- Kean, J., Wratten, S., Tylianakis, J., & Barlow, N. (2003). The population consequences of natural enemy enhancement, and implications for conservation biological control. *Ecology Letters*, **6**, 604–612.
- Koptur, S. (1979). Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. *American Journal of Botany*, **66**, 1016–1020.
- Koptur, S. (2005). Nectar as fuel for plant protectors. Pp. 75-108 In: Wäckers, F. L. van Rijn, C. J. & Bruin, J. (Eds.) *Plant-provided food for carnivorous insects: a protective mutualism and its applications*. Cambridge University Press, Cambridge, United Kingdom.
- Labeyrie, V. (1960). Contribution to the study of insect population dynamics: 1. Stimulating influence of the host, *A. assectella*, on the multiplication of an Ichneumonid wasp (*Diadromus* sp.), Doctoral dissertation, L' University de Paris, Paris, France.
- Landis, D. A., Wratten, S. D., & Gurr, G. M. (2000). Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology*, **45**, 175–201.
- Landry, J. F. (2007). Taxonomic review of the leek moth genus *Acrolepiopsis* (Lepidoptera: Acrolepiidae) in North America. *The Canadian Entomologist*, **139**, 319–353.

- Langellotto, G. A., & Denno, R. F. (2004). Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. *Oecologia*, **139**, 1–10.
- Lavandero, B., Wratten, S., Shishehbor, P., & Worner, S. (2005). Enhancing the effectiveness of the parasitoid *Diadegma semiclausum* (Helen): movement after use of nectar in the field. *Biological Control*, **34**, 152–158.
- Lavandero, B., Wratten, S. D., Didham, R. K., & Gurr, G. (2006). Increasing floral diversity for selective enhancement of biological control agents: a double-edged sword? *Basic and Applied Ecology*, **7**, 236–243.
- Lee, J. C., Menalled, F. D., & Landis, D. A. (2001). Refuge habitats modify impact of insecticide disturbance on carabid beetle communities. *Journal of Applied Ecology*, **38**, 472–483.
- Lee, J. C., Heimpel, G. E., & Leibe, G. L. (2004). Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp. *Entomologia Experimentalis et Applicata*, **111**, 189–199.
- Lee, J. C., & Heimpel, G. E. (2008). Floral resources impact longevity and oviposition rate of a parasitoid in the field. *Journal of Animal Ecology*, **77**, 565–572.
- Leius, K. (1961a). Influence of food on fecundity and longevity of adults of *Itopectis conquisitor* (Say) (Hymenoptera: Ichneumonidae). *The Canadian Entomologist*, **93**, 771–780.
- Leius, K. (1961b). Influence of various foods on fecundity and longevity of adults of *Scambus buolianae* (Htg.) (Hymenoptera: Ichneumonidae). *The Canadian Entomologist*, **93**, 1079–1084.
- Leius, K. (1967). Influence of wild flowers on parasitism of tent caterpillar and codling moth. *The Canadian Entomologist*, **99**, 444–446.
- Letourneau, D. K., Armbricht, I., Rivera, B. S., Lerma, J. M., Carmona, E. J., Daza, M. C., Escobar, S., Galindo, V., Gutiérrez, C., López, S.D., Mejía, J.L., Rangel, A.M.C., Rangel, J.H., Rivera, L., Saavedra, C.A., Torres, A.M., Trujillo, A.R. (2011). Does plant diversity benefit agroecosystems? A synthetic review. *Ecological Applications*, **21**, 9–21.
- Lewis, W. J., Stapel, J. O., Cortesero, A. M., & Takasu, K. (1998). Understanding how parasitoids balance food and host needs: importance to biological control. *Biological Control*, **11**, 175–183.

- Liu, Z., Li, D., Gong, P., & Wu, K. (2004). Life table studies of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), on different host plants. *Environmental Entomology*, **33**, 1570–1576.
- Liu, W. X., Wang, W. X., Zhang, Y. B., Wang, W., Lu, S. L., & Wan, F. H. (2015). Adult diet affects the life history and host-killing behavior of a host-feeding parasitoid. *Biological Control*, **81**, 58–64.
- Majerus, M. E. N., Geoghegan, I. E. & Majerus, T. M. O. (2000). Adaptive preferential selection of young coccinellid hosts by the parasitoid wasp *Dinocampus coccinellae* (Hymenoptera: Braconidae). *European Journal of Entomology*, **93**, 161–164.
- Mansfield, S., & Mills, N. J. (2002). Host egg characteristics, physiological host range, and parasitism following inundative releases of *Trichogramma platneri* (Hymenoptera: Trichogrammatidae) in walnut orchards. *Environmental Entomology*, **31**, 723–731.
- Mason, P. G., Appleby, M., Juneja, S., Allen, J., & Landry, J. F. (2010). Biology and development of *Acrolepiopsis assectella* (Lepidoptera: Acrolepiidae) in eastern Ontario. *The Canadian Entomologist*, **142**, 393–404.
- Mason, P. G., Weiss, R. M., Olfert, O., Appleby, M., & Landry, J. F. (2011). Actual and potential distribution of *Acrolepiopsis assectella* (Lepidoptera: Acrolepiidae), an invasive alien pest of *Allium* spp. in Canada. *The Canadian Entomologist*, **143**, 185–196.
- Mason, P. G., Brauner, A. M., Miall, J. H., & Bennett, A. M. R. (2013a). *Diadromus pulchellus* in North America: field release against leek moth and new characters to distinguish it from *Diadromus subtilicornis*, a native diamondback moth parasitoid. *Biocontrol Science and Technology*, **23**, 260–276.
- Mason, P. G., Jenner, W. H., Brauner, A., Kuhlmann, U., & Cappuccino, N. (2013b). *Acrolepiopsis assectella* (Zeller), leek moth (Lepidoptera: Acrolepiidae). Pp. 56–62 In: Mason, P. G., & Gillespie, D. R. (Eds.) *Biological control programmes in Canada 2001–2012*. CABI Publishing, Croydon, United Kingdom.
- May, R. M., & Hassell, M. P. (1981). The dynamics of multiparasitoid-host interactions. *The American Naturalist*, **117**, 234–261.
- Mayhew, P. J. (2016). Comparing parasitoid life histories. *Entomologia Experimentalis et Applicata*, **159**, 147–162.

- Meyhöfer, R., & Klug, T. (2002). Intraguild predation on the aphid parasitoid *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Aphidiidae): mortality risks and behavioral decisions made under the threats of predation. *Biological Control*, **25**, 239–248.
- Miall, J. H., Abram, P. K., Cappuccino, N., & Mason, P. G. (2014). Potential impact of the native hyperparasitoid *Conura albifrons* (Hymenoptera: Chalcididae) on the exotic biological control agent *Diadromus pulchellus* (Hymenoptera: Ichneumonidae). *Biocontrol Science and Technology*, **24**, 611–624.
- Miranda, M. M. M., Picanço, M., Zanuncio, J. C., & Guedes, R. N. C. (1998). Ecological life table of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Biocontrol Science and Technology*, **8**, 597–606.
- Morris, R. F., & Miller, C. A. (1954). The development of life tables for the spruce budworm. *Canadian Journal of Zoology*, **32**, 283–301.
- Müller, C. B., & Brodeur, J. (2002). Intraguild predation in biological control and conservation biology. *Biological Control*, **25**, 216–223.
- Nachman, G. (2006). A functional response model of a predator population foraging in a patchy habitat. *Journal of Animal Ecology*, **75**, 948–958.
- Nofemela, R. S. (2013). The effect of obligate hyperparasitoids on biological control: differential vulnerability of primary parasitoids to hyperparasitism can mitigate trophic cascades. *Biological Control*, **65**, 218–224.
- Norris, R. F., & Kogan, M. (2000). Interactions between weeds, arthropod pests, and their natural enemies in managed ecosystems. *Weed Science*, **48**, 94–158.
- Noyes, J. S. (1974). *The biology of the leek moth, Acrolepia assectella (Zeller)*, Doctoral dissertation, University of London, London, United Kingdom.
- Orr, D., & Fox, A. (2012). Augmentation and conservation of natural enemies. Pp. 58–91 In: Abrol, D. P., & Shankar, U. (Eds.) *Integrated pest management: principles and practice*. CAB International, Wallingford, United Kingdom.
- Patt, J. M., Hamilton, G. C., & Lashomb, J. H. (1997a). Impact of strip-insectary intercropping with flowers on conservation biological control of the Colorado potato beetle. *Advances in Horticultural Science*, **11**, 175–181.
- Patt, J. M., Hamilton, G. C., & Lashomb, J. H. (1997b). Foraging success of parasitoid wasps on flowers: interplay of insect morphology, floral architecture and searching behavior. *Entomologia Experimentalis et Applicata*, **83**, 21–30.

- Peck, O. (1963). A catalogue of the nearctic Chalcidoidea (Insecta: Hymenoptera). *The Memoirs of the Entomological Society of Canada*, **95**, 5–1092.
- Pedersen, B. S., & Mills, N. J. (2004). Single vs. multiple introduction in biological control: the roles of parasitoid efficiency, antagonism and niche overlap. *Journal of Applied Ecology*, **41**, 973–984.
- Pekas, A., & Wäckers, F. L. (2017). Multiple resource supplements synergistically enhance predatory mite populations. *Oecologia*, **184**, 1–6.
- Peterson, A. T. (2003). Predicting the geography of species' invasions via ecological niche modeling. *The Quarterly Review of Biology*, **78**, 419–433.
- Pfiffner, L., & Luka, H. (2000). Overwintering of arthropods in soils of arable fields and adjacent semi-natural habitats. *Agriculture, Ecosystems & Environment*, **78**, 215–222.
- Poveda, K., Gómez, M. I., & Martínez, E. (2008). Diversification practices: their effect on pest regulation and production. *Revista Colombiana de Entomología*, **34**, 131–144.
- R Core Team (2016). R: A language and environment for statistical computing. Version 3.3.2. *R Foundation for Statistical Computing*. Vienna, Austria.
- Rand, T. A., Tylianakis, J. M., & Tscharntke, T. (2006). Spillover edge effects: the dispersal of agriculturally subsidized insect natural enemies into adjacent natural habitats. *Ecology Letters*, **9**, 603–614.
- Razinger, J., Vasileiadis, V. P., Giraud, M., van Dijk, W., Modic, Š., Sattin, M., & Urek, G. (2016). On-farm evaluation of inundative biological control of *Ostrinia nubilalis* (Lepidoptera: Crambidae) by *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) in three European maize-producing regions. *Pest Management Science*, **72**, 246–254.
- Rijk, M., Yang, D., Engel, B., Dicke, M., & Poelman, E. H. (2016). Feeding guild of non-host community members affects host-foraging efficiency of a parasitic wasp. *Ecology*, **97**, 1388–1399.
- Root, R. B. (1967). The niche exploitation pattern of the blue-gray gnatcatcher. *Ecological Monographs*, **37**, 317–350.

- Root, R.B. (1973). Organization of plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecological Monographs*, **43**, 95–124.
- Rosenheim, J. A., Kaya, H. K., Ehler, L. E., Marois, J. J., & Jaffee, B. A. (1995). Intraguild predation among biological-control agents: theory and evidence. *Biological Control*, **5**, 303-335.
- Rosenheim, J. A. (1998). Higher-order predators and the regulation of insect herbivore populations. *Annual Review of Entomology*, **43**, 421–447.
- Rusch, A., Chaplin-Kramer, R., Gardiner, M. M., Hawro, V., Holland, J., Landis, D., Thies, C., Tschardtke, T., Weisser, W.W., Winqvist, C., Woltz, M., & Bommarco, R. (2016). Agricultural landscape simplification reduces natural pest control: a quantitative synthesis. *Agriculture, Ecosystems & Environment*, **221**, 198–204.
- Sastawa, B. M., Lawan, M., & Maina, Y. T. (2004). Management of insect pests of soybean: effects of sowing date and intercropping on damage and grain yield in the Nigerian Sudan savanna. *Crop Protection*, **23**, 155–161.
- Segoli, M., & Rosenheim, J. A. (2013). Spatial and temporal variation in sugar availability for insect parasitoids in agricultural fields and consequences for reproductive success. *Biological Control*, **67**, 163–169.
- Seto, M., & Shelton, A. M. (2015). Development and evaluation of degree-day models for *Acrolepiopsis assectella* (Lepidoptera: Acrolepiidae) based on hosts and flight patterns. *Journal of Economic Entomology*, **109**, 613–621.
- Sheehan, W. (1986). Response by specialist and generalist natural enemies to agroecosystem diversification: a selective review. *Environmental Entomology*, **15**, 456–461.
- Sholes, O. D. (1984). Responses of arthropods to the development of goldenrod inflorescences (Solidago: Asteraceae). *American Midland Naturalist*, **112**, 1–14.
- Siekmann, G., Tenhumberg, B., & Keller, M. A. (2001). Feeding and survival in parasitic wasps: sugar concentration and timing matter. *Oikos*, **95**(3), 425-430.
- Stamp, N. E., & Bowers, M. D. (1991). Indirect effect on survivorship of caterpillars due to presence of invertebrate predators. *Oecologia*, **88**, 325–330.
- Steidle, J. L., Rees, D., & Wright, E. J. (2001). Assessment of Australian *Trichogramma* species (Hymenoptera: Trichogrammatidae) as control agents of stored product moths. *Journal of Stored Products Research*, **37**, 263–275.

- Stephens, M. J., France, C. M., Wratten, S. D., & Frampton, C. (1998). Enhancing biological control of leafrollers (Lepidoptera: Tortricidae) by sowing buckwheat (*Fagopyrum esculentum*) in an orchard. *Biocontrol Science and Technology*, **8**, 547–558.
- Stiling, P. (1993). Why do natural enemies fail in classical biological control programs? *American Entomologist*, **39**, 31–37.
- Suarez, R. K., Darveau, C. A., Welch, K. C., O'Brien, D. M., Roubik, D. W., & Hochachka, P. W. (2005). Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. *Journal of Experimental Biology*, **208**, 3573–3579.
- Sullivan, D. J. (1987). Insect hyperparasitism. *Annual Review of Entomology*, **32**, 49–70.
- Sullivan, D. J., & Völkl, W. (1999). Hyperparasitism: multitrophic ecology and behavior. *Annual Review of Entomology*, **44**, 291–315.
- Tabone, E., Bardon, C., Desneux, N., & Wajnberg, E. (2010). Parasitism of different *Trichogramma* species and strains on *Plutella xylostella* L. on greenhouse cauliflower. *Journal of Pest Science*, **83**, 251–256.
- Tajmiri, P., Fathi, S. A. A., Golizadeh, A., & Nouri-Ganbalani, G. (2017). Strip-intercropping canola with annual alfalfa improves biological control of *Plutella xylostella* (L.) and crop yield. *International Journal of Tropical Insect Science*, **37**, 208–216.
- Tena, A., Wäckers, F. L., Heimpel, G. E., Urbaneja, A., & Pekas, A. (2016). Parasitoid nutritional ecology in a community context: the importance of honeydew and implications for biological control. *Current Opinion in Insect Science*, **14**, 100–104.
- Therneau T (2015). A Package for Survival Analysis in S. version 2.38. <https://CRAN.R-project.org/package=survival>.
- Tompkins, J. M., Wratten, S. D., & Wäckers, F. L. (2010). Nectar to improve parasitoid fitness in biological control: Does the sucrose: hexose ratio matter? *Basic and Applied Ecology*, **11**, 264–271.
- Torre-Bueno, J. R., Tulloch, G. S., & Schuh, R. T. (1989). *The Torre-Bueno glossary of entomology*. New York Entomological Society, New York, New York, United States.

- Tscharntke, T., Klein, A. M., Kruess, A., Steffan-Dewenter, I., & Thies, C. (2005). Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. *Ecology Letters*, **8**, 857–874.
- Tscharntke, T., Bommarco, R., Clough, Y., Crist, T.O., Kleijn, D., Rand, T.A., Tylianakis, J.M., van Nouhuys, S., Vidal, S., (2007). Conservation biological control and enemy diversity on a landscape scale. *Biological Control*, **43**, 294–309.
- Tscharntke, T., Karp, D. S., Chaplin-Kramer, R., Batáry, P., DeClerck, F., Gratton, C., Hunt, L., Ives, A., Johnsson, M., Larsen, A., & Martin, E. A. (2016). When natural habitat fails to enhance biological pest control—Five hypotheses. *Biological Conservation*, **204**, 449–458.
- Tschumi, M., Albrecht, M., Bärtschi, C., Collatz, J., Entling, M. H., & Jacot, K. (2016). Perennial, species-rich wildflower strips enhance pest control and crop yield. *Agriculture, Ecosystems & Environment*, **220**, 97–103.
- Turlings, T. C., Scheepmaker, J. W. A., Vet, L. E., Tumlinson, J. H., & Lewis, W. J. (1990). How contact foraging experiences affect preferences for host-related odors in the larval parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). *Journal of Chemical Ecology*, **16**, 1577–1589.
- Tylianakis, J. M., Didham, R. K., & Wratten, S. D. (2004). Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology*, **85**, 658–666.
- Ueno, T. (1997). Host age preference and sex allocation in the pupal parasitoid *Itopectis naranyae* (Hymenoptera: Ichneumonidae). *Annals of the Entomological Society of America*, **90**, 640–645.
- Ueno, T. (1998). Selective host-feeding on parasitized hosts by the parasitoid *Itopectis naranyae* (Hymenoptera: Ichneumonidae) and its implication for biological control. *Bulletin of Entomological Research*, **88**, 461–466.
- Ueno, T. (2015). Effects of host size and laboratory rearing on offspring development and sex ratio in the solitary parasitoid *Agrothereutes lanceolatus* (Hymenoptera: Ichneumonidae). *European Journal of Entomology*, **112**, 281–287.
- Ulrichs, C., & Mewis, I. (2004). Evaluation of the efficacy of *Trichogramma evanescens* Westwood (Hym., Trichogrammatidae) inundative releases for the control of *Maruca vitrata* F.(Lep., Pyralidae). *Journal of Applied Entomology*, **128**, 426–431.

- Van Driesche, R., Hoddle, M., & Center, T. (2009). *Control of pests and weeds by natural enemies: an introduction to biological control*. (Pp. 243-247). Blackwell Publishing, Malden, Massachusetts, United States.
- Van Lenteren, J. C. (2000). Success in biological control of arthropods by augmentation of natural enemies. Pp. 77–103 In: Gurr, G.M. & Wratten, S.D (Eds.) *Biological control: measures of success*. Kluwer Academic Publishers. Dordrecht, Netherlands.
- Van Lenteren, J. C. (2012). The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl*, **57**, 1–20.
- van Rijn, P. C., & Wäckers, F. L. (2016). Nectar accessibility determines fitness, flower choice and abundance of hoverflies that provide natural pest control. *Journal of Applied Ecology*, **53**, 925–933.
- Vandermeer, J. (1989). *The ecology of intercropping*. Cambridge University Press, Cambridge, United Kingdom.
- Vandermeer, J. (1995). The ecological basis of alternative agriculture. *Annual Review of Ecology and Systematics*, **26**, 201–224.
- Varenes, Y. D., Gonzalez Chang, M., Boyer, S., & Wratten, S. D. (2016). Nectar feeding increases exploratory behaviour in the aphid parasitoid *Diaeretiella rapae* (McIntosh). *Journal of Applied Entomology*, **140**, 479–483.
- Vattala, H.D., Wratten, S.D., Phillips, C.B., & Wäckers, F.L. (2006). The influence of flower morphology and nectar quality on the longevity of a parasitoid biological control agent. *Biological Control*, **39**, 179–185.
- Vinson, S.B. (2010). Nutritional ecology of insect egg parasitoids. Pp. 25–56 In: Consoli, F.L., Parra, J. R. P. & Zucchi, R. A. (Eds.) *Egg Parasitoids in Agroecosystems with Emphasis on Trichogramma. Progress in Biological Control 9*. Springer Science + Business Media B.V., Dordrecht, Netherlands.
- Wäckers, F. L. (1994). The effect of food deprivation on the innate visual and olfactory preferences in the parasitoid *Cotesia rubecula*. *Journal of Insect Physiology*, **40**, 641–649.
- Wäckers, F. L. (2001). A comparison of nectar-and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *Journal of Insect Physiology*, **47**, 1077–1084.

- Wäckers, F. L. (2005). Suitability of (extra-) floral nectar, pollen, and honeydew as insect food sources. Pp. 17-74 In: Wäckers, F.L., van Rijn, P.C.J., & Bruin, J. (Eds.) *Plant-provided food for carnivorous insects: a protective mutualism and its applications*. Cambridge University Press, New York, NY, USA.
- Wäckers, F. L., Romeis, J., & van Rijn, P. (2007). Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions. *Annual Review of Entomology*, **52**, 301-323.
- Wade, M. R., & Wratten, S. D. (2007). Excised or intact inflorescences? Methodological effects on parasitoid wasp longevity. *Biological Control*, **40**, 347–354.
- Wilkinson, T. K., & Landis, D. A. (2005). Habitat diversification in biological control: the role of plant resources. Pp. 305–325 In: Wäckers, F. L., van Rijn, P. C. J., & Bruin, J. (Eds.) *Plant Provided Food for Carnivorous Insects, a Protective Mutualism and its Applications*. Cambridge University Press, Cambridge, United Kingdom.
- Williams, L., & Hendrix, D. L. (2008). Comparing different floral resources on the longevity of a parasitic wasp. *Agricultural and Forest Entomology*, **10**, 23–28.
- Winkler, K., Wäckers, F.L., Stingli, A., & van Lenteren, J.C. (2004). *Plutella xylostella* and its parasitoid *Diadegma semiclausum* show different gustatory and longevity responses to a range of nectar and honeydew sugars. *Entomologia Experimentalis et Applicata* **115**, 187–192.
- Winkler, K., Wäckers, F., Bukovinszkine-Kiss, G., & van Lenteren, J.C. (2006) Sugar resources are vital for *Diadegma semiclausum* fecundity under field conditions. *Basic and Applied Ecology*, **7**, 133–140.
- Winkler, K., Wäckers, F. L., Kaufman, L. V., Larraz, V., & van Lenteren, J. C. (2009a). Nectar exploitation by herbivores and their parasitoids is a function of flower species and relative humidity. *Biological control*, **50**, 299-306.
- Winkler, K., Wäckers, F., & Pinto, D.M. (2009b). Nectar-providing plants enhance the energetic state of herbivores as well as their parasitoids under field conditions. *Ecological Entomology*, **34**, 221–227.
- Wolcott, George N. (1942). The requirements of parasites for more than hosts. *Science*, **96**, 317–318.
- Wratten, S. D., Gurr, G. M., Landis, D. A., Irvin, N. A., & Berndt, L. A. (2000). Conservation biological control of pests: multi-trophic-level effects. Pp. 11–12 In:

Hoddle, M.S. (Ed.) *California Conference on Biological Control II, The Historic Mission Inn*. Riverside, California, United States.

- Wratten, S., Berndt, L., Gurr, G., Tylianakis, J., Fernando, P., & Didham, R. (2002). Adding floral diversity to enhance parasitoid fitness and efficacy. Pp 211-214 In: VanDriesche, R.G. (Ed.) *Proceedings of the first international symposium on biological control of arthropods, Honolulu, Hawaii, 14-18 January 2002*. United States Department of Agriculture, Forest Service, Morgantown, West Virginia.
- Yu, J. Z., Chi, H., & Chen, B. H. (2013). Comparison of the life tables and predation rates of *Harmonia dimidiata* (F.) (Coleoptera: Coccinellidae) fed on *Aphis gossypii* Glover (Hemiptera: Aphididae) at different temperatures. *Biological Control*, **64**, 1–9.
- Zhang, Y. B., Liu, W. X., Wang, W., Wan, F. H., & Li, Q. (2011). Lifetime gains and patterns of accumulation and mobilization of nutrients in females of the synovigenic parasitoid, *Diglyphus isaea* Walker (Hymenoptera: Eulophidae), as a function of diet. *Journal of Insect Physiology*, **57**, 1045–1052.

Appendix

Appendix 1. Aerial images of 2015 and 2016 buckwheat and no-buckwheat life table study field site.



Figure A1-1. Aerial image of buckwheat and no-buckwheat life table study site showing **A.** field plot used for experimental block setups; **B.** mowed, grassy border surrounding three sides of the plot; **C.** hedgerow of *Populus* sp. trees on southern margin of the site; **D.** field site where initial release of *Diadromus pulchellus* occurred beginning in 2010. Note that this image was taken in July 2017: the year after experiments were completed. (Google^{CA}, 2018).



Figure A1-2. Wider aerial image of buckwheat and no-buckwheat life table study site showing **A.** field site used for experimental block setups; **B.** field site where initial release of *Diadromus pulchellus* occurred beginning in 2010. Note that this image was taken in July 2017: the year after experiments were completed. (Google^{CA}, 2018).

Appendix 2. Complete stage-specific life tables for second generation leek moth in 2015 and 2016 caged and uncaged, buckwheat and no-buckwheat treatments.

Table A2-1. Life table for the second generation of leek moth, *Acrolepiopsis assectella*. Data are pooled from the two uncaged, no buckwheat leek blocks in 2015. l_x = number entering stage; d_x = number dying in stage; m_x = marginal mortality; q_x = apparent mortality; r_x = real mortality.

Life stage	Mortality factor	l_x	d_x	m_x	$100q_x$	r_x	k -value	% generational mortality
Eggs	Unknown	1000.0	513.4	0.51	51.3	0.51	0.313	9.5
Neonates	Unknown	486.6	261.3	0.54	53.7	0.26	0.334	10.1
Larvae	Unknown	225.3	145.3	0.65	64.5	0.15	0.450	13.6
	Parasitism	80.0	13.5	0.09	6.0	0.01	0.080	2.4
Pupae	Unknown	66.5	57.2	1.00	86.0	0.06	0.854	25.9
	* Parasitism	9.3	9.3	1.00	14.0	0.01	1.270	38.5
Adults emerged	Sex ratio	0.0	---	---	---	---	---	---
Adult females		---						
Potential progeny **	Lost fertility	---	---	---	---	---	---	---
Realised progeny ***		0.0						
Total mortality =		100.0%				$K_g =$	3.301	
Net reproductive rate (R_0) =		0.00						

* Parasitism k -value measured with assumption of 50:50 male:female sex ratio.

** Potential progeny = (# of surviving females) x (mean potential fecundity from 2015).

*** Realised progeny = (# of surviving females) x (mean realized fecundity from 2015).

Table A2-2. Life table for the second generation of leek moth, *Acrolepiopsis assectella*. Data are pooled from the two uncaged, buckwheat leek blocks in 2015. l_x = number entering stage; d_x = number dying in stage; m_x = marginal mortality; q_x = apparent mortality; r_x = real mortality.

Life stage	Mortality factor	l_x	d_x	m_x	$100q_x$	r_x	k -value	% generational mortality
Eggs	Unknown	1000.0	558.3	0.56	55.8	0.56	0.355	10.7
Neonates	Unknown	441.7	164.3	0.37	37.2	0.16	0.202	6.1
Larvae	Unknown	277.4	177.5	0.64	64.0	0.18	0.444	13.4
	Parasitism	99.9	18.7	0.10	6.8	0.02	0.090	2.7
Pupae	Unknown	81.1	64.1	1.00	79.0	0.06	0.678	20.5
	* Parasitism	17.0	17.0	1.00	21.0	0.02	1.532	46.4
Adults emerged	Sex ratio	0.0	---	---	---	---	---	---
Adult females		---						
Potential progeny **	Lost fertility	---	---	---	---	---	---	---
Realised progeny ***		0.0						
Total mortality =		100.0%				$K_g =$	3.301	
Net reproductive rate (R_o) =		0.00						

* Parasitism k -value measured with assumption of 50:50 male:female sex ratio.

** Potential progeny = (# of surviving females) x (mean potential fecundity from 2015).

*** Realised progeny = (# of surviving females) x (mean realized fecundity from 2015).

Table A2-3. Life table for the second generation of leek moth, *Acrolepiopsis assectella*. Data are pooled from the two caged, no-buckwheat leek blocks in 2015. l_x = number entering stage; d_x = number dying in stage; m_x = marginal mortality; q_x = apparent mortality; r_x = real mortality.

Life stage	Mortality factor	l_x	d_x	m_x	$100q_x$	r_x	k -value	% generational mortality
Eggs	Unknown	1000.0	399.5	0.40	40.0	0.40	0.222	6.1
Neonates	Unknown	600.5	381.5	0.64	63.5	0.38	0.438	12.2
Larvae	Unknown	219.0	99.1	0.45	45.3	0.10	0.262	7.3
	Parasitism	119.9	0.0	0.00	0.0	0.00	0.000	0.
Pupae	Unknown	119.9	53.9	0.03	45.0	0.05	0.260	7.2
	Parasitism	65.9	23.4	0.10	19.5	0.02	2.120	58.9
Adults emerged	Sex ratio	42.6	21.3	0.50	50.0	0.02	0.301	8.4
Adult females		21.3						
Potential progeny *	Lost fertility	7267.6	5057.2	---	---	---	---	---
Realised progeny **		2210.4						
Total mortality =		95.7%				$K_g =$	3.602	
Net reproductive rate (R_o) =		2.21						

* Potential progeny = (# of surviving females) x (mean potential fecundity from 2015).

** Realised progeny = (# of surviving females) x (mean realized fecundity from 2015).

Table A2-4. Life table for the second generation of leek moth, *Acrolepiopsis assectella*. Data are pooled from the two caged, buckwheat leek blocks in 2015. l_x = number entering stage; d_x = number dying in stage; m_x = marginal mortality; q_x = apparent mortality; r_x = real mortality.

Life stage	Mortality factor	l_x	d_x	m_x	$100q_x$	r_x	k -value	% generational mortality
Eggs	Unknown	1000.0	355.7	0.36	35.6	0.36	0.191	5.3
Neonates	Unknown	644.3	369.0	0.57	57.3	0.37	0.369	10.3
Larvae	Unknown	275.3	124.6	0.45	45.3	0.12	0.262	7.3
	Parasitism	150.7	0.7	0.00	0.3	0.00	0.002	0.1
Pupae	Unknown	150.0	81.0	0.57	54.0	0.08	0.337	9.4
	Parasitism	69.0	12.8	0.12	8.5	0.01	2.140	59.4
Adults emerged	Sex ratio	56.3	28.1	0.50	50.0	0.03	0.301	8.4
Adult females		28.1						
Potential progeny *	Lost fertility	9607.4	6685.4	---	---	---	---	---
Realised progeny **		2922.0						
Total mortality =		94.4%				$K_g =$	3.602	
Net reproductive rate (R_o) =		2.92						

* Potential progeny = (# of surviving females) x (mean potential fecundity from 2015).

** Realised progeny = (# of surviving females) x (mean realized fecundity from 2015).

Table A2-5. Life table for the second generation of leek moth, *Acrolepiopsis assectella*. Data are pooled from the two uncaged, no-buckwheat leek blocks in 2016. l_x = number entering stage; d_x = number dying in stage; m_x = marginal mortality; q_x = apparent mortality; r_x = real mortality.

Life stage	Mortality factor	l_x	d_x	m_x	$100q_x$	r_x	k -value	% generational mortality
Eggs	Unknown	1000.0	411.4	0.41	41.1	0.41	0.230	6.4
Neonates	Unknown	588.6	329.1	0.56	55.9	0.33	0.356	9.9
Larvae	Unknown	259.5	146.0	0.56	56.3	0.15	0.359	10.0
	Parasitism	113.5	19.5	0.10	7.5	0.02	0.082	2.3
Pupae	Unknown	94.1	67.3	0.84	71.5	0.07	0.546	15.1
	Parasitism	26.8	17.4	0.38	18.5	0.02	1.729	48.0
Adults emerged	Sex ratio	9.3	4.7	0.50	50.0	0.00	0.301	8.4
Adult females		4.7						
Potential progeny *	Lost fertility	1569.9	1049.0	---	---	---	---	---
Realised progeny **		520.9						
Total mortality =		99.1%				$K_g =$	3.602	
Net reproductive rate (R_o) =		0.52						

* Potential progeny = (# of surviving females) x (mean potential fecundity from 2016).

** Realised progeny = (# of surviving females) x (mean realized fecundity from 2016).

Table A2-6. Life table for the second generation of leek moth, *Acrolepiopsis assectella*. Data are pooled from the two uncaged, buckwheat leek blocks in 2016. l_x = number entering stage; d_x = number dying in stage; m_x = marginal mortality; q_x = apparent mortality; r_x = real mortality.

Life stage	Mortality factor	l_x	d_x	m_x	$100q_x$	r_x	k -value	% generational mortality
Eggs	Unknown	1000.0	470.9	0.47	47.1	0.47	0.276	7.7
Neonates	Unknown	529.1	257.1	0.49	48.6	0.26	0.289	8.0
Larvae	Unknown	272.0	163.9	0.60	60.3	0.16	0.401	11.1
	Parasitism	108.1	38.8	0.20	14.3	0.04	0.193	5.4
Pupae	Unknown	69.4	47.6	0.79	68.6	0.05	0.503	14.0
	Parasitism	21.8	12.2	0.33	17.6	0.01	1.639	45.5
Adults emerged	Sex ratio	9.5	4.8	0.50	50.0	0.00	0.301	8.4
Adult females		4.8						
Potential progeny *	Lost fertility	1599.2	1068.6	---	---	---	---	---
Realised progeny **		530.6						
Total mortality =		99.0%				$K_g =$	3.602	
Net reproductive rate (R_o) =		0.53						

* Potential progeny = (# of surviving females) x (mean potential fecundity from 2016).

** Realised progeny = (# of surviving females) x (mean realized fecundity from 2016).

Table A2-7. Life table for the second generation of leek moth, *Acrolepiopsis assectella*. Data are pooled from the two caged, no-buckwheat leek blocks in 2016. l_x = number entering stage; d_x = number dying in stage; m_x = marginal mortality; q_x = apparent mortality; r_x = real mortality.

Life stage	Mortality factor	l_x	d_x	m_x	$100q_x$	r_x	k -value	% generational mortality
Eggs	Unknown	1000.0	364.4	0.36	36.4	0.36	0.197	5.5
Neonates	Unknown	635.6	375.8	0.59	59.1	0.38	0.389	10.8
Larvae	Unknown	259.8	114.3	0.44	44.0	0.11	0.252	7.0
	Parasitism	145.5	1.9	0.01	0.8	0.00	0.006	0.2
Pupae	Unknown	143.5	36.4	0.26	25.4	0.04	0.127	3.5
	Parasitism	107.1	4.4	0.04	3.0	0.00	2.331	64.7
Adults emerged	Sex ratio	102.7	51.4	0.50	50.0	0.05	0.301	8.4
Adult females		51.4						
Potential progeny *	Lost fertility	17256.4	11530.7	---	---	---	---	---
Realised progeny **		5725.8						
Total mortality =		89.7%				$K_g =$	3.602	
Net reproductive rate (R_o) =		5.73						

* Potential progeny = (# of surviving females) x (mean potential fecundity from 2016).

** Realised progeny = (# of surviving females) x (mean realized fecundity from 2016).

Table A2-8. Life table for the second generation of leek moth, *Acrolepiopsis assectella*. Data are pooled from the two caged, buckwheat leek blocks in 2016. l_x = number entering stage; d_x = number dying in stage; m_x = marginal mortality; q_x = apparent mortality; r_x = real mortality.

Life stage	Mortality factor	l_x	d_x	m_x	$100q_x$	r_x	k -value	% generational mortality
Eggs	Unknown	1000.0	358.7	0.36	35.9	0.36	0.193	5.4
Neonates	Unknown	641.3	357.0	0.56	55.7	0.36	0.353	9.8
Larvae	Unknown	284.4	137.2	0.48	48.3	0.14	0.286	7.9
	Parasitism	147.2	2.1	0.01	0.8	0.00	0.006	0.2
Pupae	Unknown	145.0	35.3	0.25	24.4	0.04	0.121	3.4
	Parasitism	109.7	2.9	0.02	2.0	0.00	2.341	65.0
Adults emerged	Sex ratio	106.7	53.4	0.50	50.0	0.05	0.301	8.4
Adult females		53.4						
Potential progeny *	Lost fertility	17930.4	11981.0	---	---	---	---	---
Realised progeny **		5949.4						
Total mortality =		89.3%				$K_g =$	3.602	
Net reproductive rate (R_o) =		5.95						

* Potential progeny = (# of surviving females) x (mean potential fecundity from 2016).

** Realised progeny = (# of surviving females) x (mean realized fecundity from 2016).