The parasitoid complex associated with the invasive swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae) in Europe: prospects for classical biological control in North America.

By

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Abstract

The swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae) is an invasive pest of crucifer (*Brassica oleracea* L.) crops in North America. In order to identify potential classical biological control agents for the swede midge, this study aimed to ascertain which natural enemies attack the pest in its native European range and to investigate their basic biology and phenological relationships with their host. Two species of parasitoids, *Synopeas myles* Walker (Hymenoptera: Platygasteridae) and *Macroglanes chalybeus* Haliday (Hymenoptera: Pteromalidae) were found throughout Europe and attacked every generation of the swede midge. Neither species consistently parasitized large proportions of host populations and neither is host-specific, according to existing literature. For these reasons, neither parasitoid species currently appears to be a promising candidate for classical biological control of the swede midge in North America.

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Preface

Although the work presented in this thesis is my own, this research was undertaken as part of a collaborative effort and each co-author played a valuable role in its completion. Chapters 3 and 4 are being prepared for submission to a refereed journal, and are co-authored by Tim Haye, Peter Mason, Naomi Cappuccino, Guy Boivin, and Ulrich Kuhlmann. U. Kuhlmann, P. Mason, and G. Boivin conceived the swede midge biological control project and the general research objectives leading to the questions addressed in this thesis. Tim Haye collected the 2008-2009 European survey data presented in Chapter 3. I collected the 2010-2011 survey data as well as the seasonal phenology data presented in Chapter 3 with help from T. Haye. I collected all the data in Chapter 4. Although I designed all the experiments, conducted all the data analysis, and wrote both chapters, each of my coauthors provided valuable guidance in all of these respects. N. Cappuccino and G. Boivin, in particular, provided useful suggestions with regard to data analysis and statistics. P. Mason, N. Cappuccino, and T. Haye helped to choose and organize the content of both chapters.

Table of Contents

Abstract	ii
Acknowledgements	iii
Preface	v
Table of Contents.....	vi
List of Tables.....	ix
List of Figures	xi
Acronyms.....	xiii
Chapter 1: General Introduction	1
Chapter 2: Literature Review	4
Invasive species and classical biological control of insect pests.....	4
<i>Ecology of biological invasions.....</i>	<i>4</i>
<i>Classical biological control of insect pests</i>	<i>6</i>
Biological control of plant-feeding gall midges	8
<i>Plant-feeding gall midges.....</i>	<i>8</i>
<i>Predators and pathogens.....</i>	<i>10</i>
<i>Parasitoids.....</i>	<i>11</i>
<i>Historical classical biological control attempts.....</i>	<i>13</i>
The swede midge, <i>Contarinia nasturtii</i>	16
<i>Host range and geographical distribution</i>	<i>16</i>
<i>Life History and Population Dynamics</i>	<i>17</i>
<i>Control Strategies.....</i>	<i>19</i>
Chapter 3: Identity, distribution, and seasonal phenology of parasitoids of the swede midge, <i>Contarinia nasturtii</i> Kieffer (Diptera: Cecidomyiidae) in Europe.	23
Abstract.....	23
Introduction	24
Materials & Methods	27
<i>Parasitoid survey.....</i>	<i>27</i>
<i>Monitoring of untreated plot</i>	<i>28</i>

<i>Statistical analyses</i>	31
Results	32
<i>Parasitoid survey</i>	32
<i>Monitoring of untreated plot</i>	34
Discussion.....	37
Tables.....	48
Figures	58
 Chapter 4: Biology of <i>Synopeas myles</i> Walker (Hymenoptera: Platygasteridae), a parasitoid of the swede midge, <i>Contarinia nasturtii</i> Kieffer (Diptera: Cecidomyiidae).....	
Abstract	64
Materials & Methods	68
<i>Insects and general methodology</i>	68
<i>Effect of sugar feeding on adult longevity</i>	69
<i>Host stage suitability</i>	70
<i>Development of immature stages</i>	71
<i>Age-specific and potential lifetime fecundity</i>	72
<i>Incidence of superparasitism in the field</i>	73
<i>Superparasitism by groups of females in the lab and effect on offspring fitness</i>	73
<i>Statistical analysis software</i>	74
Results	74
<i>Effect of sugar feeding on adult longevity</i>	74
<i>Host stage suitability</i>	75
<i>Development of immature stages</i>	75
<i>Age-specific and potential lifetime fecundity</i>	77
<i>Incidence of superparasitism in the field</i>	77
<i>Superparasitism by groups of females in the lab and effect on offspring fitness</i>	78
Discussion.....	79
Tables.....	88
Figures	92

Chapter 5: General Discussion.....	97
References.....	103

List of Tables

Table 1. Sample sizes for the determination of swede midge (<i>Contarinia nasturtii</i>) infestation rates (plant dissections) and parasitism rates (midge and parasitoid rearing).....	48
Table 2. Results of collections of swede midge (<i>Contarinia nasturtii</i>) from throughout Europe (country codes in ISO format) showing the host plant sampled (VEG = crucifer vegetable crops; VR = volunteer oilseed rape; SR = spring-planted oilseed rape), collection date, total sample size (total number of midges and parasitoids emerged), and parasitism rate by <i>Synopeas myles</i> , <i>Macroglenes chalybeus</i> , <i>Inostemma opacum</i> , and <i>S. osaces</i> . *Non-diapausing emergence only.....	49
Table 3. Other reported gall midge (Diptera: Cecidomyiidae) hosts of the swede midge (<i>Contarinia nasturtii</i>) parasitoids found in this study, and their host plants. UCD – Universal Chalcidoidea Database and references therein.....	57
Table 4. The survival and mean development time ($\pm$ SE) of <i>Synopeas myles</i> males and females at different temperatures. The time required for unparasitized adult swede midge (<i>Contarinia nasturtii</i>) to emerge is also shown. * = (N emerging midges + N emerging parasitoids/total number of swede midge larvae originally placed on soil).	88
Table 5. The distribution of parasitoid eggs among swede midge (<i>Contarinia nasturtii</i>) larvae offered to 25 single <i>Synopeas myles</i> females in the laboratory. Using a Pearson chi-squared goodness-of-fit test, each observed distribution (O) was compared to a Poisson distribution (E) with a mean and variance equal to the group's mean number of parasitoid eggs per host larva (expected values shown are rounded to the nearest integer). χ^2 values with an asterisk indicate that the observed distribution differs significantly from its fitted Poisson distribution ($p < 0.05$).....	89
Table 6. The distribution of <i>Synopeas myles</i> eggs among 3 rd -instar swede midge (<i>Contarinia nasturtii</i>) larvae collected from a garden plot in Delémont, Switzerland in 2011 compared with a Poisson distribution with the same mean. Using a Pearson chi-squared goodness-of-fit test, each observed distribution (O) was compared to a Poisson distribution (E) with a mean and variance equal to the group's mean number	

of parasitoid eggs per host larva (expected values shown are rounded to the nearest integer). χ^2 values with an asterisk indicate that the observed distribution differs significantly from its fitted Poisson distribution ($p < 0.05$).90

Table 7. The distribution of parasitoid eggs among 16 groups of swede midge (*Contarinia nasturtii*) larvae, each offered to groups of four *Synopeas myles* females in the laboratory. Using a Pearson chi-squared goodness-of-fit test, each observed distribution (O) was compared to a Poisson distribution (E) with a mean and variance equal to the group's mean number of parasitoid eggs per host larva (expected values shown are rounded to the nearest integer). χ^2 values with an asterisk indicate that the observed distribution differs significantly from its fitted Poisson distribution ($p < 0.05$).91

List of Figures

- Figure 1. Areas of Europe surveyed for parasitoids of the swede midge, *Contarinia nasturtii*. Single points indicate single sampling sites; ellipses indicate sampling areas containing several sites. The number of collections from each area/site is indicated. Country codes are in two-letter ISO format.....58
- Figure 2. The distribution of total parasitism levels of the swede midge, *Contarinia nasturtii*, and the distribution of the levels attributable to each of the two dominant parasitoids, *Synopeas myles* and *Macroglanes chalybeus*, among samples collected in a survey of Europe.....59
- Figure 3 The proportion of swede midge, *Contarinia nasturtii*, diapausing in samples collected from 2008-2010 plotted against the proportion of *Synopeas myles* and *Macroglanes chalybeus* diapausing. Correlation coefficients and p-values are from Spearman's rank correlation analysis.60
- Figure 4. Swede midge (*Contarinia nasturtii*) infestation levels ($\pm$ SE of the total number of swede midge/plant), swede midge pheromone trap catches, and percent parasitism by *Synopeas myles* and *Macroglanes chalybeus* in collections made weekly from a garden plot of summer oilseed rape in Delémont, Switzerland in 2010.....61
- Figure 5. The proportions of diapausing swede midge (*Contarinia nasturtii*) larvae their parasitoids *Synopeas myles* and *Macroglanes chalybeus* in field collections made weekly in the summer of 2010 from a plot of summer oilseed rape in Delémont, Switzerland.62
- Figure 6. Swede midge (*Contarinia nasturtii*) infestation levels ($\pm$ SE of the total number of swede midge/plant), swede midge pheromone trap catches, and percent parasitism by *Synopeas myles* and *Macroglanes chalybeus* in collections made weekly from a garden plot of summer oilseed rape in Delémont, Switzerland in 2011.....63
- Figure 7. The number of offspring emerging from swede midge, *Contarinia nasturtii*, offered to female *Synopeas myles* at different ages (days after oviposition at 22.5°C). The life stages of the hosts on each day are shown below the x-axis. Boxes are bounded by the 25th and 75th percentiles with the line inside indicating the median; whiskers extend to the 5th and 95th percentiles.92

Figure 8. Life stages of <i>Synopeas myles</i> : (A) single egg inside swede midge, <i>Contarinia nasturtii</i> , larva, (B) superparasitized host containing five eggs, (C) encapsulated eggs, (D) newly-hatched first-instar larva, (E) partially engorged first-instar larva, (F) second-instar larva close to pupation, (G) pupa extracted from host cocoon, (H) adult female. e – <i>S. myles</i> egg, mg – host midgut, fb – host fat bodies, en – encapsulated egg, m – mandible of <i>S. myles</i> larva.	93
Figure 9. The mean number of eggs ($\pm$ SE) laid by female <i>Synopeas myles</i> in swede midge (<i>Contarinia nasturtii</i>) larvae on each day of their life, with the number of females surviving to each day shown.	94
Figure 10. The relationship between the number of <i>Synopeas myles</i> eggs laid in swede midge (<i>Contarinia nasturtii</i>) larvae and A. number of surviving <i>S. myles</i> offspring per host larva ($y = 0.005x^3 - 0.051x^2 + 0.143x + 0.215$), and B. percent of emerging <i>S. myles</i> offspring that were female ($y = -1.193x^2 + 18.01x + 33.50$).	95
Figure 11. The relationship between the number of <i>Synopeas myles</i> eggs laid in swede midge (<i>Contarinia nasturtii</i>) larvae and A. right hind tibia (RHT) length of female ($y = 0.1459x^2 - 0.9016x + 23$) and male ($y = -1.0698x^3 + 7.9683x^2 - 17.778x + 33.374$) <i>S. myles</i> offspring, and B. egg to adult development time of female ($y = -0.0484x^2 + 0.7479x + 21.469$) and male ($y = 0.3918x^2 - 0.9373x + 22.288$) <i>S. myles</i> offspring.	96

Acronyms

Bt – *Bacillus thuringiensis*

CFIA – Canadian Food Inspection Agency

ISO – International Organization for Standardization

L:D – light:dark

RH – relative humidity

SE – standard error

UCD – Universal Chalcidoidea Database

Chapter 1: General Introduction

Non-indigenous invasive species are a threat to native biodiversity and can cause serious economic damage in their invasive range (Clavero and Garcia-Berthou 2005; Pimentel et al. 2005). The defining characteristics of invasive species—high relative abundance, competitive dominance and rapid spread—are often attributed to a release from mortality due to the specialized natural enemies they have left behind in their native range (Keane and Crawley 2002; Mack et al. 2000). Indeed, rates of attack by natural enemies in the area of introduction are typically lower than in the native range of invaders, and native natural enemy communities in the area of introduction tend to be dominated by generalists that may occasionally attack invasive species but provide little control (Torchin et al. 2003). Classical biological control, which typically involves the importation of one or more of an invasive species' specialized natural enemies from its native range to reunite them with their host in the area of introduction, is often considered as a management option when an invasive species becomes an economic or ecological concern (Van Den Bosch 1971).

The goal of classical biological control is to provide self-sustaining suppression of invader populations below levels where they are economically or ecologically harmful while minimizing harmful effects to native ecosystems (Bigler et al. 2006; Van Driesche and Bellows 1996). For example, natural enemies that are able to kill large proportions of the host population and are specialized on the target host are more desirable than generalist species attacking relatively small proportions of the target host population (Hawkins and Cornell 1994; Mason et al. 2008; Waage 1990). To assess how well potential biological control agents meet these and other important criteria, it is essential

to possess a thorough understanding of their biology and their impact on host populations in their native range.

The swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae), native to Eurasia, has a wide range of host plants within the family *Brassicaceae* and is an occasional pest of cole crops in several European countries (Hallett 2007; Readshaw 1966). The presence of swede midge in Ontario, Canada, was first confirmed in 2000 (Hallett and Heal 2001). While newly invasive populations began to cause serious economic damage to cole crops in Ontario, surveys found that swede midge was spreading to other regions of Canada and the northeastern United States (CFIA 2008; Chen et al. 2009b). A 2006 survey of swede midge populations in Québec found no parasitoids attacking swede midge, suggesting that the pest's associated natural enemies did not accompany it to North America when it was introduced (Corlay et al. 2007). If the lack of attack by natural enemies is indeed responsible for the rapid increase in the distribution and abundance of swede midge populations, classical biological control by a specialized natural enemy from the midge's native range may prove to be an effective management strategy that could complement those currently practiced.

Very little is known about the swede midge's natural enemy community in its native range. While there have been a few reports of hymenopteran parasitoids attacking the swede midge in Europe, identifications are infrequently to the species level and the biology of these parasitoids has received virtually no attention (Bovien and Knudsen 1950; Dry 1915; Readshaw 1966; Rogerson 1963). A thorough knowledge of the composition of the swede midge's native natural enemy community and the biology of its members will be critical for the development of a successful classical biological control

program against this potentially serious pest. The objectives of this research were therefore to:

- 1) Determine the identity of the parasitoids attacking the swede midge throughout its European range and evaluate their impact on host populations.
- 2) Investigate the biology of any potentially specialist parasitoids, focusing on attributes relevant to host population suppression.

Europe comprises most of the accepted native range of the swede midge, where there has presumably been thousands or even millions of years for close host-parasitoid relationships to evolve. It is therefore hypothesized that the parasitoid assemblage attacking swede midge in Europe will contain co-evolved specialist parasitoids. It is expected that these parasitoids will:

- (i) Represent a significant mortality factor for the swede midge in Europe, consistently attacking host populations at a relatively high rate.
- (ii) Exhibit phenological synchronization with the swede midge; parasitism levels should be highest during generational peaks in swede midge abundance.
- (iii) Be host-specific, with no other host records existing in the scientific literature. If other host records do exist, they should describe only occasional attack of putative secondary host species at low rates.

The assessment of the impact of these candidate specialist species on swede midge populations and the elucidation of their physiological and ecological relationships to their host will begin to shed light on the potential for classical biological control against the swede midge in North America.

Chapter 2: Literature Review

Invasive species and classical biological control of insect pests

Ecology of biological invasions

Although biological invasions have likely always played a role in ecosystem structure and function, the relatively recent increases in the human-mediated global transport of organisms, usually unintentionally, has resulted in a vast increase in the rate of introductions of non-indigenous organisms to many areas of the world (Mack et al. 2000; Pimentel et al. 2005). When a non-indigenous organism is introduced into a new area, there are several possible outcomes. While the vast majority of such introductions do not result in establishment, some non-indigenous organisms manage to survive and reproduce in their introduced range. Of those that do establish, a subset undergoes rapid population growth and geographical range expansion, with local abundances sometimes far exceeding those in a given species' native range (Mack et al. 2000; Sakai et al. 2001). When these conditions are met, the non-indigenous species is said to have become invasive. The genetic variation present in founding populations may be important in determining whether an introduced species becomes invasive. There is evidence that low amounts of genetic variation in introduced populations may lower the potential for evolutionary adaptation to conditions in the area of introduction, or cause inbreeding depression, decreasing the chances that the introduced species will persist (Sakai et al. 2001). High amounts of genetic variation in introduced populations, due to, for example, gene flow between populations resulting from multiple introductions from different

source areas, may increase the potential for adaptive evolution and increase the chance that the introduced species will become invasive (Sakai et al. 2001).

Non-indigenous invasive species can have potentially drastic ecological and economic impacts. They can alter the structure of native ecosystems, sometimes driving native species to extinction (Clavero and Garcia-Berthou 2005; Sakai et al. 2001). Some invasive species become serious agricultural pests, causing large economic losses and prompting increased use of chemical pesticides (Pimentel et al. 2005). If environmentally conscious, ecologically based management strategies for invasive pests are to be effectively developed and employed, it is first important to attempt to understand why some non-indigenous species are so successful in their introduced range.

The Enemy Release Hypothesis states that a major reason for the success of invaders in their introduced range is that they have left behind their co-evolved natural enemies from their area of origin during the introduction process, and thus escaped the mortality or decreased fitness they cause (Colautti et al. 2004; Keane and Crawley 2002; Liu and Stiling 2006). In support of this hypothesis, invasive species are often introduced without a large proportion of their parasites, herbivores, or pathogens from their native range (Liu and Stiling 2006; Mitchell and Power 2003; Torchin et al. 2003). Research has found that they also suffer lower relative rates of attack by those that do accompany them to their introduced range (Liu and Stiling 2006; Mitchell and Power 2003; Torchin et al. 2003). In addition, native natural enemies that attack the invader in its introduced range usually do so at relatively low rates, and are typically generalists rather than specialists (Cornell and Hawkins 1993; Liu and Stiling 2006; Mitchell and Power 2003; Torchin et al. 2003). There is evidence that attack rates by native natural enemies may

increase over time, but this is likely to occur on evolutionary timescales given that it may require behavioural and/or physiological adaptation (Blaustein et al. 1983; Cornell and Hawkins 1993). This framework provides the rationale for classical biological control, which aims to expedite the process of natural population suppression of non-indigenous invasive species by importing specialist natural enemies from the invader's native range (Van Den Bosch 1971).

Classical biological control of insect pests

Classical biological control can be distinguished from other forms of biological control (e.g. conservation, inundative) in that it involves the importation of an exotic natural enemy to provide long-term control of invasive pest populations, rather than the enhancement of control provided by natural enemy species already present in the area of introduction, or the mass release of a natural enemy to provide temporary control (Eilenberg et al. 2001). While the success of classical biological control introductions to manage insect pests has been variable (Greathead and Greathead 1992), biological control agents that have successfully established and provided self-sustaining control of their target pest have become important components of many integrated pest management programs worldwide (Bale et al. 2008; Caltagirone 1981).

Once it is established that native natural enemies in the invaded range are not providing satisfactory control of an invasive pest (ie. suppressing population levels below economic thresholds), the next step in a biological control program is to compile a list of candidate control agents, relying on the knowledge of natural enemy fauna present in the target pest's native range (Van Driesche and Bellows 1996). The need for comprehensive and up-to-date taxonomic information on the pest's natural enemy

community often necessitates a rigorous survey of its native range (Legner and Bellows 1999). This is followed by obtaining basic information on the biology of natural enemies discovered, including the host stage they attack, their reproductive and developmental biology, and their seasonal population dynamics relative to their hosts, if not already known (Van Driesche and Hoddle 1997). Life table studies should then be undertaken in the pest's area of origin to directly measure the importance of the natural enemies as a mortality factor for pest populations (Kidd et al. 2005). These investigations pave the way for subsequent evaluation of the natural enemies designed to select the single species, or combination of multiple species, which would provide the safest and most effective control of the target pest.

In an effort to improve the rate of biological control success, there has been substantial effort directed towards improving the selection process for biological control agents. Evaluation of potential agents now integrates studies of population biology, systematics, ecology, behaviour, and molecular genetics (Hopper et al. 1993; Luck 1990; Murdoch and Briggs 1996; Unruh and Woolley 1999; Van Driesche and Bellows 1996). Synchronization with the target pest, high power of increase relative to hosts, importance as a natural mortality factor for the target pest in its native range, efficient host finding behaviour, dispersal ability, genetic diversity, and tolerance of climatic conditions in the potential area of introduction are examples of attributes considered potentially important predictors of a biological control agent's chance of establishing and effectively suppressing host populations (Crawley et al. 1986; Hawkins and Cornell 1994; Hopper et al. 1993; Mason et al. 2008; Waage 1990).

More recent concerns about the possible risks of biological control have added a new dimension to natural enemy evaluation. In addition to effectively suppressing the target pest, agents must also pose negligible risk to economically or ecologically important 'non-target' species in the area of introduction (Follett et al. 2000; Howarth 1983; Howarth 1991; Simberloff and Stiling 1996). For example, parasitoid biological control agents for insect pests must now undergo 'host range' or 'non-target' testing in an effort to predict what, if any, non-target insect species in the area of introduction could potentially be attacked if the agent were introduced (Bigler et al. 2006; Van Driesche and Hoddle 1997). Ecological studies are also necessary, since the impact of parasitoids on native species can also be indirect, for example by altering food web structure by competing with native parasitoids (Parry et al. 2009; Schellhorn et al. 2002).

When the introduction of a particular biological control agent is being considered, the potential ecological and economic risks of introducing the agent to the target area are weighed against its perceived benefits based on the information made available by the above studies (Louda et al. 2003; Thomas and Willis 1998). It is anticipated that this approach, while more conservative than historical methods, will lead to a higher success rate for biological control and reduce the number of introductions that have unforeseen negative consequences (Bigler et al. 2006; Louda et al. 2003).

Biological control of plant-feeding gall midges

Plant-feeding gall midges

The diversity of plant-feeding gall midges in the family Cecidomyiidae is considerable, with more than 900 species having been recorded in North America alone (Gagné 1989).

The host plant range of gall midges is typically restricted to one species or a few species within a genus, almost never extending beyond a single family of plants (Yukawa and Rohfritsch 2005). Adult midges lay their eggs on or inside the tissue of their host plants. Once the eggs have hatched and larvae are established at a feeding site, their salivary secretions induce cell growth, which results in the formation of a partially or entirely closed feeding chamber, or gall, in their host plant's tissue (Ananthakrishnan 1984). Depending on the species of midge, the larval feeding and subsequent gall induction may occur in the roots, stems, buds, leaves, rosettes, flowers, or fruits (Barnes 1946a; Barnes 1946b; Barnes 1948; Barnes 1956; Gagné 1989). The structure of galls is diverse, and exhibits a large range of complexity. The gall midges *Contarinia tritici* Kirby (Diptera: Cecidomyiidae) and *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae) do not form a conspicuous gall while feeding on the developing grain of wheat and barley (Affolter 1990; Barnes 1956). In contrast, feeding by the larvae of *Contarinia medicaginis* Kieffer (Diptera: Cecidomyiidae) and *Contarinia pisi* Winnertz (Diptera: Cecidomyiidae) causes severe swelling at the base of the flowers of their host plants, alfalfa and peas respectively, which creates a partially enclosed chamber within which they feed (Barnes 1946a; Barnes 1946b). Other species of gall midges can form comparatively complex, multi-chambered galls on their host plants. *Rhopalomyia tripleurospermi* Skuhrava & Hinz (Diptera: Cecidomyiidae) can induce galls with up to 80 separate chambers, each containing a single larva, on various meristematic tissues of its host plant *Triplospermum perforatum* Mérat (Asteraceae) (Hinz 1998).

Attack by gall midges can have a substantial negative effect on a plant's growth and reproductive output. Galls formed in flowers or buds can have direct detrimental

effects on host plant reproduction (Hinz and Muller-Scharer 2000; Schowalter and Haverty 1989; Smiley et al. 2004), while galls elsewhere can alter plant architecture and act as a sink for nutrients and energy, decreasing the overall vigor of the host and sometimes negatively affecting reproductive output (Fernandes et al. 2010; Hartley 1998; Hartnett and Abrahamson 1979; Marini-Filho and Fernandes 2011; Raman and Abrahamson 1995). Because of the deleterious effects they have on their host plants, some species of plant-feeding cecidomyiids are serious agricultural pests in economically important crops worldwide (Barnes 1946a; Barnes 1946b; Barnes 1948; Barnes 1956; Gagné 1989). For the same reasons, several species of gall midges have also been utilized in the biological control of weeds (Julien and Griffiths 1998). The economic and ecological importance of plant-feeding gall midges has stimulated considerable interest in the biology and ecology of their natural enemies, particularly their hymenopteran parasitoids.

Predators and pathogens

Gall midges are attacked by a variety of predators, including other cecidomyiids in the genus *Lestodiplosis*, which attack gall midges larvae inside their galls (Gagné 1989). Mendonca and Romanowski (2002) reported three species of ants attacking the galls of *Eugeniomyia dispar* Maia (Diptera: Cecidomyiidae), causing up to 41% mortality. *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae) falls prey to spider webs as an adult and several species of ground beetles (Coleoptera: Carabidae) as a larva when it drops to the soil to pupate (Holland et al. 1996). Sampson et al. (2002) found that the larvae of *Toxomerus geminatus* Say (Diptera: Syrphidae) were important early-season predators of the blueberry gall midge, *Dasineura oxycoccana* Johnson (Diptera:

Cecidomyiidae) in the United States. Vertebrate predators such as birds are also an important mortality factor for some species of gall midges, and can interfere with the density-dependant effect of parasitoids on midge populations (Tscharntke 1992). Fungal pathogens can sometimes invade galls and cause the death of gall midge larvae. In 1983, an epidemic of *Entomophthora brevinucleata* (Zygomycetes: Entomophthoraceae) spread across Europe, causing widespread mortality of several species of gall midges (Keller and Wilding 1985).

Parasitoids

Plant feeding gall midges are most commonly parasitized by members of the Hymenopteran families Platygasteridae, Eulophidae, Torymidae, Pteromalidae, Eurytomidae, and less commonly Braconidae (Adair et al. 2006; Ananthakrishnan 1984; Barnes 1956; Rogers 1977; Waring and Price 1989; Yukawa and Rohfritsch 2005). These species may be either strict specialists (with a host range containing only one species) or broad generalists (with a host range containing several species) (Hawkins and Goeden 1984).

Ectoparasitoids of cecidomyiids, which feed externally on host larvae inside galls, are represented by all of the aforementioned families except for the Platygasteridae (Askew and Ruse 1974; Mendonca and Romanowski 2002; Moore 1989; Stiling et al. 1992; Tabuchi and Amano 2003; Tscharntke et al. 1991). These ectoparasitoids can be solitary, with only one parasitoid offspring emerging from each host, or gregarious, with several parasitoids emerging from a single host (Askew and Ruse 1974; Stiling et al. 1992; Tabuchi and Amano 2003; Tscharntke et al. 1991). Some of the gregarious species, if host resources have been depleted, are able to complete their development by

consuming plant gall tissue (facultative herbivory) or the larvae of other parasitoids (facultative hyperparasitism) (Stiling et al. 1992; Tschamntke et al. 1991).

Endoparasitoids of plant-feeding gall midges can also be solitary or gregarious, but are mostly made up of solitary koinobiont platygastriids, pteromalids, and eulophids (Affolter 1990; Askew and Ruse 1974; Tabuchi and Amano 2003; Tschamntke et al. 1991). In contrast to idiobionts (typically ectoparasitic), whose offspring commonly develop on paralyzed hosts, koinobionts (typically endoparasitic) complete part of their development inside living, active hosts that are often still developing themselves. Platygastriids, specifically in the subfamily Platygastriinae, are perhaps the group of endoparasitoids most specialized on cecidomyiids. Members of this taxon are typically very host-specific, attacking a single species or few very closely related species of gall midges (Vlug 1995). Unfortunately, the biology of platygastriids is poorly known, only having been studied in a handful of species (Austin et al. 2005). From the few studies conducted so far, it is known that females oviposit inside the eggs or early-instar larvae of their hosts (Affolter 1990; Austin et al. 2005; Clausen 1940; Ogah et al. 2010). Embryonic development is then delayed until host larval development is completed, the adult parasitoid later emerging from the host puparium (Kim et al. 2011; Sandanayaka and Ramankutty 2007). The majority of species are solitary, their free-living larvae often possessing sickle-shaped mandibles used to eliminate competitors in the event of super- or multiparasitism (Affolter 1990; Clausen 1940; Sampson et al. 2006). However, a few species, such as *Platygaster diplosisae* Risbec (Hymenoptera: Platygastriidae) and *Platygaster szelenyii* Huggert (Hymenoptera: Platygastriidae), are gregarious (Ogah et al. 2010; Tschamntke et al. 1991); one field investigation by Tschamntke et al. (1991) showed

that an average of 14.7 *P. szelenyii* emerged per parasitized *Giraudiella sp.* host larva. *Platygaster hiemalis* Forbes (Hymenoptera: Platygasteridae), a parasitoid of *Mayetiola destructor* Say (Hymenoptera: Cecidomyiidae) exhibits a form of development termed polyembryony, with single eggs giving rise to “twin” parasitoid larvae (Patterson 1927).

The above-described hymenopteran parasitoids are often cited as an important mortality factor for natural populations of plant-feeding gall midges (Askew and Ruse 1974; Bullock et al. 2004; Feijen and Schulten 1983; Mendonca and Romanowski 2002; Stiling et al. 1992; Waring and Price 1989). Accordingly, in the few instances where invasive cecidomyiids have become an economic concern, parasitoids from their native ranges have been introduced to the affected area as a means of classical biological control.

Historical classical biological control attempts

Overall, there have been very few attempts to suppress populations of cecidomyiid agricultural pests using classical biological control agents. Of 5,670 records in the BIOCAT database of biological control introductions of arthropods to control arthropods (Greathead and Greathead 1992, updated 2005), only 13 have targeted cecidomyiids. Out of these introductions, only 5 have resulted in agent establishment, and none of these are listed as having provided complete or even substantial control of the target pest. Three of the most well documented examples of classical biological control against cecidomyiids are summarized here, with updates and assessments of their success.

There have been several introductions of parasitoids targeting the Hessian fly, *Mayetiola destructor* Say (Diptera: Cecidomyiidae), in the United States and New Zealand, where it has become a serious pest of cereal crops after its arrival in both

countries from Europe during the 19th century (Bullock et al. 2004; Cameron et al. 1989; Smiley et al. 2004). *Platygaster hiemalis* (Hymenoptera: Platygastriidae) and *Pediobius epigonus* Walker (Hymenoptera: Eulophidae) were introduced to New Zealand in 1894, although it was later determined that *P. hiemalis* already occurred in New Zealand at the time of the introduction and was probably introduced along with *M. destructor* (Cameron et al. 1989). *Platygaster hiemalis* and *P. epigonus* are now responsible for most parasitism of *M. destructor* in New Zealand (albeit at relatively low levels), however improved cultural practices since the 1940s have been primarily responsible for reducing economic losses (Cameron et al. 1989). *Pediobius epigonus*, *Platygaster pleuron* Walker (Hymenoptera: Platygastriidae), and *Trichasis remulus* Walker (Hymenoptera: Platygastriidae) were introduced to the United States between 1894 and 1939, but only *P. epigonus* successfully established (Clausen 1978). *Pediobius epigonus* has since spread throughout the country, but its parasitism levels are extremely low and it is doubtful that it contributes to the control of *M. destructor* (Bullock et al. 2004; Clausen 1978). Clausen (1978) attributes its lack of success to competition with the 42 other native and introduced species of *M. destructor* parasitoids already present in the United States, especially competitively superior generalist ectoparasitoids that parasitize a large proportion of the host population and are sometimes facultatively hyperparasitic.

From 1925 to 1926, *Platygaster demades* Walker (Hymenoptera: Platygastriidae) was introduced to New Zealand from France to control apple leafcurling midge, *Dasineura pyri* Bouché (Diptera: Cecidomyiidae) and pear leafcurling midge, *Dasineura mali* Kieffer (Diptera: Cecidomyiidae) in fruit orchards, and subsequently established. Some investigators have stated that asynchrony in the emergence of the second

generation of the parasitoid and its hosts has, at least in part, prevented effective control of leafcurling midge populations (Sandanayaka and Ramankutty 2007). A larger and more comprehensive study by Zhao He and Wang (2011), however, found evidence that *P. demades* is actually very well-synchronized with *D. mali*, with parasitism rates rising and host density decreasing with each generation. These authors suggested that the high fecundity and long life of *P. demades* may help to overcome any asynchrony that may exist between its emergence and that of *D. mali* (Zhao He and Wang 2011).

One of the most recent introductions of a classical biological control agent targeting a cecidomyiid pest was in 1993, when *Platygaster tuberosula* Kieffer (Hymenoptera: Platygastriidae) was introduced at one site in Saskatchewan, Canada, against the orange wheat blossom midge, *Sitodiplosis mosellana* (Doane et al. 2002). Surveys conducted from 1996 to 2001 showed that *P. tuberosula* had established at the release site where its numbers were quickly increasing, and was also present in several adjacent fields (Olfert et al. 2003). However, *Macroglenes penetrans* Kirby (Hymenoptera: Pteromalidae), probably unintentionally introduced from Europe at the same time as *S. mosellana*, continues to be the most important natural enemy of the midge with typical parasitism levels between 30 and 80 percent (Doane et al. 2002; Olfert et al. 2003). Although it is too early to conclude that *P. tuberosula* will not at some point contribute to the control of *S. mosellana* in Canada, the work of Olfert et al. (2003) suggests that the success of *P. tuberosula* may be hampered by low dispersal ability or a low tolerance to the chemical insecticides typically applied to wheat fields.

Since there are so few examples of classical biological control against cecidomyiids, it is difficult to assess whether problems such as inability of agents to

compete with native parasitoids, poor synchronization with host population dynamics, or poor dispersal ability are shortfalls that affect parasitoids of this target group more than others. Additionally, lack of post-release research or of its publication leaves the reasons for the unsuccessful establishments of many species unclear. In the future, it will be important to conduct rigorous post-release research on agents released against cecidomyiids to identify if there are any trends in the causes of successful and unsuccessful host population suppression, and whether these can inform future evaluation of potential agents.

The swede midge, *Contarinia nasturtii*

Host range and geographical distribution

The swede midge is a plant-feeding cecidomyiid whose hosts, which are restricted to the family Brassicaceae, include economically important crucifer crops such as broccoli (*Brassica oleracea* var. *italica*), cauliflower (var. *botrytis*), cabbage (var. *capitata*), Brussels sprouts (var. *gemmifera*) and canola (*Brassica napus* L. and *Brassica rapa* L.), as well as many cruciferous weeds (Barnes 1946b; Chen et al. 2009b; Darvas et al. 2000; Hallett 2007; Stokes 1953). The swede midge's geographical range is mostly Palaearctic, including a large area in Europe; extending north to Scandinavia, south to the Mediterranean, west to Britain, and east to Turkey and Western Russia (Darvas et al. 2000; Fauna 2011; Skuhravá 1997). Reports of the midge as a sporadic or serious pest of crucifer crops exist from the Netherlands, Switzerland, England, Sweden, Norway, Northern France, Germany, Denmark, and Slovenia (Olfert et al. 2006; Readshaw 1966). In 2000, the swede midge was first positively identified in Southern Ontario, Canada,

although there were reports of damage symptoms in plantings of crucifer crops as early as 1996; these were at first attributed to a nutrient deficiency (Hallett and Heal 2001). Not long after its detection, crop losses of up to 85% were reported in Southern Ontario (Hallett and Heal 2001). The midge has since been detected in Quebec, Nova Scotia, Manitoba, and Saskatchewan, as well as five American states: New York, Massachusetts, New Jersey, Connecticut, and Ohio (CFIA 2008; Chen et al. 2009b). Modeling of the swede midge's distribution and abundance based on climate has shown that populations could spread further and potentially become established throughout a large proportion of Canada and the United States (Mika et al. 2008; Olfert et al. 2006). As swede midge populations spread westward, there is some concern that high production spring-planted canola crops could also be attacked, especially in years when crops are seeded later than usual and plants are still in a susceptible phenological stage when the first generation of swede midge emerges (Hallett and Baute 2007).

Life History and Population Dynamics

Adult females mate soon after emerging from the soil, and lay eggs in batches of 2-50 on the actively growing tissue of their host plant; most often on heart leaves near the central growing tip (Readshaw 1966). Larvae hatch 1-3 days later and begin feeding gregariously by extraintestinal digestion, whereupon salivary secretions liquefy the host tissue (Readshaw 1966). Larval feeding induces gall-like structures in the growing tips of host plants, causing leaf distortion and twisting, the development of compensatory side-shoots, and the prevention of proper inflorescence development (Barnes 1946b; Darvas et al. 2000; Hallett and Heal 2001). Larval feeding also leaves corky scars, makes plants more susceptible to bacterial rot, and may limit seed production (Barnes 1946b; Hallett et

al. 2007; Hallett and Heal 2001; Kikkert et al. 2006). Upon completion of their development, full-grown larvae, 3-4 mm long and whitish yellow, fall or 'jump' from their host plant into the soil within which they construct a cocoon (Barnes 1946b; Readshaw 1966). Some larvae then pupate inside their cocoons and emerge as adults 1-3 weeks later (depending on temperature and soil moisture), while others will enter diapause and emerge the following spring with the proportion diapausing increasing throughout the growing season (Readshaw 1966). Diapause is likely triggered by decreasing temperature and day length and could last several years in some individuals, although these presumptions have never been directly tested (Chen et al. 2009a; Readshaw 1966)

In its native range, the swede midge normally has 2-4, but occasionally 5 generations depending on seasonal temperature conditions, with the first generation typically emerging in late May or early June and the last generation extending into September or October (Dry 1915; Mesnil 1938; Olombel 1931; Roesler 1937; Rygg and Braekke 1963). Typically, it is the second and third generation larvae that cause the most damage to crucifer crops (Barnes 1946b). In southern Ontario, 3-4 generations of swede midge with two overwintering emergence phenotypes have been reported (Hallett et al. 2007; Hallett et al. 2009b). Similarly, Corlay & Boivin (2008) found evidence for 3-4 overlapping generations in Quebec based on larval counts and pheromone trap data. In Canada, similar to Europe, first emergence of adult swede midge usually occurs in mid- to late May, with overwintering emergence peaking in early to mid-June (Corlay and Boivin 2008; Hallett et al. 2007; Hallett et al. 2009b).

Control Strategies

The application of synthetic insecticides is the primary method of swede midge control where the pest causes economic damage to crucifer crops (Chen et al. 2007; Hallett et al. 2009a; Wu et al. 2006). In the laboratory, treatment of broccoli plants with the neonicotinoid class of insecticides by soil drenching, seed treatment, or foliar application results in >95% mortality of swede midge larvae (Wu et al. 2006). Use of these insecticides has been shown to be particularly effective when they are applied to seedlings before swede midge oviposition occurs, rather than to plants already infested with late-instar larvae (Chen et al. 2007). In the field, however, the efficacy of chemical control is more variable, and is complicated by the high degree of overlap between swede midge generations and difficulty in achieving adequate spray coverage (Hallett et al. 2009a). Hallett et al. (2009a) found that keeping swede midge damage below marketable limits when infestation levels are high is particularly difficult, and recommended supplementing seedling insecticide treatment prior to transplanting with subsequent well-timed foliar applications and appropriate cultural control.

In conjunction with chemical control, cultural control methods can potentially play an important role in swede midge management. Crop rotation may be the single most effective method, and informal observations have documented lower levels of swede midge infestation in areas of its invasive range where rotation systems including non-crucifer crops are practiced (various personal communications). Similarly, simulated crop rotation systems have shown that as little as one season without suitable hosts can provide complete control of the swede midge, although these experiments did not incorporate the possibility of adults undergoing an extended diapause, which may occur

in field systems (Chen et al. 2009a). Management of brassicaceous weeds bordering fields may also be important in crop rotation systems, as they can serve as alternate hosts for the swede midge when their preferred, crucifer crop hosts are not present (Chen et al. 2009a). Since burying swede midge pupae with more than 5 cm of soil in the laboratory reduced and delayed adult emergence, deep tilling of fields was suggested as a potential management technique (Chen and Shelton 2007), but this strategy appears to be of minimal effectiveness at present (Chen et al. 2011). Exclusion fences, which disrupt the flight path of swede midge adults, were shown to reduce damage in Swiss crucifer plantings by up to 79% relative to controls, which was greater than the damage reduction from standard insecticide applications (Wyss and Daniel 2004). This strategy, however, remains untested as a swede midge control technique in North American production systems.

At present, there are no widely used and effective biological control strategies for the swede midge. Published reports of the effective use of natural enemies to cause swede midge mortality are, thus far, only from laboratory studies. At a rate of 241.5 g (AI)/ha, the biological insecticide *Bacillus thuringiensis* Berliner var. *israeliensis* (Bacillales: Bacillaceae) (Bt) caused significant mortality of swede midge larvae relative to controls in the laboratory (Wu et al. 2006). Results concerning the efficacy of Bt under field conditions have not been published, but its relative efficacy would presumably be reduced in light of the aforementioned problems with insecticidal control. Corlay et al. (2007) found that the entomopathogenic soil nematode *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae), at a concentration of 1000 initial infective juveniles/larva, caused 80-100% mortality of swede midge larvae in four

different types of soil, compared to 15-45% in control treatments. No published reports, however, exist concerning the efficacy of entomopathogenic nematodes against swede midge in the field. Corlay et al. (2007) also observed that two species of generalist coccinellid predators, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), consumed swede midge larvae under artificial conditions (in a clear plastic cups), but not under more natural conditions (on infested broccoli plants), making their potential as a biological control agent minimal.

In the field, the impact of predators on natural swede midge populations is not known, apart from a few observations of generalist predators feeding on swede midge adults and larvae (Dry 1915). There have been no studies comprehensively describing the parasitoid assemblage attacking the swede midge across its native range, but several investigators have reported parasitism of swede midge larvae by various hymenopterous parasitoids. This includes reports of *Synopeas* spp. (Platygastridae) (Readshaw 1966; Rogerson 1963), *Macroglenes eximius* Haliday (Pteromalidae) ((Bovien and Knudsen 1950)), *Platygaster* sp. (Readshaw 1966), and an unknown species in the family Proctotrupidae (Dry 1915). Buhl and Notton (2009) reported several instances of *Synopeas myles* (Hymenoptera: Platygastridae) being reared from swede midge throughout Great Britain, and a single record *Synopeas ventrale* (Hymenoptera: Platygastridae) emerging from larvae collected in Hertfordshire, England. The only attempt to describe the biology of the parasitoids in any detail was made by Readshaw (1966). He found that the three species of parasitoids studied (*Platygaster* sp. and two *Synopeas* spp.) laid their eggs inside feeding swede midge larvae. After their host had left

the plant, constructed a cocoon in the soil, and was about to pupate, the parasitoid larva hatched and consumed their host (Readshaw 1966). The parasitoid larvae then pupated, and adults emerged from the soil about 10 days after unparasitized swede midge adults from the same cohort (Readshaw 1966).

Following the detection of the swede midge in North America, Corlay et al. (2007) conducted a survey for swede midge parasitoids in Québec, Canada. They collected a total of 5,142 swede midge larvae from two field sites over two years (2005-2006) but recorded no parasitoid emergence, indicating that the swede midge's associated parasitoids were probably not introduced to North America along with their host, and no native parasitoids were attacking the midge (Corlay et al. 2007).

Chapter 3: Identity, distribution, and seasonal phenology of parasitoids of the swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae) in Europe.

Abstract

The swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae) is an invasive gall midge of Eurasian origin that has recently become a pest of crucifer (*Brassica oleracea* L.) crops in North America. In order to identify possible candidates for the classical biological control of this pest, the first ever extensive survey of Europe to determine what species of parasitoids attack the swede midge was conducted. In addition, weekly monitoring of an oilseed rape plot in Northwestern Switzerland allowed the observation of the seasonal phenological relationships between the swede midge and its parasitoids. *Synopeas myles* Walker (Hymenoptera: Platygasteridae) and *Macroglenus chalybeus* Haliday (Hymenoptera: Pteromalidae) were found to be the two primary parasitoid species, present throughout the surveyed range and, in Switzerland, attacking every generation of the swede midge. In the survey and the monitoring of the oilseed rape plot, total percent parasitism of samples ranged from 0-40%, but was typically quite low (<10%), possibly due in part to phenological asynchrony between host and parasitoid populations. Both *S. myles* and *M. chalybeus* have been reported to attack several other species of gall midges in Europe, casting doubt on their host specificity. However, before classical biological control of the swede midge in North America using its parasitoids from Europe can be ruled out, more research is needed to measure their importance as a mortality factor for natural swede midge populations and to assess their host specificity.

Introduction

The swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae) is an invasive pest of crucifer crops in North America. The midge is native to Europe and southwest Asia, where it is occasionally a serious pest of economically important crops such as broccoli, cabbage, cauliflower, kohlrabi, kale, and Brussels sprouts (*Brassica oleracea* L.[Brassicaceae]) (Barnes 1946; Darvas et al. 2000; Readshaw 1966; Stokes 1953).

Feeding by the midge larvae in the central growing tip of their host plant causes distortion and twisting of heart leaves, corky scarring, and bacterial rot, thereby preventing proper inflorescence development and resulting in reduced marketability, or in severe cases, complete crop loss (Barnes 1946; Hallett 2007; Kikkert et al. 2006).

Since its first official detection in Ontario, Canada, in 2000 (Hallett and Heal 2001) surveys have confirmed the presence of swede midge in Québec, Nova Scotia, Manitoba, and Saskatchewan (CFIA 2008). The midge was first detected in New York State in 2004, and has since been found in Massachusetts, New Jersey, Connecticut, and Ohio (Chen et al. 2009). Bioclimatic models have predicted that the midge could spread further and become established in every Canadian province and a large area of the United States (Mika et al. 2008; Olfert et al. 2006). There is concern that, if the swede midge becomes widespread in western Canada, it could become a pest of spring-planted canola, an extremely valuable and widely cultivated crop (Chen et al. 2011).

Not long after its initial detection, the swede midge caused up to 85% loss of broccoli crops southern Ontario (Hallett and Heal 2001). In subsequent years, the presence of the midge in some areas forced the abandonment of previously developed integrated pest management programs in favour of regular spraying schedules (Chen et

al. 2011). Though substantial effort has been directed towards developing effective insecticidal treatment regimes to control the swede midge (Chen et al. 2007; Hallett et al. 2009; Wu et al. 2006) reliable control of this pest has proven difficult, especially when infestation levels are high (Chen and Shelton 2010; Hallett et al. 2009). Cultural control methods such as deep soil tillage have also been tested, but appear to have minimal efficacy (Chen and Shelton 2007; Chen et al. 2011).

Currently, there are no effective biological control options for the swede midge. Corlay et al. (2007) tested the efficacy of entomopathogenic nematodes and coccinellid predators against the swede midge in the laboratory and determined that both could cause high mortality under certain conditions, but their potential efficacy in the field remains questionable. The same investigators were also unable to find any parasitoids attacking the swede midge in Québec, suggesting parasitoids associated with the swede midge in its native range were probably not introduced to Canada with the pest (Corlay et al. 2007) and that native North American parasitoids did not find the swede midge to be a suitable host. If the swede midge's rapid spread and high local abundances are in part due to a release from its co-evolved natural enemies (Keane and Crawley 2002), the introduction of a parasitoid from its native range could contribute to biologically-based swede midge management efforts in North America.

Given that the swede midge is such a widespread and sometimes serious pest in Europe, surprisingly little is known about its parasitoids in its native range. Bovien and Knudsen (1950) observed that swede midge in Denmark was attacked by *Macroglenes eximius* Haliday (Hymenoptera: Pteromalidae). In England, two *Synopeas* spp. and one *Platygaster* sp. (Hymenoptera: Platygasteridae) have been reared from swede midge larvae

(Readshaw 1966; Rogerson 1963). Buhl and Notton (2009) reported several instances of *Synopeas myles* Walker (Hymenoptera: Platygasteridae) being reared from swede midge throughout Great Britain, and a single record of *Synopeas ventrale* Westwood (Hymenoptera: Platygasteridae) emerging from larvae collected in Hertfordshire, England. Given that records are still lacking from most areas of the swede midge's native range, a more comprehensive survey was needed to identify potential classical biological control agents for introduction into North America. In addition, no studies have yet examined the importance of individual parasitoid species in regulating the swede midge in Europe, or their temporal relationships to host populations.

The study objectives were to (i) identify the parasitoids that attack the swede midge throughout Europe and at what rates, and (ii) evaluate the temporal relationships between these parasitoids and their host in the field. An extensive four-year survey for swede midge parasitoids was conducted throughout the midge's European range, complemented by two years of intensive monitoring of a summer oilseed rape plot in northwestern Switzerland. Results of these investigations will provide a basis for evaluation of the potential for classical biological control of the swede midge in North America.

Materials & Methods

Parasitoid survey

From 2008 to 2011, a total of 128 collections of swede midge-infested shoots were made from sites throughout Europe (Figure 1). Collections of plant parts (growing tips, bud clusters, and leaves) showing signs of infestation with swede midge larvae (Barnes 1946; Darvas et al. 2000; Hallett and Heal 2001) were made from *Brassica* spp. vegetable crops (*Brassica oleracea* L.var. *botrytis*, *italica*, *capitata*, *gemmifera*) or oilseed rape (*Brassica napus* L.) at each site. Sampling was conducted from May to September. Infested plant parts were placed in ventilated plastic cylinders (diameter: 10 cm, height: 15 cm) containing a moist soil/vermiculite mix and with fine gauze (150 meshes/inch) stretched over the top to prevent insect escape. Containers were stored at 50-60 % RH and $22.5 \pm 1^\circ\text{C}$ with a 16h: 8h (L:D) photoperiod. Plant material was removed from containers once swede midge larvae had left their feeding sites to construct cocoons in the substrate. Emergence of adult midges and parasitoids was scored daily, with the species and sex of each parasitoid recorded. Once no insects had emerged from containers for 10 days, samples were placed in a subterranean insectary for overwintering (in the dark, at ambient temperatures and humidity), and the substrate was kept moist. For the 2008-2010 samples, post-diapause emergence was scored the following spring beginning in April after samples had been incubated at room temperature. Eight large samples from 2008 and 2009 which had particularly high rates of first-year diapause were overwintered a second time to test whether swede midge and/or its parasitoids are capable of undergoing extended diapause.

Midge identifications were based on their host plant association (e.g., the swede midge is the only cecidomyiid known to feed on the *Brassicaceae* plant parts collected) and confirmed using the morphological characteristics described in Hallett and Heal (2001). In 2008 and spring 2009, all parasitoids were identified to family and sent to taxonomic experts for species confirmation: Pteromalidae were identified by Dr. Gary Gibson (Agriculture and Agri-Food Canada, Ottawa), and Platygasteridae by Peter N. Buhl (Zoological Museum, Department of Entomology, University of Copenhagen, Denmark). In 2009-2011, once the identity of the most common parasitoids was known, only unknown specimens and subsamples of the common species were given to taxonomists to confirm their identification.

The percent parasitism in each sample was calculated by dividing the number of emerging parasitoids by the total number of emerging parasitoids and midges. Host plants were classified as (i) crucifer vegetable plantings (Broccoli, cauliflower, Brussels sprouts, beets, kohlrabi), (ii) spring-planted oilseed rape, or (iii) 'volunteer' oilseed rape in harvested oilseed rape fields, other crop fields, or various disturbed sites. The percent of midges and of each parasitoid species entering diapause was calculated by dividing post-diapause emergence counts by total emergence counts. All means are quoted $\pm$ SE.

Monitoring of untreated plot

In 2010 and 2011, untreated summer oilseed rape (*B. napus*, JUMBO summer oilseed rape, W. von Borrier – Eckendorf GmbH & co. KG, Switzerland) plots were seeded biweekly from April to July at the CABI Europe-Switzerland centre in Delémont, Switzerland where a population of swede midge and its parasitoids occurred naturally. Oilseed rape was used as a host plant due to the ease with which large numbers of plants

can be cultivated and its long period of susceptibility to swede midge attack. Each 5 m x 3 m plot contained four (2010) or five (2011) evenly spaced 20 cm x 5 m rows of plants seeded at 500 seeds/m². Although plots were seeded every two weeks, several plots were destroyed in the seedling stage by slugs and/or flea beetles. To monitor the relative abundance of adult swede midge, one swede midge pheromone trap (Andermatt Biocontrol, Switzerland) was set up next to the field in the second week of April of each year, according to the manufacturer's instructions, and checked weekly until November. The pheromone lure was changed every 4-5 weeks. The week after the first catch of swede midge in the pheromone trap, June 7 in 2010 and May 16 in 2011, plants were randomly sampled from each plot weekly to monitor the host plant growth stage, swede midge infestation levels, and parasitism levels.

In 2010, 20 plants were randomly sampled from each of four rows in each plot on each sampling date (80 plants/plot/sampling date), beginning when most seedlings had more than three true leaves and ending once seed development was completed. The growth stages of the primary and secondary racemes on each plant were recorded (Canola Council of Canada 2011) and each plant was dissected under a dissecting microscope at 200x magnification and the number of swede midge larvae and eggs was recorded. Larvae were classified as 'early' (1st-2nd instar) if they did not have a sternal spatula, and 'late' (3rd instar) if a sternal spatula was present (Mamaev and Krivosheina 1993). Initially, swede midge larvae from dissected plants were transferred with a wet paintbrush to the tip of a new shoot on soil/vermiculite mix in ventilated 7.5 cm x 9 cm plastic cups to rear midges and parasitoids to adulthood and determine percent parasitism (see survey methods) in each plot on each sampling date. Containers were stored at 50-60

% RH, $22.5 \pm 1^\circ\text{C}$, and 16h: 8h (L:D). This method, however, caused high mortality of the midge larvae, particularly early instars. Therefore, starting the week of July 26 a random subsample of 10 plants from each row were dissected (40 plants/plot/sampling date) while infested shoots from the remaining 10 plants were placed directly in rearing cups. After emergence of midges and parasitoids was complete, rearing cups were overwintered as above with emergence scored the following spring.

In 2011, methods were the same as above except for the following alterations to increase the sample sizes for the determination of parasitism rates. At the outset, sampling of each plot consisted of randomly selecting three of five rows in each plot and removing 10 plants for dissection at random (30 plants/plot/sampling date), while non-random collections of 10 plants showing symptoms of current larval infestation were made from each of the remaining two rows on each sampling date. If 10 infested plants could not be found in a given row, as many as were present were sampled. Infested material from these plants (20 plants/plot/sampling date) was used to rear midges and parasitoids to determine parasitism rate of non-diapausing individuals.

To assess changes in swede midge infestation over time in each year, the mean ($\pm\text{SE}$) number of larvae per plant was calculated for each sampling date, with plants from all plots pooled. Rearing data were also pooled and percent parasitism on each date by each parasitoid species was calculated. Plots were excluded from the above analyses if their median plant growth stage (as determined by the most advanced raceme on each plant) was prior to bud formation or after pod maturation had begun, since it was observed that plants in these plots had considerably reduced infestation levels relative to plants in plots in all other growth stages (unpublished observations). Total sample sizes

and those used in the determination of infestation levels (number of plants dissected) and percent parasitism (total number of midges and parasitoids emerged from rearing) on each sampling date in 2010 and 2011 are shown in Table 1.

Statistical analyses

Comparisons of rank scores of parasitism rates between Swedish volunteer and oilseed rape were made using a Wilcoxon test. The same test was used to compare diapause rates between early-season (May-July) and late season (August-September) collections. Spearman rank correlations were used to test whether there was a significant relationship between the proportion of midges and the proportion of each parasitoid species entering diapause in each sample. For parasitoid species, only samples containing that species and from which at least one diapausing midge or parasitoid emerged were included in the analysis. A chi-squared test of independence was used to determine if the sex ratio of each parasitoid differed significantly from 50:50. In all cases where non-parametric tests were used, the distribution of the data was severely non-normal and could not be normalized using transformations. All analyses were performed using JMP 8.0 (SAS Institute, 2008).

Results

Parasitoid survey

Table 2 shows the results of the 128 collections of swede midge from throughout Europe between 2008 and 2011. In total, 91,632 midges and 7,098 parasitoids emerged from the collections. Four species of parasitoids were identified: *Macroglenes chalybeus* Haliday (Hymenoptera: Pteromalidae), *S. myles*, *Inostemma opacum* Thomson (Hymenoptera: Platygasteridae), and *Synopeas osaces* Walker (Hymenoptera: Platygasteridae). The swede midge is a previously unreported host for *M. chalybeus*, *S. osaces*, and *I. opacum*.

Synopeas myles and *M. chalybeus* were by far the most widespread and common species, reared from 76 and 46 collections, respectively. *Inostemma opacum* and *S. osaces* were only found in single collections from Switzerland and Slovenia, respectively. *Synopeas myles* was reared from collections made in Switzerland, Germany, France, Belgium, Slovenia, Great Britain (England), Sweden, and Denmark. *Macroglenes chalybeus* was found in all of the same countries as *S. myles* except for England and France. No parasitoids emerged from the four Hungarian collections. Typically, adult swede midge and parasitoids began to emerge from samples 10 and 20 days after the collection was made, respectively, with the emergence period of each typically extending over 5-10 days.

The average total percent parasitism among samples was 4.60 ± 0.66 %. Average parasitism by *S. myles* was 2.94 ± 0.45 %, and 1.67 ± 0.51 % by *M. chalybeus*. The distribution of parasitism rates among all collections is shown in Figure 2. Collections from which no parasitoids emerged at all were relatively common – parasitism was completely absent in 32.8% of samples. In the majority of the samples from which

parasitoids did emerge (68/86), the total parasitism was less than 10%. Total parasitism of 30% or more was recorded in only 3 collections, with a maximum overall parasitism level of 40.79% in a 2008 Swiss collection. Although *S. myles* emerged from more samples than *M. chalybeus* (see above), parasitism by *S. myles* was never more than 28.66%, whereas in three samples parasitism by *M. chalybeus* was higher than 30% (maximum 40.13%).

Although the non-systematic nature of the survey precluded detailed analyses of the overall effects of host plant, collection time, and geographic region on parasitism rates or parasitoid species compositions, several trends were noted. First, *M. chalybeus* appeared to be the dominant species in collections from crucifer vegetable crops, where this species accounted for the majority of the parasitism in 71% (17/24) of samples in which parasitism occurred. *Synopeas myles*, on the other hand, parasitized a larger proportion of swede midge than *M. chalybeus* in 88% (51/58) of samples collected from volunteer oilseed rape (in which parasitism was recorded). This trend could be at least partially attributable to geographic region; there was higher parasitism by *S. myles* than *M. chalybeus* in 84% (21/25) of collections from Northern Europe (Northern Germany, Sweden, and Denmark) in which parasitism was recorded, none of which were from crucifer vegetable crops. In a large survey of Southern Sweden and Denmark in June 2011, the total parasitism rates were significantly higher in volunteer oilseed rape than in cultivated spring-planted oilseed rape ($S = 243$, $Z = 2.00$, $p = 0.047$, $n = 17, 13$). There was no apparent trend in the seasonal presence of either parasitoid species; both species were found in samples collected from May to September.

Diapause of midges and/or parasitoids occurred in 63% (54/86) of samples collected between 2008 and 2010, with an average of 10.94 ± 2.46 % of midges and 6.26 ± 1.72 % of parasitoids diapausing among those samples. The average rank-score of the percentage of insects that entered diapause was significantly higher in samples collected in August and September than those collected in May, June, and July ($S = 632$, $Z = -2.98$, $p = 0.0029$, $n = 21, 65$). *Synopeas myles* and *M. chalybeus* entered diapause in 12.5% and 18.7% of samples, respectively, whereas overwintering emergence of *I. opacum* and *S. osaces* was not recorded. A significant positive correlation was found between the proportion of swede midge and the proportion of *S. myles* that entered diapause ($\rho = 0.56$, $p = 0.0002$, $n = 40$), but not between the diapausing proportions of midges and *M. chalybeus* ($\rho = 0.27$, $p = 0.18$, $n = 26$) (Figure 3).

Out of eight samples that were overwintered for a second year, midges subsequently emerged from three of them. The rate of this extended diapause was extremely low (<0.1%) in all three cases. Extended diapause by parasitoids was not recorded.

The overall male:female sex ratios of *S. myles* and *M. chalybeus* were 1.05:1 ($n = 5,155$) and 1.37:1 ($n = 2,014$), respectively. The sex ratio of *S. myles* did not differ significantly from 50:50 ($\chi^2 = 0.064$, $p = 0.80$, $df = 1$), nor did that of *M. chalybeus* ($\chi^2 < 0.0001$, $p = 0.99$, $df = 1$). All emerging *S. osaces* ($n = 2$) and *I. opacum* ($n = 32$) were female.

Monitoring of untreated plot

Infestation and parasitism levels in the plot of untreated summer oilseed rape on each sampling date in 2010 are shown in Figure 4. Based on the number of eggs per plant,

first generation oviposition by swede midge began in early June. Together, pheromone trap catches and relative abundances of swede midge eggs and larvae indicate that at least four generations of swede midge occurred in 2010, with egg/larval abundances peaking in early June, mid-July, mid-August, and mid to late-September. Pheromone trap catches corresponded to the peaks of the 1st, 3rd, and 4th generations, but not the highest peak of eggs and larvae (3.28 ± 0.61 swede midge/plant) in mid-July.

Synopeas myles and *M. chalybeus* were the only two species of parasitoids reared from swede midge, with both species present during every host generation sampled. *Macroglanes chalybeus* emerged from samples collected as early as June 21, and *S. myles* first emerged from samples collected one week later. Total parasitism in 2010 (Figure 4) ranged from 0-28.8%, with parasitism peaking at the end of the first swede midge generation at the end of June. Percent parasitism of swede midge by *S. myles* and *M. chalybeus* averaged 4.22 ± 1.65 and $1.59 \pm 0.55\%$, respectively, and the highest recorded parasitism (27.7%) was by *S. myles*. Parasitism by *M. chalybeus* never exceeded 6.8%. There were peaks in total parasitism during the first, second, and fourth generation peak periods of swede midge infestation, but not during the third generation in mid-August.

The diapause rates of the swede midge, *S. myles*, and *M. chalybeus* throughout the sampling period in 2010 are shown in Figure 5. Unparasitized swede midge and *M. chalybeus* began to enter diapause in samples collected as early as July 19. The percent of midges entering diapause increased from the middle of August onwards, reaching levels of 36.5% on September 20. A large proportion of *M. chalybeus* (50-83%) entered diapause in samples collected from September 6 onwards. Diapausing *S. myles* emerged

from samples collected as early as September 6, although at much lower rates (2-6%) than *M. chalybeus* or unparasitized swede midge.

In 2011, presumably due to unusually warm spring temperatures, swede midge oviposition in the plot was first recorded in the middle of May (Figure 6). Pheromone trap catches and infestation levels indicated that at least 5 generations of swede midge occurred in 2011, with egg/larval abundances peaking in late May, late June, early August, late August, and mid-September. In general, pheromone trap catches were indicative of relative infestation levels and peaked within a week of maxima in egg/larval abundances. Swede midge abundance was the highest on August 8 (18.87 ± 3.66 swede midge/plant), and was consistently higher than in 2010, a trend also apparent in the relative numbers of males caught in pheromone traps during generational peaks in each year (Figures 4 and 6).

As in 2010, *S. myles* and *M. chalybeus* were the only parasitoids reared from swede midge collected from the plot in 2011, and were again found to parasitize every host generation (Figure 6). Both *S. myles* and *M. chalybeus* were first reared from samples collected on May 30. Total parasitism rates ranged from 0-41%, peaking at the end of the first generation (as in 2010) at the beginning of June, and again at the end of the third/start of the fourth generation of swede midge in the middle-end of August. Average parasitism by *S. myles* and *M. chalybeus* was 3.94 ± 1.03 and 8.46 ± 2.48 %, respectively. The highest recorded parasitism by an individual species (36.6%) was by *M. chalybeus*, and parasitism by this species exceeded 15% on three sampling dates. Parasitism by *S. myles* never exceeded 11.9%. There were peaks in total parasitism in the middle of the first and the beginning of the fourth generations of swede midge, but not during the

second or third generations. There was an additional rise in total parasitism (to 18.6%) during the fifth generation of swede midge at the end of September.

Discussion

The recent invasion by the swede midge in North America, the absence of effective natural enemies, and the growing concern about its impact on *Brassica* sp. crop production necessitated a thorough evaluation of what potential classical biological control options might exist, focusing on the pest's parasitoids in its area of origin. An extensive survey of the swede midge's European range and more detailed monitoring of a single population in Switzerland determined that two species of Hymenopteran parasitoids, *S. myles* and *M. chalybeus*, attack the midge throughout the surveyed range and appear to be present during each of the midge's four to five generations. Two other species, *I. opacum* and *S. osaces* were also found attacking the midge, but only at single sites (Birrhards, Switzerland and Jablje, Slovenia respectively) (Table 2).

This study confirmed previous reports (Buhl and Notton 2009) that *S. myles* is a parasitoid of the swede midge; this parasitoid was reared from more samples than any other species and was responsible for the most parasitism. Although a comprehensive survey of England was not conducted, we were unable to corroborate the single existing record of *S. ventrale* as a swede midge parasitoid (Buhl and Notton 2009), nor did this species emerge from any samples collected throughout the rest of Europe. *Macroglenes eximius* (then *Pirene eximia*), reportedly reared from the swede midge in Denmark (Bovien and Knudsen 1950), was probably misidentified; it is likely that the species reported by these authors was actually *M. chalybeus* (then *Pirene chalybea*). Although not previously known as such, *M. chalybeus* was actually found to be quite a common

parasitoid of the swede midge in Europe, and it was occasionally found parasitizing host populations at relatively high rates (>20%) in Switzerland and Slovenia. Both *S. myles* and *M. chalybeus* are known to be solitary koinobiont endoparasitoids parasitizing the larvae of their hosts, their larvae hatching and consuming the swede midge larva once it has constructed a cocoon in the soil (Keller and Schweizer 1994; Readshaw 1966). Based on the typical biology of the Platygasteridae, the two other species reared in this study, *I. opacum* and *S. osaces*, are probably also koinobiont endoparasitoids, attacking swede midge eggs or early-instar larvae.

The rate of attack of a host population by natural enemies in a pest's area of origin is, to some degree, a predictor of its success as a biological control agent for the pest in its introduced range. Hawkins and Cornell (1994) found a significant positive relationship between the rated success of 787 parasitoid introductions and the maximum parasitism rate reported from the species' native range. According to their study, parasitoids causing less than 32% parasitism are predicted to have little impact (Hawkins and Cornell 1994). Although parasitism by *S. myles* and *M. chalybeus* could reach levels of up to 28 and 40% in the survey samples, respectively, average parasitism by both species was much lower (< 5%) and was zero in many samples (Table 2). Van Driesche (1983) pointed out that single collections from insect populations are unlikely to result in the accurate estimation of the generational mortality inflicted on host populations by their parasitoids. Parasitism can be under- or over-estimated depending on the timing of the collection in relation to the phenological state of the host and parasitoid populations (Van Driesche 1983), a notion further re-inforced when examining the results of our weekly monitoring of a single swede midge population. On certain sampling dates during each

swede midge generation, even when infestation rates were high, parasitism levels could be very low or even zero (Figures 4 and 6). However, the overall parasitism of a given generation might be much higher than indicated on one sampling date, for example, due to relatively high parasitism on a different sampling date within the same generation (Figures 4 and 6). Despite this more intensive monitoring, however, a similar range of parasitism (0-40%) was observed during the weekly sampling, and average parasitism by the two major parasitoid species was still less than 10% in both years. This weekly monitoring also showed that large portions of several generations of swede midge in both years were not parasitized. This could have been partially due to the fact that all swede midge life stages were reared to maturity and included in the percent parasitism calculations. If earlier host stages (eggs, for example) were not susceptible to attack by the parasitoids, percent parasitism would be underestimated. Alternatively, if, for example, parasitized larvae remained in plants for longer than unparasitized larvae, generational parasitism could be overestimated by a single collection (Van Driesche 1983). In order to accurately estimate the mortality inflicted by these parasitoids on swede midge populations, a life table study examining the mortality inflicted by parasitoids on closely monitored cohorts of swede midge during each generation would be necessary (Kidd et al. 2005).

Phenological synchronization between host and parasitoid populations is considered very important for the ability of a given parasitoid species to effectively suppress host populations (Kidd et al. 2005). When parasitoids are able to begin ovipositing soon after emergence, they are said to be synchronized with their hosts in a given generation if their emergence peaks at the same time as the susceptible host stage

(Kidd et al. 2005). In contrast, asynchrony can result in some proportion of the host population escaping parasitism (Godfray et al. 1994). In the monitoring of the oilseed rape plot in this study, although the flight of adult parasitoids were not directly monitored, peaks in parasitism likely indicated peaks in adult parasitoid presence since these parasitoids are short-lived (usually 2-10 days in the laboratory) and have short pre-oviposition periods (<24 h) (Chapter 4; unpublished data). Furthermore, given the short generational time of insects in this system, most parasitism recorded on each date probably took place in the week prior to sampling. In both years, large proportions (>80%) of several swede midge generations were unparasitized, particularly the 2nd-4th generations in 2010 and the 2nd and 3rd generations in 2011 (Figures 4 and 6). In some well-synchronized multivoltine host-parasitoid systems, parasitism builds throughout the season, accompanied by a drop in host density in later generations (presumably due to high parasitism in preceding generations) (Zhao He and Wang 2011). These trends were not observed in either year of our study. For example, there was an increase in swede midge density and a decrease in percent parasitism from the first to second generation in 2010, despite relatively high percent parasitism at the end of the first generation (Figure 4). Therefore, it is plausible that there was phenological asynchrony between populations of the swede midge and its parasitoids, and this may have been an important reason why parasitism never exceeded moderate levels. Asynchrony can sometimes be tempered by parasitoids having a long lifespan or a high lifetime fecundity (Sandanayaka and Ramankutty 2007; Zhao He and Wang 2011), but there is no indication that this is the case for either *S. myles* or *M. chalybeus* (Chapter 4; unpublished data).

The relative rates on entry into diapause could also be relevant for the synchronization between host and parasitoid populations (Brodeur and McNeil 1989). From the rates of diapause of parasitoids and midges in our survey samples (Figure 3), we found evidence that the proportion of *S. myles* diapausing is significantly positively correlated with the proportion of its hosts diapausing. This was not observed for *M. chalybeus*, although its relative diapause rates were more similar to those of swede midge than *S. myles* diapause rates were in the samples collected from our oilseed rape plot in 2010 (Figure 5). Parasitoids sometimes coordinate entry into diapause with their hosts using host cues, however they may also enter diapause at higher or lower rates, depending on a complex combination of intrinsic and extrinsic factors (Brodeur and McNeil 1989; Zhao He and Wang 2011). More research needs to be conducted to determine what diapause cues are utilized by *M. chalybeus* and *S. myles*, and what the implications of relative diapause rates are for host-parasitoid population dynamics in the following season.

When screening potential biological control agents, host specificity is an important consideration. Parasitoids that are more specifically adapted to the target pest's physiology and ecology may be more likely to provide reliable control of the target pest and less likely to attack non-target species (Bigler et al. 2006). All of the parasitoid species found in this survey have been reported to attack other species of gall midges (Table 3). *Synopeas myles* has reportedly been reared from a number of other gall midges on a variety of host plants, including pests of wheat, alfalfa, and willow. *Macroglanes chalybeus* has also reportedly been reared from several other hosts, and is known to be the most important parasitoid of the pea midge, *Contarinia pisi* Winnertz

(Diptera: Cecidomyiidae), in Switzerland, sometimes parasitizing more than 63% of host populations (Keller and Schweizer 1994). By obtaining voucher material and having it identified by current taxonomic experts, it was confirmed that the parasitoids from the studies by Affolter (1990) (*S. myles* reared from *C. tritici*) and Keller and Schweizer (2004) (*M. chalybeus* reared from *C. pisi*) were correctly identified. It was not possible to obtain voucher material for the other host records, however at least one, the report of *M. chalybeus* being reared from *C. tritici* and *Sitodiplosis mosellana* Géhin (Diptera: Cecidomyiidae), seems unlikely. Affolter (1990) extensively studied the parasitoid complex of *C. tritici* and *S. mosellana* in Switzerland over several years (1985-1989) but never reported parasitism by *M. chalybeus*, despite the fact that this parasitoid, as shown by the results of the current study, is common in Switzerland. Because of the difficulty associated with distinguishing species of these groups, especially for researchers lacking taxonomic expertise, and the age of many of these records, the remainder of these reports should not be considered definite and need to be confirmed by re-identification of past and new collection material by current taxonomic experts.

Given that *I. opacum* and *S. osaces* were only found at one site each and parasitism levels were very low in both cases, it is likely that swede midge is the not the primary host of these species, and that these instances of attack were opportunistic (i.e. perhaps populations of more suitable host species were not available when they emerged, or they happened upon swede midge-infested plants by chance). This is unlikely in the case for *S. myles* and *M. chalybeus*, although that they are specialized, host-specific parasitoids of the swede midge seems unlikely, given the numerous reports of attack of other host species, some of which are well-substantiated. In the case of *S. myles*, limited

information is available on how commonly it attacks the other species of gall midges, or whether it parasitizes large proportions of their populations. Keller and Schweizer (1994) reported rearing only a single *S. myles* from *C. pisi* despite the collection of thousands of larvae. A species complex including *S. myles* was shown to occasionally parasitize *C. tritici* by Affolter (1990), but at levels typically less than 5%. Romankow and Dankowska (1993, 1994) reported that *Contarinia medicaginis* Kieffer (Diptera: Cecidomyiidae) and *Dasineura ignorata* Wachtl (Diptera: Cecidomyiidae) were parasitized by *S. myles* at rates between 2-16% and 1-9%, respectively, and that *S. myles* was sometimes responsible for the majority of the parasitism of *C. medicaginis*. The low parasitism reported in these studies do not definitively indicate that any of these other hosts are necessarily the primary host species for *S. myles*. Without any more information from other putative host species, it can be concluded that either, (i) the primary host species of *S. myles* has yet to be determined, or (ii) *S. myles* is a generalist species and attacks many species of gall midges within the subfamily Cecidomyiinae on several different host plants at moderate rates. It would be similarly difficult to argue that *M. chalybeus* is a specialized and host-specific parasitoid of the swede midge since this species is known to be a closely associated and important parasitoid of another gall midge, *C. pisi* (Keller and Schweizer 1994). However, in this study, parasitism of swede midge by *M. chalybeus* was common and sometimes at a high rate despite the absence of nearby pea fields that could harbour populations of *C. pisi* (for example, in the closely monitored field plot of oilseed rape). Therefore, *M. chalybeus* seems capable of sustained population maintenance using swede midge alone as a host. Whether *M. chalybeus* also commonly attacks other species of gall midges at high rates, including

those shown in Table 3, is currently unknown. More research involving the collection of these and other gall midges species could help to resolve this issue as it relates to both *S. myles* and *M. chalybeus*. Given the aforementioned difficulties in distinguishing species of these groups, it should also be considered that one or both of these species could in fact be composed of several cryptic subspecies (Smith et al. 2006). This possibility could be tested by comparing variability in DNA sequences between parasitoids reared from different hosts with the species-wide (i.e. geographical) variation present within species reared from the same host.

It is interesting to consider what might affect parasitoid species composition in populations of swede midge. Since our survey was not conducted in a systematic manner, we were unable to directly test hypotheses concerning the effect of geographic region, collection time, or host plant. However, several interesting patterns emerged that may implicate habitat stability as a factor affecting species composition. Firstly, *Macroglenes chalybeus* appeared to be more common than *S. myles* in crucifer vegetable crops overall, while the opposite seemed to be true in volunteer oilseed rape. Crucifer vegetable plantings sampled tend to be planted in the same area or even the same plot year to year; swede midge is such a serious pest in some areas of Europe in large part because effective crop rotation is seldom practiced (various personal communications). This means that plants were available to swede midge populations, and hosts available to parasitoids, from year to year in crucifer vegetable fields. Volunteer oilseed rape sites, in contrast, were likely to be more ephemeral due to weed removal ploughing of fields at the end of each year. It is possible that *S. myles* is the first to parasitize new host populations, or is able to tolerate habitat instability better than *M. chalybeus*. This could

be in part due to the reported ability of *S. myles* to parasitize several other hosts that infest plants (wheat, alfalfa) often in close proximity to fields in which oilseed rape grows as a volunteer plant. *Macroglenes chalybeus* may be the more common species in fields where host populations have existed for several years, but may take longer to find new populations of hosts or establish. This hypothesis is further supported by the monitoring of an untreated plot of oilseed rape in Switzerland. In these trials, *S. myles* was the most common species in 2010 (Figure 4), the second year that the plot was infested with swede midge, whereas *M. chalybeus* was the dominant species the following year (Figure 6). Keller and Schweizer (1994) similarly reported a rise in parasitism of *C. pisi* by *M. chalybeus* over the first several years of *C. pisi* population establishment in pea fields. The hypothesis that *M. chalybeus* eventually rises to dominance over *S. myles* in stable habitats is still, however, highly speculative and needs to be tested directly and its potential mechanisms explored.

An additional finding of this work was that the swede midge was commonly found to be attacking volunteer oilseed rape throughout Europe. Plants growing among other crops or that had grown from spilled seed after harvest of winter oilseed rape in July were often infested. Oilseed rape plants present in the spring were continually attacked by swede midge throughout the year; compensatory side shoots resulting from the attack provided oviposition sites for subsequent generations. Although previously unstudied, the use of these plants by swede midge is likely to play an important role in the build-up and maintenance of the pest's populations in Europe. It could also help to maintain parasitoid populations; it was also found that average percent parasitism was significantly higher in volunteer oilseed rape than in cultivated spring-planted oilseed

rape (which was likely treated with insecticides) in Sweden in 2011. Management efforts for the swede midge in Europe and North America should take the control of volunteer canola seriously, as this may affect the infestation levels in nearby fields of crucifer vegetable crops. Also, if the swede midge populations were to increase in abundance and spread in Western Canada, the presence of widely abundant and herbicide-resistant volunteer canola could act as a reservoir for swede midge populations in concert with cruciferous weeds (Chen et al. 2009). This could increase the possibility of the swede midge becoming a pest of consequence in spring-planted canola in Western Canada. This becomes even more of a concern given the finding that swede midge infestation of spring-planted oilseed rape in Sweden was a relatively common occurrence, with at least some infestation found in nearly every field. In some fields, particularly along borders, the infestation appeared quite severe. This attack is not usually considered economically important in Sweden (Andersson 2005), although the actual impact of swede midge on spring-planted oilseed rape has not been directly studied. The perceived lack of economic consequence of swede midge attack may be in part due to the fact that the damage is difficult to recognize, and can be mistaken for nutritional deficiencies or mechanical damage. However, even if yield is not affected by swede midge infestation, heavy attack could cause delay or asynchrony in flowering and subsequent seed development (Andersson 2005; P. Abram, unpublished observations). If the swede midge does become established and abundant in Western Canada, a detailed assessment of its impact in spring-planted canola will be needed.

Based on the information at hand, it does not appear that the swede midge's parasitoid complex contains any strictly host-specific species; both species have been

reported to attack several other cecidomyiids in nature on a variety of host plants. Moreover, although both *S. myles* and *M. chalybeus* are geographically widespread and parasitize every generation of the swede midge, neither seems to consistently parasitize a large proportion of host populations, and there is evidence that phenological asynchrony may exist in this system. The decision of whether or not to introduce either *S. myles* and/or *M. chalybeus* to North America for control of the swede midge will need to be supported by a more rigorous evaluation of the potential risks and benefits. Life table studies in several European locations are needed to accurately quantify the population-level mortality inflicted on the swede midge by its parasitoids, and to identify whether there are other more important mortality factors. In addition, the evaluation of the host range of each of these species, both from field collections of other putative hosts and laboratory tests with native North American gall midges, will need to be undertaken. Unless these investigations can, contrary to the evidence to date, definitively show that (i) one or both of these parasitoid species are important biotic factors suppressing swede midge populations in Europe, and (ii) one or both of the parasitoid species are unlikely to attack non-target gall midges in the area of introduction either because of physiological or ecological incompatibility, it would be difficult to justify the introduction of either species to North America

Tables

Table 1. Sample sizes for the determination of swede midge (*Contarinia nasturtii*) infestation rates (plant dissections) and parasitism rates (midge and parasitoid rearing)

Year	Sampling Date	Number of plants dissected	Total number of midges and parasitoids emerged from rearing
2010	7-Jun	80	7
	14-Jun	160	3
	21-Jun	160	54
	28-Jun	240	90
	5-Jul	240	6
	12-Jul	320	73
	19-Jul	400	287
	26-Jul	200	0
	2-Aug	120	114
	9-Aug	160	203
	16-Aug	200	561
	23-Aug	160	166
	30-Aug	120	88
	6-Sep	120	328
	13-Sep	120	337
	20-Sep	120	487
2011	16-May	30	0
	23-May	30	0
	30-May	60	544
	6-Jun	60	226
	13-Jun	60	657
	20-Jun	90	181
	27-Jun	90	429
	4-Jul	120	304
	11-Jul	120	389
	18-Jul	120	303
	25-Jul	120	383
	1-Aug	90	511
	8-Aug	30	440
	15-Aug	30	119
	22-Aug	30	118
	29-Aug	60	801
	5-Sep	60	214
	12-Sep	60	119
	19-Sep	60	188*

Table 2. Results of collections of swede midge (*Contarinia nasturtii*) from throughout Europe (country codes in ISO format) showing the host plant sampled (VEG = crucifer vegetable crops; VR = volunteer oilseed rape; SR = spring-planted oilseed rape), collection date, total sample size (total number of midges and parasitoids emerged), and parasitism rate by *Synopeas myles*, *Macroglenes chalybeus*, *Inostemma opacum*, and *S. osaces*. *Non-diapausing emergence only.

Year	Site name	GPS Coordinates	Country	Host plant	Collection Date	Total N	% parasitism				
							<i>Synopeas myles</i>	<i>Macroglenes chalybeus</i>	<i>Inostemma opacum</i>	<i>Synopeas osaces</i>	Total
2008	Wädenswil	N 47°13'20'' E 08°40'09''	CH	VEG	09 Jul	1369	0.00	0.51	0.00	0.00	0.51
	Müntschemier 2	N 47°00' 27'' E 07°10'59''	CH	VEG	15 Jul	324	0.00	0.31	0.00	0.00	0.31
	Galmiz	N 46°59'38'' E 07°07'27''	CH	VEG	15 Jul	40	0.00	0.00	0.00	0.00	0.00
	Nübel	N 54°34'45'' E 09°36'15''	DE	VR	17 Jul	194	1.03	1.03	0.00	0.00	2.06
	Saint-Martin-des-Champs	N 48°33'14'' E 03°51'17''	FR	VEG	31 Jul	2225	0.00	0.00	0.00	0.00	0.00
	Müntschemier 1	N 46°59'22'' E 07°09'35''	CH	VEG	04 Aug	59	0.00	0.00	0.00	0.00	0.00
	Müntschemier 2	N 46°59'22'' E 07°09'35''	CH	VEG	04 Aug	1444	0.97	29.50	0.00	0.00	30.47
	Galmiz	N 46°59'38'' E 07°07'27''	CH	VEG	04 Aug	421	0.24	2.85	0.00	0.00	3.09
	Wädenswil	N 47°13'20'' E 08°40'09''	CH	VEG	05 Aug	3003	0.00	14.79	0.00	0.00	14.79
	Salenstein	N 47°39'43'' E 09°08'59''	CH	VEG	07 Aug	88	0.00	0.00	0.00	0.00	0.00
	Galmiz	N 46°59'38'' E 07°07'27''	CH	VEG	11 Aug	42	0.00	4.76	0.00	0.00	4.76
	Courroux	N 47°21'53'' E 07°22'29''	CH	VR	16 Aug	176	13.07	0.00	0.00	0.00	13.07
	Müntschemier 1	N 46°59'22'' E 07°09'35''	CH	VEG	21 Aug	307	0.00	0.00	0.00	0.00	0.00
	Galmiz	N 46°59'38'' E 07°07'27''	CH	VEG	21 Aug	726	0.00	0.96	0.00	0.00	0.96
	Nussbaumen	N 47°36'59'' E 08°50'56''	CH	VEG	25 Aug	2616	6.08	0.42	0.00	0.00	6.50
	Courroux	N 47°21'53'' E 07°22'29''	CH	VR	27 Aug	78	8.97	0.00	0.00	0.00	8.97

Table 2. cont'd

Year	Site name	GPS Coordinates	Country	Host plant	Collection Date	Total N	% parasitism				Total
							<i>Synopeas myles</i>	<i>Macroglenes chalybeus</i>	<i>Inostemma opacum</i>	<i>Synopeas osaces</i>	
2008	Wädenswil	N 47°13'20'' E 08°40'09''	CH	VEG	28 Aug	1070	2.62	1.31	0.00	0.00	3.93
	Courtetelle	N 47°20'29'' E 07°19'38''	CH	VR	15 Sep	38	5.26	0.00	0.00	0.00	5.26
2009	Wädenswil	N 47°13'20'' E 08°40'09''	CH	VEG	25 Jun	152	0.66	40.13	0.00	0.00	40.79
	Alle	N 47°25'55'' E 07°06'17''	CH	VR	21 Jul	380	6.05	0.00	0.00	0.00	6.05
	Wädenswil	N 47°13'20'' E 08°40'09''	CH	VEG	24 Jul	54	0.00	5.56	0.00	0.00	5.56
	Basadingen	N 47°39'52'' E 08°47'43''	CH	VEG	24 Jul	171	1.17	4.09	0.00	0.00	5.26
	Feldi	N 47°34'49'' E 08°47'41''	CH	VEG	24 Jul	855	0.00	0.00	0.00	0.00	0.00
	Steinegg	N 47°37'02'' E 08°52'01''	CH	VEG	24 Jul	198	0.00	0.00	0.00	0.00	0.00
	Hendschiken 1	N 47°23'23'' E 08°12'53''	CH	VEG	29 Jul	63	0.00	0.00	0.00	0.00	0.00
	Tagerwilen	N 47°39'49'' E 09°08'54''	CH	VEG	30 Jul	1939	0.52	2.63	0.00	0.00	3.15
	Kerseach	N 48° 35' 40'' E 03° 56' 26''	FR	VEG	30 Jul	1439	0.21	0.00	0.00	0.00	0.21
	Oedheim	N 49°13'34'' E 09°16'18''	DE	VEG	03 Aug	9178	0.73	1.42	0.00	0.00	2.15
	Heiligenhafen	N 54°21'50'' E 10°57'30''	DE	VR	06 Aug	2691	8.44	0.00	0.00	0.00	8.44
	Klaustorf	N 54°21'30'' E 11°00'36''	DE	VR	06 Aug	1098	2.09	0.27	0.00	0.00	2.28
	Gremersdorf	N 54°19'45'' E 10°55'49''	DE	VR	07 Aug	607	14.50	0.00	0.00	0.00	14.50
	Wangels	N 54°16'26'' E 10°46'22''	DE	VR	07 Aug	720	3.89	0.14	0.00	0.00	4.03
	Rastorf	N 54°16'51'' E 10°17'12''	DE	VR	07 Aug	1303	3.22	5.83	0.00	0.00	9.06
	Bendfeld	N 54°21'50'' E 10°25'33''	DE	VR	07 Aug	329	0.61	0.91	0.00	0.00	1.52

Table 2. cont'd

Year	Site name	GPS Coordinates	Country	Host plant	Collection Date	Total N	% parasitism				Total
							<i>Synopeas myles</i>	<i>Macroglenes chalybeus</i>	<i>Inostemma opacum</i>	<i>Synopeas osaces</i>	
2009	Sint-Katelijne-Waver	N 51°04'10 E 04°30'24	BE	VEG	12 Aug	229	0.87	0.44	0.00	0.00	2.18
	Alle	N 47°25'55 E 07°06'17	CH	VR	18 Aug	209	10.53	0.00	0.00	0.00	10.53
	Alle 2	N 47°25'41 E 07°07'20	CH	VR	18 Aug	698	22.92	0.14	0.00	0.00	23.07
	Courroux	N 47°21'53 E 07°22'29	CH	VR	18 Aug	315	10.79	0.32	0.00	0.00	11.11
	Feldi	N 47°34'49 E 08°47'41	CH	VEG	20 Aug	949	3.27	0.32	0.00	0.00	3.58
	Rickenbach	N 47°32'55 E 08°47'15	CH	VR	20 Aug	799	3.25	1.50	0.00	0.00	4.76
	Rheinfelden	N 47°33'04 E 07°48'53	CH	VR	20 Aug	48	18.75	2.08	0.00	0.00	20.83
	Wintertur	N 47°31'49 E 08°42'44	CH	VR	20 Aug	156	0.64	0.00	0.00	0.00	0.64
	Laupersdorf	N 47°18'42 E 07°39'25	CH	VR	25 Aug	695	2.16	0.43	0.00	0.00	2.59
	Hendschiken 2	N 47°23'02 E 08°13'28	CH	VR	25 Aug	708	11.30	6.64	0.00	0.00	17.94
	Rothrist	N 47°18'36 E 07°54'05	CH	VR	25 Aug	249	8.43	0.40	0.00	0.00	8.84
	Wädenswil	N 47°13'20 E 08°40'09	CH	VEG	27 Aug	195	0.00	0.00	0.00	0.00	0.00
	Aesch	N 47°28'40 E 07°33'35	CH	VR	27 Aug	1061	6.60	0.00	0.00	0.00	6.60
	Tägerwilen	N 47°39'49 E 09°08'54	CH	VEG	01 Sep	60	0.00	0.00	0.00	0.00	0.00
	Alle	N 47°25'55 E 07°06'17	CH	VR	20 Sep	126	4.76	0.00	0.00	0.00	4.76
2010	Dörflingen	N 47°70'31 E 08°71'76	CH	VR	08 Jun	58	0.00	0.00	0.00	0.00	0.00
	Solothurn	N 47°12'42 E 07°30'31	CH	VR	23 Jun	78	3.85	0.00	0.00	0.00	3.85
	Heiligenhafen	N 54°21'50 E 10°57'30	DE	VR	04 Jul	581	1.03	0.00	0.00	0.00	1.03

Table 2. cont'd

Year	Site name	GPS Coordinates	Country	Host plant	Collection Date	Total N	% parasitism				
							<i>Synopeas myles</i>	<i>Macroglenes chalybeus</i>	<i>Inostemma opacum</i>	<i>Synopeas osaces</i>	Total
2010	Kondoros	N 46°44'10 E 20°48'59	HU	VR	09 Jul	235	0.00	0.00	0.00	0.00	0.00
	Hmvhely 1	N 46°26'29 E 20°19'59	HU	VR	09 Jul	28	0.00	0.00	0.00	0.00	0.00
	Hmvhely 2	N 46°27'01 E 20°23'21	HU	VR	09 Jul	84	0.00	0.00	0.00	0.00	0.00
	Karduskut	N 46°26'25 E 20°43'35	HU	VR	09 Jul	286	0.00	0.00	0.00	0.00	0.00
	Albertsdorf	N 54°25'49 E 11°06'58	DE	VR	08 Aug	75	2.67	0.00	0.00	0.00	2.67
	Heiligenhafen	N 54°21'50 E 10°57'30	DE	VR	08 Aug	166	0.60	0.00	0.00	0.00	0.60
	Klaustorf	N 54°21'30 E 11°00'36	DE	VR	08 Aug	165	0.00	0.00	0.00	0.00	0.00
	Neuteschendorf	N 54°20'57 E 10°52'20	DE	VR	08 Aug	130	14.62	0.00	0.00	0.00	14.62
	Hornby	N 54°20'29 E -1°39'47	GB	VR	08 Aug	1915	0.16	0.00	0.00	0.00	0.16
	Bern	N 47°17'03 E 07°53'24	CH	VR	09 Aug	315	7.94	0.00	0.00	0.00	7.94
	Münchenbuchsee	N 47°03'66 E 07°41'98	CH	VR	09 Aug	85	5.88	0.00	0.00	0.00	5.88
	Bedale	N 54°19'42 E -1°41'04	GB	VR	09 Aug	650	0.00	0.00	0.00	0.00	0.00
	Ravensworth	N 54°47'75 E -1°77'93	GB	VR	09 Aug	106	0.00	0.00	0.00	0.00	0.00
	North Richmond	N 54°25'00 E -1°44'40	GB	VR	09 Aug	79	0.00	0.00	0.00	0.00	0.00
	Liebenswiler	N 47°30'03 E 7°27'40	FR	VR	11 Aug	1508	2.92	0.00	0.00	0.00	2.92
	Alle	N 47°25'55 E 07°06'17	CH	VR	11 Aug	1397	12.38	0.00	0.00	0.00	12.38
	Kovor 1	N 46°20'34 E 14°17'05	SI	VEG	17 Aug	267	0.00	22.10	0.00	0.00	22.10
	Kovor 2	N 46°20'34 E 14°17'05	SI	VEG	17 Aug	253	0.00	3.16	0.00	0.00	3.16

Table 2. cont'd

Year	Site name	GPS Coordinates	Country	Host plant	Collection Date	Total N	% parasitism				
							<i>Synopeas myles</i>	<i>Macroglenes chalybeus</i>	<i>Inostemma opacum</i>	<i>Synopeas osaces</i>	Total
2010	Kovor 3	N 46°20'34 E 14°17'05	SI	VEG	17 Aug	392	0.00	0.51	0.00	0.00	0.51
	Jezica	N 46°05'14 E 14°31'40	SI	VEG	18 Aug	236	0.00	0.00	0.00	0.00	0.00
	Jablje	N 46°08'39 E 14°34'04	SI	VR	18 Aug	1427	3.71	28.80	0.00	0.14	32.52
	Mengeš	N 46°09'20 E 14°34'33	SI	VR	18 Aug	151	0.66	1.32	0.00	0.00	1.99
	Feldi	N 47°28'52 E 07°35'29	CH	VEG	26 Aug	554	1.99	0.00	0.00	0.00	1.99
	Basadingen	N 47°40'12 E 08°43'41	CH	VR	26 Aug	1645	3.59	5.29	0.00	0.00	8.88
	Müntschemier	N 46°59'17 E 07°09'05	CH	VEG	02 Sep	138	0.00	0.00	0.00	0.00	0.00
	Galmiz 2	N 46°56'51 E 07°08'51	CH	VEG	02 Sep	955	0.31	0.00	0.00	0.00	0.31
	Gränichen	N 47°20'50 E 08°07'02	CH	VR	09 Sep	1089	3.21	0.00	0.00	0.00	3.21
	Rüstenschwil	N 47°13'71 E 08°22'16	CH	VR	09 Sep	171	8.19	0.00	0.00	0.00	8.19
	Neudorf	N 47°11'17 E 08°12'26	CH	VR	09 Sep	2398	4.34	0.00	0.00	0.00	0.00
	Birrhard	N 47°25'65 E 08°14'76	CH	VR	09 Sep	1553	5.47	1.61	2.06	0.00	9.14
	Courroux	N 47°21'53 E 07°22'29	CH	VR	10 Sep	609	0.99	0.00	0.00	0.00	0.99
	Alle	N 47°25'55 E 07°06'17	CH	VR	10 Sep	190	6.84	0.53	0.00	0.00	7.37
	Aesch	N 47°28'52 E 07°35'29	CH	VR	14 Sep	2446	0.94	0.00	0.00	0.00	0.94
	Heiligenhafen	N 54°21'50 E 10°57'30	DE	VR	19 Sep	695	16.40	0.14	0.00	0.00	16.55
2011*	Alle	N 47°26'22 E 07°08'51	CH	VR	07 May	31	0.00	0.00	0.00	0.00	0.00
	Bonfol	N 47°26'91 E 07°08'26	CH	VR	27 May	558	0.54	0.00	0.00	0.00	0.54

Table 2. cont'd

Year	Site name	GPS Coordinates	Country	Host plant	Collection Date	Total N	% parasitism				Total
							<i>Synopeas myles</i>	<i>Macroglenes chalybeus</i>	<i>Inostemma opacum</i>	<i>Synopeas osaces</i>	
2011*	Courroux	N 47°21'53 E 07°22'29	CH	VR	27 May	9123	28.66	0.54	0.00	0.00	29.20
	Bonfol	N 47°26'91 E 07°08'26	CH	VR	01 Jun	4828	2.98	0.02	0.00	0.00	3.00
	Wädenswil	N 47°13'20 E 08°40'09	CH	VEG	02 Jun	211	0.00	0.47	0.00	0.00	0.47
	Wöhlen	N 47°21'72 E 08°15'18	CH	VR	02 Jun	391	14.32	3.58	0.00	0.00	17.90
	Bonfol	N 47°26'91 E 07°08'26	CH	VR	06 Jun	1165	2.92	0.00	0.00	0.00	2.92
	Lönnstorp	N 55°40'04 E 13°07'06	SE	SR	17 Jun	523	0.00	0.00	0.00	0.00	0.00
	Kävlinge 2	N 55°48'46 E 13°04'45	SE	SR	18 Jun	2161	0.14	0.19	0.00	0.00	0.32
	Alstad 2	N 55°26'40 E 13°11'04	SE	SR	18 Jun	696	0.00	0.00	0.00	0.00	0.00
	Staffanstorp	N 55°39'76 E 13°12'62	SE	SR	18 Jun	349	0.57	0.00	0.00	0.00	0.57
	Bjuv	N 56°06'00 E 12°53'40	SE	SR	19 Jun	792	0.00	0.00	0.00	0.00	0.00
	Asmundstorp	N 55°52'80 E 12°57'60	SE	SR	19 Jun	402	0.25	0.00	0.00	0.00	0.25
	Eslöv	N 55°51'48 E 13°22'38	SE	SR	19 Jun	39	0.00	0.00	0.00	0.00	0.00
	Helsingborg	N 56°05'40 E 12°51'60	SE	SR	19 Jun	334	0.00	0.00	0.00	0.00	0.00
	Hörby	N 55°45'00 E 13°37'80	SE	SR	19 Jun	41	0.00	0.00	0.00	0.00	0.00
	Haljarp	N 55°52'20 E 12°56'40	SE	SR	19 Jun	175	0.00	0.00	0.00	0.00	0.00
	Kungabacken	N 55°47'10 E 13°35'16	SE	VR	19 Jun	2182	4.08	0.00	0.00	0.00	4.08
	Futterkamp	N 54°17'20 E 10°38'27	DE	VR	20 Jun	188	2.13	0.53	0.00	0.00	2.66
	Vällered	N 55°36'00 E 13°43'20	SE	SR	20 Jun	396	0.00	0.00	0.00	0.00	0.00

Table 2. cont'd

Year	Site name	GPS Coordinates	Country	Host plant	Collection Date	Total N	% parasitism				Total
							<i>Synopeas myles</i>	<i>Macroglenes chalybeus</i>	<i>Inostemma opacum</i>	<i>Synopeas osaces</i>	
2011*	Gärsnäs	N 55°32'40 E 14°12'60	SE	SR	20 Jun	80	0.00	0.00	0.00	0.00	0.00
	Mossby	N 55°25'20 E 13°36'00	SE	SR	20 Jun	36	0.00	0.00	0.00	0.00	0.00
	Nöbbelev	N 55°30'60 E 14°18'00	SE	VR	20 Jun	1100	0.09	0.00	0.00	0.00	0.09
	Simrishamn	N 55°30'60 E 14°18'00	SE	VR	20 Jun	291	0.00	0.00	0.00	0.00	0.00
	Klagstorp	N 55°22'80 E 13°19'80	SE	VR	20 Jun	78	0.00	0.00	0.00	0.00	0.00
	Fuglie	N 55°25'80 E 13°04'02	SE	VR	20 Jun	687	0.73	0.00	0.00	0.00	0.73
	Hoby	N 55°28'20 E 14°12'60	SE	VR	20 Jun	255	2.35	0.00	0.00	0.00	2.35
	Mjohult 2	N 56°09'00 E 12°40'80	SE	SR	21 Jun	283	0.00	0.00	0.00	0.00	0.00
	Lomma	N 55°44'40 E 13°04'80	SE	SR	21 Jun	158	0.00	0.00	0.00	0.00	0.00
	Ängelsbäcksstrand	N 56°21'60 E 12°46'20	SE	SR	21 Jun	111	0.00	0.00	0.00	0.00	0.00
	Lomma 2	N 55°45'00 E 13°04'80	SE	VR	21 Jun	637	1.10	0.00	0.00	0.00	1.10
	Mjohult	N 56°10'80 E 12°40'20	SE	VR	21 Jun	91	7.69	0.00	0.00	0.00	7.69
	Holbæk	N 55°68'18 E 11°73'12	DK	SR	22 Jun	284	2.11	0.00	0.00	0.00	2.11
	Køge	N 55°47'18 E 11°59'00	DK	VR	22 Jun	54	0.00	0.00	0.00	0.00	0.00
	Solrød	N 55°32'14 E 12°07'01	DK	VR	22 Jun	129	0.00	0.00	0.00	0.00	0.00
	Ringsted	N 55°38'24 E 11°83'24	DK	VR	22 Jun	613	6.36	0.16	0.00	0.00	6.53
	Lejre	N 55°37'49 E 11°57'35	DK	VR	22 Jun	127	0.00	0.00	0.00	0.00	0.00
	Veddelev	N 55°67'36 E 12°10'00	DK	VR	22 Jun	257	0.00	0.00	0.00	0.00	0.00

Table 2. cont'd

Year	Site name	GPS Coordinates	Country	Host plant	Collection Date	Total N	% parasitism				Total
							<i>Synopeas myles</i>	<i>Macroglenes chalybeus</i>	<i>Inostemma opacum</i>	<i>Synopeas osaces</i>	
2011*	Neustadt	N 54°06'17 E 10°50'80	DE	VR	23 Jun	112	2.68	11.61	0.00	0.00	14.29
	Sint-Katelijne-Waver	N 51°04'10 E 04°30'24	BE	VEG	28 Jun	91	0.00	3.30	0.00	0.00	3.30
	Bonfol	N 47°26'91 E 07°08'26	CH	VR	01 Jul	3543	2.77	0.00	0.00	0.00	2.77
	Sønderborg	N 54°54'88 E 09°49'89	DK	VR	01 Aug	124	0.81	0.00	0.00	0.00	0.81

Table 3. Other reported gall midge (Diptera: Cecidomyiidae) hosts of the swede midge (*Contarinia nasturtii*) parasitoids found in this study, and their host plants. UCD – Universal Chalcidoidea Database and references therein.

Parasitoid species	Gall midge species	Host plant(s)	Reference(s)
<i>Synopeas myles</i> Walker (Hym.: Platygastriidae)	<i>Contarinia tritici</i> Kirby	<i>Triticum</i> sp. (Poaceae)	Affolter (1990), Buhl (2009)
	<i>Dasineura marginemtorquens</i> Bremi	<i>Salix viminalis</i> L. (Salicaceae)	Strong and Larsson (1992)
	<i>Contarinia pisi</i> Winnertz	<i>Pisium sativum</i> L. (Fabaceae)	Keller and Schweizer (1994)
	<i>Contarinia medicaginis</i> Kieffer	<i>Medicago sativa</i> L. (Fabaceae)	Romankow and Dankowska (1993)
	<i>Dasineura ignorata</i> Wachtl	<i>Medicago sativa</i> L. (Fabaceae)	Romankow and Dankowska (1994)
	<i>Dasineura viciae</i> Kieffer	<i>Vicia tetrasperma</i> L. (Fabaceae)	Buhl (2009)
<i>Macroglenes chalybeus</i> Haliday (Hym.: Pteromalidae)	<i>Contarinia pisi</i> Winnertz	<i>Pisium sativum</i> L. (Fabaceae)	Keller and Schweizer (1994), UCD (2011)
	<i>Contrarinia tritici</i> Kirby	<i>Triticum</i> sp. (Poaceae)	UCD (2011)
	<i>Sitodiplosis mosellana</i> Géhin	<i>Triticum</i> sp. (Poaceae)	UCD (2011)
	<i>Contarinia thermopsisii</i> Kieffer	<i>Thermopsis turcestanica</i> (Fabaceae)	UCD (2011)
	<i>Dasineura viciae</i> Kieffer	<i>Vicia tetrasperma</i> L. (Fabaceae)	UCD (2011)
<i>Inostemma opacum</i> Thomson (Hym.: Platygastriidae)	<i>Contarinia medicaginis</i> Kieffer	<i>Medicago sativa</i> L. (Fabaceae)	Romankow and Dankowska (1993)
	<i>Dasineura ignorata</i> Wachtl	<i>Medicago sativa</i> L. (Fabaceae)	Romankow and Dankowska (1994)
<i>Synopeas osaces</i> Walker (Hym.: Platygastriidae)	<i>Contarinia medicaginis</i> Kieffer	<i>Medicago</i> sp. (Fabaceae)	Sundholm (1963), Ferron (1964)

Figures

Figure 1. Areas of Europe surveyed for parasitoids of the swede midge, *Contarinia nasturtii*. Single points indicate single sampling sites; ellipses indicate sampling areas containing several sites. The number of collections from each area/site is indicated. Country codes are in two-letter ISO format.

Figure 2. The distribution of total parasitism levels of the swede midge, *Contarinia nasturtii*, and the distribution of the levels attributable to each of the two dominant parasitoids, *Synopeas myles* and *Macroglanes chalybeus*, among samples collected in a survey of Europe.

Figure 3 The proportion of swede midge, *Contarinia nasturtii*, diapausing in samples collected from 2008-2010 plotted against the proportion of *Synopeas myles* and *Macroglenes chalybeus* diapausing. Correlation coefficients and p-values are from Spearman's rank correlation analysis.

Figure 4. Swede midge (*Contarinia nasturtii*) infestation levels ($\pm$ SE of the total number of swede midge/plant), swede midge pheromone trap catches, and percent parasitism by *Synopeas myles* and *Macroglanes chalybeus* in collections made weekly from a garden plot of summer oilseed rape in Delémont, Switzerland in 2010.

Figure 5. The proportions of diapausing swede midge (*Contarinia nasturtii*) larvae their parasitoids *Synopeas myles* and *Macroglanes chalybeus* in field collections made weekly in the summer of 2010 from a plot of summer oilseed rape in Delémont, Switzerland.

Figure 6. Swede midge (*Contarinia nasturtii*) infestation levels ($\pm$ SE of the total number of swede midge/plant), swede midge pheromone trap catches, and percent parasitism by *Synopeas myles* and *Macroglanes chalybeus* in collections made weekly from a garden plot of summer oilseed rape in Delémont, Switzerland in 2011.

Chapter 4: Biology of *Synopeas myles* Walker (Hymenoptera: Platygasteridae), a parasitoid of the swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae).

Abstract

Synopeas myles Walker (Hymenoptera: Platygasteridae) is a parasitoid of the swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae), in Europe, and is a candidate for classical biological control of this invasive pest in North America. We conducted the first thorough investigation of this parasitoid's biology, including longevity, fecundity, host stage suitability, immature development, and host discrimination. The effect of superparasitism on offspring fitness was also examined. We found that sugar feeding increased the longevity of adults, but longevity was considerably reduced in the presence of unlimited hosts. Contrary to the biology reported for all platygasterids to date, exposure of late-instar larvae to parasitism as opposed to eggs or early larvae tended to yield more *S. myles* offspring. Superparasitism was relatively common in the field and among groups of females in the laboratory, and was much less common among single females, providing preliminary evidence for self-discrimination. Inspection of immature stages of *S. myles* revealed that supernumerary larvae in superparasitized hosts were probably eliminated by physical combat soon after hatching. With increasing levels of superparasitism, sex ratios of offspring became more female-biased and their mean development time increased. The probability that an offspring would emerge from a host and the size of offspring were unaffected by increasing levels of superparasitism. It is suggested that *S. myles* and the swede midge

represent an ideal parasitoid-host system for future studies of the behavioural ecology of parasitoids.

Introduction

The Platygasteridae (Hymenoptera: Platygastroidea) are small parasitic wasps with over 5,300 species known worldwide (Hymenoptera Online Database 2011). The level of host specificity within subfamilies of this group is one of the highest among the parasitic Hymenoptera, with each subfamily or tribe associated with a single host family, subfamily, or tribe (Austin et al. 2005). Members of the subfamily Platygastriinae, the best-studied group of platygasterids, are only known to parasitize cecidomyiid gall midges (Vlug 1995), with some species parasitizing large proportions of host populations (Affolter 1990; Duso et al. 2011; Sampson et al. 2006; Williams et al. 1999; Zhao He and Wang 2011). Several species have been utilized in classical biological control programs for gall midge pests, with variable levels of success (Cameron et al. 1989; Clausen 1978; Olfert et al. 2003; Sandanayaka and Ramankutty 2007; Zhao He and Wang 2011).

Despite their incredible diversity and importance as natural enemies for many species of phytophagous gall midges, very little is known about the biology of the Platygastriinae. Among the species studied so far, several are gregarious, sometimes with polyembryonic development (Clausen 1940; Kim et al. 2011; Ogah et al. 2010; Patterson 1927). Most species, however, are solitary koinobiont endoparasitoids, ovipositing inside the eggs or newly hatched larvae of their hosts (Austin et al. 2005; Clausen 1940). In these parasitoid species, embryonic development of the parasitoid is usually delayed until the host larva has completed its development; the parasitoid larva then hatches and consumes its host (Kim et al. 2011). Larval morphology is diverse, and the number of

larval instars can range from one to three (Clausen 1940; Kim et al. 2011; Ogah et al. 2010). The first instar larvae of several species are cyclopiform, possessing large sickle-shaped mandibles which are used to eliminate competitors in the event of superparasitism, where a parasitoid lays an egg in an already-parasitized host (Affolter 1990; Clausen 1940; Sampson et al. 2006).

Superparasitism of hosts by solitary endoparasitoids is actually a relatively common phenomenon (Van Alphen and Visser 1990), despite the fact that each host normally yields only one parasitoid offspring. This results in competition between immature parasitoids, which can be in the form of direct physical combat or physiological suppression (Chau and Maeto 2008; Fisher 1971; Ueno 1997). The costs associated with physical combat/physiological suppression and the consumption of host resources by competitors prior to their death may negatively affect the fitness of the surviving individual (Harvey et al. 1993; Mackauer 1990; Ueno 1997). There have, however, been a few cases described in which superparasitism is beneficial, helping to overcome host immune defences (Hegazi and Khafagi 2008) or manipulating host behaviour to increase resource availability, thereby increasing the fitness of surviving parasitoid offspring (Bai and Mackauer 1992). Presumably because superparasitism reduces the chance of a given parasitoid egg surviving to adulthood and can sometimes reduce the fitness of offspring if they do survive, many species of parasitoids have been found to be less likely to lay eggs in hosts already parasitized by themselves, conspecifics, or heterospecific species, a phenomenon which has been termed host discrimination (Godfray 1994; Mackauer 1990). Host discrimination and the consequences of superparasitism for offspring fitness have not, to our knowledge, been studied in the Platygasterinae, although a few authors

have reported superparasitism of gall midges by platygastrinid parasitoids in the field (Affolter 1990; Sampson et al. 2006). The host-parasitoid systems used in the past to study host discrimination, superparasitism, and the outcomes of these experiments have often been limited, and/or their results potentially affected by the fact that time-consuming observations or destructive dissections of hosts were required to count the number of eggs inside host larvae (Bai and Mackauer 1992; Gonzalez et al. 2007; Harvey et al. 1993; Hegazi and Khafagi 2008). Platygastrinid parasitoids and their gall midge hosts may represent ideal systems to study these phenomena, since immature stages of the parasitoids can be easily observed inside living hosts, which are largely transparent (Sampson et al. 2006).

Synopeas myles Walker (Hymenoptera: Platygastridae) is a common and widespread parasitoid of the swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae) in its native European range (see Chapter 3). The swede midge has become a serious invasive pest of crucifer (*Brassica* spp.) crops in Canada and the northeastern United States since its detection in Canada in 2000 (Hallett and Heal 2001). Current management methods for the swede midge are inadequate and rely on heavy use of insecticides, while invasive populations continue to spread (Chen et al. 2011). Corlay et al. (2007) found no parasitoids attacking the swede midge in Québec, suggesting that the increases in the distribution and abundance of the midge could in part be due to a release from specialized natural enemies in its native range (Enemy Release Hypothesis). Pending further study, the introduction of *S. myles* to North America could be considered as a means of classical biological control. However, at the time of its discovery as a

common parasitoid of the swede midge in Europe, the biology of *S. myles* was virtually unknown, making its evaluation as a candidate biological control agent difficult.

This study is focussed on *S. myles* and is the first to comprehensively describe the biology of a parasitoid of the swede midge. We examined the (i) effect of sugar feeding on adult longevity, (ii) lifetime fecundity of adult females, (iii) host stage suitability, (iv) development of immature stages, (v) incidence of superparasitism in the field, and (vi) effect of superparasitism on offspring survival and fitness in laboratory trials. We conducted a preliminary test of the utility of the swede midge-*S. myles* system for studying host discrimination and the consequences of superparasitism, hypothesizing that increasing levels of superparasitism would negatively affect survival and fitness of surviving *S. myles* offspring.

This information will be crucial for further assessment of this parasitoid as a biological control agent of the swede midge, particularly for host range testing and mass rearing efforts. It will also contribute to the currently limited information available on the biology of platygastriids, an ecologically and economically important group of hymenopteran parasitoids.

Materials & Methods

Insects and general methodology

Laboratory colonies of swede midge and *S. myles* were established and periodically replenished using insects from field collections in Germany and Switzerland. The swede midge colony was maintained on cauliflower (*Brassica oleracea* L. var. *botrytis*), adapting the methods of Chen and Shelton (2007). Shoots were cut 7-8 days after

oviposition (at 22.5°C) and placed on soil/vermiculite mix (1:3 ratio) in sealed plastic 10 cm x 15 cm cylinders to allow larvae to enter the soil and pupate, with subsequently emerging adults used for the next set of infestations. These separate containers were used in place of plant pots for pupation to conserve space and to make manipulation (ie. delaying adult emergence with lower temperatures if necessary) of the insects more convenient. Growing tips containing larvae were taken from the swede midge colony for experiments as needed.

The *S. myles* colony was maintained by continuously offering cauliflower growing tips to 24-48h-old, mated and sugar-fed (5% sucrose solution) *S. myles* females (2 per growing tip) eight days after the plants were exposed to ovipositing swede midge, when 3rd-instar larvae were present. After 24h, growing tips as well as any larvae that had left the shoot during the parasitization period were transferred to a 10 cm x 15 cm plastic cylinder containing a soil/vermiculite mix to allow immature parasitoids to complete development.

In all experiments, *S. myles* were transferred between containers using a wet paintbrush. Unless otherwise stated: (a) female *S. myles*, when newly emerged (<24h old), were first transferred to a container with sugar water and males for 24h and then immediately used for experiments, (b) all laboratory experiments and rearing took place at 50-60 % RH, 22.5±1°C, and 16h: 8h (L:D). All mean values in the text are presented ± SE.

Effect of sugar feeding on adult longevity

To estimate the longevity of *S. myles* under laboratory conditions, newly emerged (<24h old) male and female wasps were placed individually in ventilated 2 cm x 6 cm plastic

vials with fine (150 meshes/inch) gauze stretched under the lid. Since females always emerged in the presence of males, and mating was observed to take place almost immediately following emergence, females used in experiments were assumed to be mated. Parasitoids were given access to a cotton wick soaked with either water (25 males, 25 females) or sucrose solution (25 males, 25 females). Survival was assessed daily by checking whether the wasps were dead (not moving). Cotton wicks were replaced every 2-3 days. Mean longevity in each of the treatments for each sex were compared with a t-test (assuming unequal variance), as were male and female longevity within treatments.

Host stage suitability

To examine which stage of swede midge is most suitable for *S. myles* attack, we offered female parasitoids cauliflower shoots 1 to 9 days after swede midge oviposition under no-choice conditions. Preliminary observations showed that swede midge eggs hatched after 2-3 days, 1st and 2nd-instar larvae were present from days 3-7, and mature (3rd-instar) larvae were present from days 7-9 and began to leave the growing tips on the 9th day. Growing tips were cut from the plant and placed in large (9.5 cm x 2 cm) plastic Petri dishes with moist filter paper and fine gauze stretched under the lid. One mated and sugar-fed female *S. myles* was then transferred to the base of the growing tip and removed 24 h later. To avoid any potential artificial selection for host stage brought on by laboratory rearing techniques, only *S. myles* emerging from field collections were used. After 24 h, parasitoids were removed from the Petri dishes, growing tips were wrapped in moist filter paper, and the swede midge larvae inside were allowed to complete their development. Using this methodology, we were able to rear mature swede

midge larvae from earlier stages, even newly laid eggs, with relatively low mortality.

When mature larvae began to emerge, the growing tip and any free larvae were transferred to soil/vermiculite mix in a ventilated plastic cup. Each of the nine host age treatments was replicated 10 times. Cups were checked daily for the emergence of adult insects, and the number of emerging adult swede midge and parasitoids was recorded. As the distributions of the number of offspring produced by females in each host age treatment were non-normal, a Kruskal-Wallis test was used to test if the means of the ranked values varied significantly among treatments.

Development of immature stages

To observe immature stages of *S. myles* inside swede midge larvae, 3rd-instar swede midge larvae were taken from growing tips used in the maintenance of the parasitoid colony after they had been exposed to parasitoids for 24h. Rather than allowing the larvae to enter soil to construct cocoons, they were kept in small (5 cm x 1.5 cm) Petri dishes containing moist filter paper and with fine gauze stretched under the lid. Each day, larvae were examined under a compound microscope at 600x magnification. Living host larvae were transferred to glass slides with a paintbrush and compressed gently with a coverslip, which allowed the observation of *S. myles* eggs and larvae inside (adapted from Sampson et al. 2006). On each day, a subsample of host larvae containing immature stages of *S. myles* were cleared, stained, and mounted on glass slides using the methods of Sampson et al. (2006). The mandible length and width, as well as the head capsule width and length of mounted specimens were measured using the Leica Application Suite (Leica Microsystems, Germany) with a Leica DFC 425C camera, and means ($\pm$ SE) were calculated for each measurement.

To examine the effect of temperature on the time required for *S. myles* to complete egg to adult development, groups of 150 swede midge larvae, after 24 hours of exposure to parasitoids, were transferred to soil/vermiculite mix in plastic cylinders. Cylinders were stored at 9.5 ± 1.0 , 13.5 ± 1.0 , 20.0 ± 1.0 , 22.5 ± 1.0 , or $30.0 \pm 1.0^\circ\text{C}$. Emergence of unparasitized swede midge and *S. myles* adults was scored daily, and average egg-adult development times were calculated for each temperature. Male and female development times at each temperature were compared with t-tests (assuming equal variances).

Age-specific and potential lifetime fecundity

Twenty-five newly emerged, lab-reared *S. myles* females were allowed to feed on sucrose solution for 15 min in the presence of males, which always resulted in mating within the first 5 min. To estimate potential lifetime fecundity, single female wasps were then placed in small Petri dishes with a piece of swede midge-damaged cauliflower leaf and 50 3rd-instar swede midge larvae. Each day until their death, parasitoids were offered 50 new swede midge larvae and were given the opportunity to feed on sucrose solution. Two days after being offered to a parasitoid (when parasitoid eggs had increased in size sufficiently to be observed), swede midge larvae were examined at 600x magnification (see methods above) to count the number of eggs inside. This allowed the calculation of age-specific fecundity (the average number of eggs laid by parasitoids on each day of their lives). To examine whether individual females distributed their eggs randomly among hosts, the distributions for each female were compared to Poisson distributions with the same mean using a Pearson chi-squared goodness-of-fit test.

A separate subsample of newly-emerged females ($n = 26$) were also dissected at 400x magnification in a drop of water to estimate their egg load. The mean number of eggs in these females was compared to the mean total number laid by females in the above experiment using a t-test (assuming equal variances).

Incidence of superparasitism in the field

In 2011, weekly collections of plants were made from an untreated plot in Delémont, Switzerland with staggered plantings of summer oilseed rape (*Brassica napus* L.) (see Chapter 3). From July to September, third-instar larvae from plant dissections were kept in small Petri dishes for 2 days and then examined at 600x magnification to count the number of *S. myles* eggs inside. Rarely, *S. myles* larvae had already hatched after the two day incubation period but were scored as eggs since they would not have hatched at the time of collection. The overall percentage of parasitized hosts that were superparasitized was determined, and the distribution of eggs among hosts was compared to a Poisson distribution with a Pearson chi-squared goodness-of-fit test.

Superparasitism by groups of females in the lab and effect on offspring fitness

To examine the effect of superparasitism on survival and fitness of parasitoid offspring, 20 groups of four lab-reared female *S. myles* were offered 100 3rd-instar swede midge larvae for 24h in a small Petri dish (with fine gauze stretched under the lid) containing a small piece of swede midge-damaged cauliflower leaf. Two days after parasitoids were removed from the dish, host larvae were examined and the number of eggs inside each was counted. For each replicate (group of female parasitoids), host larvae were separated by the number of parasitoid eggs they contained and placed on a soil/vermiculite mix in

ventilated plastic vials. In some cases, a few larvae died before examination, preventing the determination of the number of eggs they contained; these were excluded from analysis. Adult parasitoids emerging from the plastic vials were counted and sexed daily. The right hind tibia (RHT) length of each wasp was measured using an ocular micrometer at 400x magnification and was used as an index of body size (Sagarra et al. 2001).

For each replicate (group of females), the distribution of eggs among larvae was compared to a Poisson distribution with a Pearson chi-squared goodness-of-fit test. For the experiment testing the effect of superparasitism on offspring fitness, the number of *S. myles* emerging per host (ie. the proportion of hosts yielding a parasitoid offspring in a solitary endoparasitoid system) and the percent female offspring of replicates containing the given treatment were calculated for each treatment (level of superparasitism, ie. number of eggs in hosts). For the analysis of the effect of the number of eggs in a host on offspring RHT length and development time, all replicates of each treatment were pooled and separated by the sex of offspring. The relationship between the number of eggs and each of the above parameters was assessed using regression analysis. Regression models were chosen using the Akaike Information Criterion (Akaike 1974).

Statistical analysis software

All statistical analyses were performed using JMP 8.0 (SAS Institute, 2008).

Results

Effect of sugar feeding on adult longevity

Sugar-fed females lived an average of 12.1 ± 1.8 days, significantly longer than females fed only water, which lived only 2.0 ± 0.2 days on average ($t = -5.55$, $p < 0.0001$, $n = 25$,

25). The same significant trend was observed for males, which lived an average of 8.0 ± 5.0 days when sugar-fed and 2.8 ± 1.0 days when fed only water ($t = -5.18$, $p < 0.0001$, $n = 25$, 25). There was no significant difference in male and female survival when wasps were sugar-fed ($t = -1.97$, $p = 0.057$), however males lived significantly longer than females when given only water ($t = 2.91$, $p < 0.01$).

Host stage suitability

The number of *S. myles* offspring emerging varied significantly with host age at exposure ($\chi^2 = 50.77$, $p < 0.0001$, $df = 8$) (Figure 7). Parasitoids emerged from hosts exposed as early as 3 days after host eggs were laid, corresponding to the time when eggs begin to hatch. No parasitoid offspring emerged from swede midge exposed when they were 1 or 2 days old (eggs), while the most parasitoid offspring tended to emerge from hosts exposed at 6-9 days old (2nd-3rd instar larvae).

Development of immature stages

Synopeas myles eggs, usually deposited next to the midgut of the 3rd-instar host larva (Figure 8A, 8B), became easily visible two days after parasitization, when they had swollen to $255.9 \pm 43.6 \mu\text{m}$ ($n = 25$) and the still-transparent, developing parasitoid larva could barely be seen enclosed by the embryonic trophamnion. When eggs were laid in 1st or 2nd-instar swede midge larvae, the egg swelled slightly, until just barely visible, and then ceased development until the host larva reached the third instar. Some eggs were encapsulated by host larvae (Figure 8C), a feature distinguishable by dark melanization, although this was relatively uncommon ($< 5\%$ of eggs). Three to four days after parasitization, corresponding to the time when the host larva would have finished

constructing its cocoon in the soil, first-instar *S. myles* larvae (Figure 8D) emerged from their trophamnion. First instars were cyclopiform, with a large head capsule (width: $142.3 \pm 2.5 \mu\text{m}$, length: $207.3 \pm 4.2 \mu\text{m}$, $n = 48$) and sickle-shaped mandibles (width: $31.2 \pm 0.9 \mu\text{m}$, length: $92.3 \pm 2.0 \mu\text{m}$, $n = 48$). In superparasitized hosts (Figure 8B), newly emerged larvae were often found directly next to each other inside the host. First instar larvae became partially engorged (Figure 8E) 4-6 days after parasitization and fully engorged 6-7 days after parasitization, by which time only one larva was alive in each host. In superparasitized hosts, supernumary larvae had apparently been killed by the surviving individual (they had stopped developing and were not moving). Direct physical combat of the parasitoid larvae was not observed in living hosts, but larvae were much more active (movement of mandibles and furca) while in close proximity to each other than when they were alone in a host. Some first-instar larvae, upon hatching, did not develop any further - even in singly parasitized hosts. Eight to nine days after parasitization, second instar larvae (Figure 8F), hymenopteriform and lacking mandibles, occupied the majority of the inside of the host. Pupation occurred soon after (9-11 days after parasitization) the developing wasp then gradually taking form (Figure 8G). After 10-12 days (21 days after parasitization), the mature *S. myles* adult would emerge (Figure 8H).

The observed range of egg-adult development times (Table 4) for *S. myles* in this study was from ~25 days at 22.5°C to ~75 days at 13.5°C. Only 4.7% of all insects survived at 30.0°C, all of which were midges. No insects emerged at 9.5°C, even after 100 days. An attempt to recover cocoons from the container kept at 9.5°C was unsuccessful, so it is not known whether viable larvae were still present. Mean male

development time was significantly less than that for females at 20.0°C ($t = -2.06$, $p = 0.047$, $n = 13, 24$), but not at 13.5°C ($t = -1.93$, $p = 0.062$, $n = 15, 19$) or 22.5°C ($t = -0.60$, $p = 0.55$, $n = 13, 25$).

Age-specific and potential lifetime fecundity

When newly emerged *S. myles* females were continually offered swede midge larvae on each day of their lives, they had a mean potential lifetime fecundity of 67.2 ± 5.9 eggs, which was not significantly different from the average egg load of 59.7 ± 4.7 mature eggs contained in newly emerged females ($t = 1.00$, $p = 0.32$, $n = 25, 26$). Females in these trials lived an average of only 2.8 ± 0.2 days. Mean age-specific fecundity declined continuously, from 32.9 ± 2.4 eggs on the first day to 12.5 ± 3.1 on the fourth day, when only 6 out of 25 females were still alive (Figure 9).

Of the swede midge larvae parasitized by individual *S. myles*, an average of 6.0 ± 1.1 % were superparasitized, all of which contained two eggs. The distribution of eggs among hosts (Table 5) was non-random (was significantly different from a Poisson distribution with the same mean) for 18 out of 25 females ($p < 0.05$). The number of hosts containing one egg tended to be higher than expected if the distribution were random, while the number containing two eggs tended to be lower than expected.

Incidence of superparasitism in the field

The distribution of *S. myles* eggs among field-collected swede midge larvae on each of 13 collections dates in the summer of 2011 is shown in Table 6. Overall, 11.4% (199/1748) of swede midge larvae were parasitized by *S. myles*. Of these, 19.1% (38/199) were superparasitized. Swede midge larvae were found to contain up to eight *S. myles* eggs,

although the average number of eggs per host was much lower, ranging from 0.09 to 0.72. The distribution of eggs among hosts was significantly different from random in 7 out of 13 weeks ($p < 0.05$). There was no apparent trend in the distribution of eggs among hosts; in some weeks levels of superparasitism were higher than expected, while they were lower than expected in other weeks.

Superparasitism by groups of females in the lab and effect on offspring fitness

Table 7 shows the distribution of *S. myles* eggs among groups of swede midge larvae offered to groups of four *S. myles* females in the laboratory. Superparasitism was very common, occurring in every replicate; swede midge larvae contained up to nine *S. myles* eggs and the mean number of eggs per host larva ranged from 1.06 to 2.53. The distribution of eggs among hosts was significantly different from random in 11 out of 16 replicates ($p < 0.05$). Similar to the tests with single females, the number of hosts containing one egg tended to be higher than expected if the distribution were random.

When hosts containing different numbers of *S. myles* eggs were reared to adulthood, there was not a significant relationship between the level of superparasitism and the mean number of *S. myles* adults emerging per host larva ($R^2 = 0.01$, $p = 0.76$) (Figure 10A). However, there was a significant positive relationship between the level of superparasitism and the percent of emerging offspring that were female ($R^2 = 0.33$, $p < 0.001$), best described by a logarithmic regression (Figure 10B). There was no significant relationship between level of superparasitism and RHT length of males ($R^2 = 0.01$, $p = 0.22$) or females ($R^2 < 0.01$, $p = 0.99$) (Figure 11A). There was a significant positive relationship between the level of superparasitism and development time for both female

($R^2 = 0.20$, $p < 0.001$) and male ($R^2 = 0.17$, $p < 0.001$) offspring, best described by logarithmic regressions (Figure 11B).

Discussion

Effective screening of potential biological control agents depends on a thorough understanding of their basic biology. When exploration in a pest's native range identifies species that are members of an understudied taxonomic group such as the Platygasteridae, studies of basic biology are particularly important. Here we made significant progress in understanding the biological relationships between *S. myles* and its host, while demonstrating the potential utility of this system for the study of the behavioural ecology of solitary endoparasitoids.

We found that adult *S. myles* were relatively short-lived (2-12 days in the laboratory), but that the longevity of both males and females was increased if they had access to sugar. An increase in longevity with access to sugar has been reported for most Hymenopteran parasitoids (Duncan et al. 2007; Jervis and Kidd 1986; Keller and Schweizer 1994; Onagbola et al. 2007) and suggests that, in nature, female *S. myles* are likely to search for both hosts and carbohydrate resources (i.e. nectar from flowering plants). The availability of these resources could affect their realized fecundity, the number of hosts they are able to parasitize over their lifetime (Desouhant et al. 2005). The findings that adult *S. myles* females are capable of laying eggs immediately upon emergence (Figure 9) and that their lifetime fecundity is equal to their egg complement at emergence indicate that this species is probably to a large degree proovigenic (emerges with fully developed eggs and does not develop any during its adult life) (Jervis et al. 1991), although females were not dissected after their deaths to confirm that they did not

contain additional eggs. Other platygastriids studied to date, although very few, have been shown to be almost completely proovigenic (Jervis et al. 2001). Evidence for host-feeding, the uptake of host haemolymph, was not observed. This is not surprising, since host-feeding is only known to occur in largely synovigenic species where the protein ingested is required for the development of new eggs (Jervis and Kidd, 1986). Female *S. myles* had a considerably shorter lifespan when given unlimited access to hosts. A trade-off between reproduction and somatic function (ex. lifespan) has been observed in many parasitoid species, although this should, in theory, only apply to synovigenic species that still have to invest resources in egg development during their adult lives (Bai and Smith 1993; Jervis et al. 2008). It is possible that female *S. myles*, when deprived of hosts in the longevity experiment, resorbed unused eggs which increased their longevity relative to females who had virtually unlimited access to hosts, although ovisorption is rarely observed in proovigenic species (but see Agboka et al. 2002). It is also possible that the energy reserves in females with access to hosts was depleted more quickly due to the energy required to search for and parasitize hosts.

Knowledge of the stage of a host most susceptible to parasitism is critical for the study and interpretation of host-parasitoid population dynamics in the field (Van Driesche 1983) as well as, in a biological control context, the development of mass rearing and host range testing programs. All platygastriids previously studied are reported to parasitize the eggs or newly hatched larvae of their hosts, but this is often assumed (Affolter 1990; Buhl et al. 2008; Sampson et al. 2006; Vlugg 1995), rather than directly tested (but see Ogah et al. 2010). Contrary to published records on other platygastriids, we found that more offspring were produced by *S. myles* females when they were offered

second or third-instar swede midge larvae on cauliflower growing tips than when they are offered eggs or 1st-instar larvae (Figure 7). Our trials of host stage suitability did not directly test the host stage preference of the female parasitoids. Rather, these trials only tested whether hosts exposed at different ages would yield different numbers of parasitoid offspring, so parasitoids may have oviposited in eggs or 1st-instar larvae that subsequently failed to yield a parasitoid offspring due to mortality of the immature parasitoid. However, the observation that egg development is delayed until the host larva reaches maturity probably means that the observed differences in host susceptibility were more likely due to differing levels of acceptance by the female parasitoids rather than differences in offspring survival when eggs were laid in differently-aged hosts (for example, mortality caused by larvae hatching in young hosts where insufficient resources were available). Although the apparent tendency of *S. myles* females to produce more offspring when host larvae are mature could be due to higher levels of acceptance by ovipositing females, the observed trend could also be due to the fact that mature swede midge larvae are more accessible; it is possible that earlier instars would be accepted at similar rates given equal accessibility. When swede midge larvae hatch from their eggs, they immediately move into the centre of the growing tip, concealed by tightly packed young leaves. After about 5-6 days at 22.5°C, corresponding to the age at which the number of hosts parasitized by *S. myles* increases, swede midge larvae move to the outer regions of the growing tip, becoming more exposed.

The development of the immature stages of the Platygasteridae has received some attention (Clausen 1940; Kim et al. 2011; Ogah et al. 2010; Sampson et al. 2006) but remains as a whole understudied, particularly in the genus *Synopeas*. The characteristics

of immature development of *S. myles* were found to generally parallel those of species described previously. There was evidence for only two larval instars, the first cyclopiform with large mandibles and the second hymenopteriform and amandibulate. There was no indication of an intermediate, mandibulate, hymenopteriform instar as described in the *Synopeas* spp. studied by Sampson et al. (2006). The number of larval instars in the Platygasteridae is known to be quite variable, however, even in closely related species (Clausen 1940; Kim et al. 2011; Ogah et al. 2010; Sampson et al. 2006). In superparasitized swede midge larvae, the fact that first-instar larvae were often found in close proximity after hatching and that all but one individual was typically dead soon after indicates that physical combat between the larvae occurred, and this may be a part of the mechanism of supernumary elimination. In other species, it has been shown that physical combat between immature parasitoid larvae need not necessarily result in death; it may only result in injury of one or both of the individuals which then succumb to the host's immune system (Hegazi et al. 1991). While this remains untested, this host-parasitoid system presents a unique opportunity to directly study the behaviour of immature parasitoid larvae inside living hosts, an area of the behavioural ecology of parasitoids that is virtually unknown.

Temperature is an important factor guiding the population dynamics of parasitoids and their hosts (Porter 1983; Sandanayaka and Ramankutty 2007). In this study, we assessed the egg to adult development time of *S. myles* as well as the larval-adult development time of unparasitized swede midge larvae. The development time curves of both *S. myles* and unparasitized swede midges showed a predictable negative relationship between 13.5 and 22.5°C, and survival between these temperatures was approximately

equal (Table 4). No insects, however, emerged at 9.5°C, and only a few midges emerged at 30°C. Sustained high temperatures have been shown many times to increase the mortality of developing insects (ex. Mann et al. 1990; Miller and Gerth 1994), and it appears that 30°C is unsuitable for the development of the swede midge and *S. myles*. The fact that no insects at all emerged at 9.5°C may indicate that this temperature is below the developmental threshold for both species. Readshaw (1966) found that the larval-adult development time of the swede midge at an average temperature of 10°C was about 34 days, but this was at fluctuating temperatures outdoors where temperatures may have periodically exceeded the swede midge's developmental threshold. More trials at different temperatures between 9.5 and 13.5°C, and between 22.5 and 30°C are needed to determine the lower and upper developmental thresholds of the swede midge and *S. myles*. A detailed picture of the relative development rates of *S. myles* and its host across the full range of temperatures could allow the investigation of physiological asynchrony between the two species (i.e. their development rates are different at the same temperatures). If this is true, it may help to explain what appears to be a lack of synchronization between host and parasitoid populations in the field (Chapter 3).

Since *S. myles* is a solitary species (only one of its offspring can survive in each parasitized host larva), it was interesting to consider what the fitness consequences might be for its offspring if hosts were superparasitized, an occurrence which was relatively common among parasitized hosts in the field (Table 6). It was hypothesized that the host resources consumed by competitors and the time and energy required to engage in physical combat might decrease the chances of completing development, reduce the size, or delay the development of the 'winning' *S. myles* individual. Increasing levels of

superparasitism did not, in fact, affect either the probability that a parasitoid would emerge from a host or the size of emerging individuals. However, the proportion of emerging offspring that were female and the development time of both male and female offspring increased with increasing levels of superparasitism (Figures 10 and 11). Some past research has also found that development time of solitary endoparasitoid offspring increases in superparasitized hosts, without an effect on offspring size or per-host survival (Eller et al. 1990; Harvey et al. 1993), although different trends have been found in other studies (Potting et al. 1997; Ueno 1997). The relatively longer development times of offspring in superparasitized hosts could be due to the time required to seek out and kill conspecifics, which would result in a loss of feeding time. The finding that more females emerge from superparasitized hosts, which has also been previously reported in other species, could be explained by superior competitive ability of female larvae (Darrouzet et al. 2003; Van Baaren et al. 1999). It is also possible that this trend is due to discrimination of ovipositing females in favour of high quality host larvae (Montoya et al. 2011). The cues that female *S. myles* use to assess host quality are unknown, but it is possible that the most important is host size, which was not strictly controlled for in this experiment. Host size is often an indicator of quality (the amount of resources available for developing offspring) and parasitoids have been shown to be more likely to oviposit in larger hosts (Godfray 1994; Li and Mills 2004). In addition, haplodiploid hymenopteran parasitoids are more likely to lay female (fertilized) eggs in higher-quality hosts and male eggs in lower-quality hosts (King 1988). Therefore, it is proposed that larger (higher quality) larvae in this experiment were superparasitized at a higher level, and also contained more fertilized eggs that would give rise to female offspring. This

explanation is only valid, however, if females were unable to detect conspecific parasitism, which remains unclear (see below). If they could detect the eggs of other females inside hosts, they would presumably have perceived already-parasitized hosts as lower quality (Godfray 1994) and be more likely to lay an unfertilized (male) egg, male-biasing the offspring sex ratio in superparasitized hosts. Whether *S. myles* discriminates between smaller and larger hosts, both in terms of whether they are likely to superparasitize these hosts and whether their allocation of female vs. male eggs differs, was not directly tested here and should be the subject of future study.

Past studies of solitary endoparasitoids have shown that discrimination against self-superparasitism is common (although not universal), as is discrimination against conspecific parasitism (Godfray 1994). There was a strong indication that single *S. myles* females avoided self-superparasitism, as evidenced by typically lower-than-expected numbers of superparasitized hosts and higher-than expected numbers of singly parasitized hosts (Table 5). It also appeared that discrimination sometimes took place in groups of four females, although it is not known to what degree this was a result of avoiding self-superparasitism as opposed to conspecific superparasitism (some proportion of hosts encountered by a female would contain their own eggs). The evidence for discrimination from field collections was even more equivocal, with non-random distributions of eggs among hosts sometimes observed but without a discernable overall pattern. This is likely due to a suite of complicating factors influencing the oviposition choices of female *S. myles* in the field, including the state of the parasitoids themselves (ex. age, egg limitation, the ability to recognize self- and conspecific-superparasitism) as well as extrinsic biotic (ex. host density and patchiness, host quality) and abiotic (ex.

temperature, weather conditions) factors (Fellowes et al. 2005). To supplement the preliminary evidence presented here, more laboratory studies are needed to directly test whether *S. myles* discriminates against self and conspecific superparasitism. Field investigations are also needed to identify factors that influence levels of superparasitism and what the consequences might be for the ability of *S. myles* to cause mortality in swede midge populations.

The use of the swede midge and *S. myles* as a host-parasitoid system for studies of behavioural ecology, although previously unused, is ideal due to the short generation times of the insects (20-30 days), their ease of manipulation, and the ease of observation of immature parasitoids in living hosts without the need for dissections. The inclusion of knowledge derived from the current investigation as well as future studies of this system into the body of work currently available concerning the behavioural ecology of parasitoids will be useful, since almost no studies to date have utilized platygastriids as models. More information on the behavioural ecology of *S. myles* will also be informative as its evaluation as a potential biological agent for the swede midge in North America continues. Properly conducted host range testing (Bigler et al. 2006) for example, requires a comprehensive knowledge of a parasitoid's basic biology and behaviour. In addition, this study's finding that superparasitism causes female-biased sex ratios could be used to inform mass-rearing of *S. myles* should it be approved for release in North America. In mass rearing, large numbers of males are not desirable; manipulations of relative host/parasitoid densities could be used to increase the incidence of superparasitism and bias the sex ratio towards females (Montoya et al. 2011).

This study has provided the first detailed biological description of a swede midge parasitoid. The information gathered by our experiments and observations will be useful for the evaluation of *S. myles* as a potential biological control agent of the swede midge, and will also provide a basis for future work focussed on its behavioural and evolutionary ecology.

Tables

Table 4. The survival and mean development time ($\pm$ SE) of *Synopeas myles* males and females at different temperatures. The time required for unparasitized adult swede midge (*Contarinia nasturtii*) to emerge is also shown. * = (N emerging midges + N emerging parasitoids/total number of swede midge larvae originally placed on soil).

Temperature (°C)	overall % survival*	Egg-adult development time of <i>S. myles</i>		3rd instar- adult (midges)
		male	female	
9.5	0.0	---	---	---
13.5	68.0	75.4 $\pm$ 0.6 (n = 15)	77.0 $\pm$ 0.5 (n = 19)	32.5 $\pm$ 0.2 (n = 68)
20.0	72.7	30.2 $\pm$ 0.5 (n = 13)	31.4 $\pm$ 0.3 (n = 24)	14.2 $\pm$ 0.1 (n = 72)
22.5	67.3	24.5 $\pm$ 0.6 (n = 13)	24.8 $\pm$ 0.3 (n = 25)	10.7 $\pm$ 0.1 (n = 63)
30.0	4.7	---	---	8.0 $\pm$ 0.0 (n = 7)

Table 5. The distribution of parasitoid eggs among swede midge (*Contarinia nasturtii*) larvae offered to 25 single *Synopeas myles* females in the laboratory. Using a Pearson chi-squared goodness-of-fit test, each observed distribution (O) was compared to a Poisson distribution (E) with a mean and variance equal to the group's mean number of parasitoid eggs per host larva (expected values shown are rounded to the nearest integer). χ^2 values with an asterisk indicate that the observed distribution differs significantly from its fitted Poisson distribution ($p < 0.05$).

Female	N Eggs in swede midge larvae (frequency)			Mean # eggs/host larva	Pearson χ^2
	0	1	2		
	O	O	O		
1	93	99	4	0.55	38.76*
2	85	109	3	0.58	53.85*
3	117	83	0	0.41	27.59*
4	115	73	6	0.44	11.60*
5	54	42	2	0.47	11.35*
6	58	40	1	0.41	11.24*
7	71	23	3	0.30	0.45
8	31	63	3	0.71	40.49*
9	31	65	2	0.70	45.40*
10	56	76	9	0.67	26.35*
11	65	79	3	0.58	35.27*
12	38	110	1	0.75	99.60*
13	64	80	3	0.59	36.79*
14	53	31	8	0.51	1.68
15	20	70	5	0.84	58.51*
16	45	5	0	0.10	0.27
17	82	58	5	0.47	10.49*
18	66	32	1	0.34	5.69
19	54	72	12	0.70	20.51*
20	107	39	2	0.29	3.56
21	150	48	1	0.25	5.68
22	28	50	11	0.81	17.95*
23	110	88	1	0.45	31.66*
24	97	51	1	0.36	11.24*
25	80	16	2	0.20	0.19

Table 6. The distribution of *Synopeas myles* eggs among 3rd-instar swede midge (*Contarinia nasturtii*) larvae collected from a garden plot in Delémont, Switzerland in 2011 compared with a Poisson distribution with the same mean. Using a Pearson chi-squared goodness-of-fit test, each observed distribution (O) was compared to a Poisson distribution (E) with a mean and variance equal to the group's mean number of parasitoid eggs per host larva (expected values shown are rounded to the nearest integer). χ^2 values with an asterisk indicate that the observed distribution differs significantly from its fitted Poisson distribution ($p < 0.05$).

Sampling Date	Number of <i>S. myles</i> eggs in swede midge larvae (frequency)										Mean # eggs/host larva	Pearson χ^2
	0	1	2	3	4	5	6	7	8			
	O	O	O	O	O	O	O	O	O			
1	45	3	1	0	2	1	0	0	1	0.72	11217.77*	
2	79	8	1	0	0	0	0	0	0	0.11	0.60	
3	50	13	2	0	0	0	0	0	0	0.26	0.20	
4	54	5	1	0	0	0	0	0	0	0.12	1.38	
5	37	3	2	0	0	0	0	0	0	0.17	6.13	
6	184	32	4	1	4	0	0	0	0	0.26	466.95*	
7	316	32	2	1	0	0	0	0	0	0.36	12.25*	
8	86	9	3	1	1	0	0	0	0	0.22	136.07*	
9	25	4	0	0	0	0	0	0	0	0.14	0.01	
10	466	32	4	1	0	0	0	0	0	0.09	23.47*	
11	59	8	1	1	0	0	0	0	0	0.19	14.54*	
12	77	9	1	0	0	0	0	0	0	0.13	0.32	
13	71	3	1	1	0	0	0	0	0	0.11	76.78*	

Table 7. The distribution of parasitoid eggs among 16 groups of swede midge (*Contarinia nasturtii*) larvae, each offered to groups of four *Synopeas myles* females in the laboratory. Using a Pearson chi-squared goodness-of-fit test, each observed distribution (O) was compared to a Poisson distribution (E) with a mean and variance equal to the group's mean number of parasitoid eggs per host larva (expected values shown are rounded to the nearest integer). χ^2 values with an asterisk indicate that the observed distribution differs significantly from its fitted Poisson distribution ($p < 0.05$).

Replicate	Number of <i>S. myles</i> eggs in swede midge larvae (frequency)											Mean # eggs/host larva	Pearson χ^2
	0	1	2	3	4	5	6	7	8	9			
	O	O	O	O	O	O	O	O	O	O			
1	9	67	11	0	1	0	0	0	0	0	1.06	61.17*	
2	2	40	28	17	6	0	0	0	0	0	1.84	21.44*	
3	8	70	15	3	0	1	0	0	0	0	1.18	58.11*	
4	5	16	32	14	12	3	3	1	0	0	2.44	8.10	
5	13	46	29	6	1	0	0	0	0	0	1.33	16.82*	
6	2	31	20	13	7	8	0	0	0	0	2.20	19.34*	
7	1	25	45	12	10	3	0	0	0	0	2.14	28.33*	
8	3	49	34	11	1	1	0	0	0	0	1.61	31.38*	
9	7	38	27	14	4	3	0	0	0	0	1.77	10.75	
10	16	30	28	16	8	1	1	0	0	0	1.77	1.48	
11	4	32	30	9	7	8	7	1	1	1	2.53	36.37*	
12	5	38	29	16	4	3	0	0	0	0	1.84	13.56	
13	8	56	26	6	3	0	0	0	0	0	1.39	29.05*	
14	1	33	30	24	4	2	0	0	0	0	2.03	21.34*	
15	3	24	36	21	5	2	0	0	0	0	2.08	16.62	
16	17	46	16	3	1	1	0	0	0	0	1.14	14.7*	

Figures

Figure 7. The number of offspring emerging from swede midge, *Contarinia nasturtii*, offered to female *Synopeas myles* at different ages (days after oviposition at 22.5°C). The life stages of the hosts on each day are shown below the x-axis. Boxes are bounded by the 25th and 75th percentiles with the line inside indicating the median; whiskers extend to the 5th and 95th percentiles.

Figure 8. Life stages of *Synopeas myles*: (A) single egg inside swede midge, *Contarinia nasturtii*, larva, (B) superparasitized host containing five eggs, (C) encapsulated eggs, (D) newly-hatched first-instar larva, (E) partially engorged first-instar larva, (F) second-instar larva close to pupation, (G) pupa extracted from host cocoon, (H) adult female. e – *S. myles* egg, mg – host midgut, fb – host fat bodies, en – encapsulated egg, m – mandible of *S. myles* larva.

Figure 9. The mean number of eggs ($\pm$ SE) laid by female *Synopeas myles* in swede midge (*Contarinia nasturtii*) larvae on each day of their life, with the number of females surviving to each day shown.

Figure 10. The relationship between the number of *Synopeas myles* eggs laid in swede midge (*Contarinia nasturtii*) larvae and A. number of surviving *S. myles* offspring per host larva and B. the percent of emerging *S. myles* offspring that were female ($y = 26.05\ln(x) + 48.84$).

Figure 11. The relationship between the number of *Synopeas myles* eggs laid in swede midge (*Contarinia nasturtii*) larvae and A. right hind tibia (RHT) length of female and male *S. myles* offspring, and B. egg to adult development time of female ($y = 1.05\ln(x) + 22.14$) and male ($y = 1.15\ln(x) + 21.64$) *S. myles* offspring.

Chapter 5: General Discussion

This is the first study to comprehensively describe the parasitoid complex of the swede midge in Europe, as well as investigate in depth the biology of one of its parasitoids.

This work has set the stage for future evaluation of the European parasitoids as candidates for classical biological control of the swede midge in North America. Four species were found attacking the swede midge in Europe, all of which were solitary endoparasitoids. However, for the most part the parasitoid complex was made up of two species, *S. myles* and *M. chalybeus*. Neither appears to be specialized exclusively on the swede midge, and there was no evidence that they consistently have a large impact on host populations. These characteristics of the swede midge's European parasitoid complex may be influenced by the life history of the swede midge itself as well as its ecological history in Europe.

The species richness of the swede midge's European parasitoid complex is not necessarily atypical. A review including 191 species of cecidomyiids in their native ranges found that they were attacked by an average of 4.2 species of parasitoids, although some are attacked by as many as 14 species (Hawkins and Gagné 1989). Hawkins and Gagné (1989) attempted to explain why some species have more species-rich parasitoid complexes than others, and whether species that are attacked by more species of parasitoids suffer greater mortality from them. They found that several life history traits significantly affected the size of a gall midge species' parasitoid complex and that the species richness of a gall midge species' parasitoid complex is positively correlated with the percentage of the host population that is parasitized (Hawkins and Gagné 1989). What these authors did not consider was that the life history of some gall midges may

prevent exploitation by ectoparasitoids, and that this may explain their less species-rich parasitoid complexes. The parasitoid complexes of many gall midge species include ectoparasitoids, which are sometimes responsible for high levels of parasitism (Feijen and Schulten 1983; Mendonca and Romanowski 2002; Stiling et al. 1992). Here we found that there are no ectoparasitoids attacking the swede midge in Europe. This is not an uncommon occurrence; there are several other species of gall midges whose parasitoid complexes do not contain ectoparasitoids (ex. Affolter 1990; Keller and Schweizer 1994; Romankow and Dankowska 1993; Romankow and Dankowska 1994; Sampson et al. 2006). This may be because of the life history and gall structure of these species. Hawkins and Gagné (1989) found that gall midges that pupate in the soil are attacked by significantly less parasitoid species than those that pupate *in situ* (in the plant), and hypothesized that this may be because they are available to parasitoids for a shorter period of time, and tend to form galls that are more visually apparent. Although not stated by these authors, the decrease in species richness may be due to the fact that midges that pupate in the soil are unsuitable for attack by ectoparasitoids, which typically attack maturing larvae or pupae of gall midges (Feijen and Schulten 1983; Mendonca and Romanowski 2002; Stiling et al. 1992; Tschamntke et al. 1991). The pupae of these species may not be available for attack (because it is in the soil), and the gall structure of these species may not be suitable for larval ectoparasitoids to successfully complete their development inside. For example, swede midge galls typically rot soon after mature larvae have left the plant, and any remaining larvae with ectoparasitoid larvae (which typically feed on paralyzed hosts) would likely decay as well. These factors have probably prevented the exploitation of hosts that pupate in the soil by ectoparasitoids, and

may therefore explain the lower average species richness of parasitoid complexes attacking these species. To test this prediction, a comprehensive review would need to be conducted to confirm that cecidomyiid species that pupate in the soil and have less apparent/complex galls are less likely to be attacked by ectoparasitoids.

The success of invasive species is often attributed to a release from specialized natural enemies in their area of origin (Keane and Crawley 2002). Following the establishment and spread of an invasive species, its associated natural enemy complexes often contain a high proportion of generalists that attack the invasive species at a low rate relative to the natural enemy complexes in the invader's area of origin (Cornell and Hawkins 1993; Mitchell and Power 2003; Torchin et al. 2003). The fact that the swede midge does not appear to have any specialized parasitoids attacking it at a high rate in its native range is somewhat surprising, since there has presumably been thousands or even millions of years for these relationships to evolve, and the swede midge is an extremely widespread and abundant resource available for exploitation by natural enemies. The parasitoid complex of the swede midge in Europe, which for the most part is comprised of two species which, at present, appear to be generalists attacking the swede midge at low to moderate rates, shows some resemblance to a parasitoid assemblage expected of a pest in its invasive range. Although the swede midge was first identified in Europe in 1888 and is reported in the literature as a pest of crucifer crops as early as the early 1900s (Dry 1915), it is possible that western and northern Europe, the areas where it is sometimes a pest and where this study's survey took place, is not the true native range of this species. The ancestral varieties of modern crucifer crops were first cultivated in the Mediterranean in approximately 600-650 BCE, with downstream varieties subsequently

spreading across Europe (Hodgkin 1995; McKinlay 1992). It is possible that this sudden increased availability of high-quality host plants precipitated a rapid increase in the distribution and abundance of the swede midge throughout Europe, leaving its specialized parasitoids behind in its area of origin. Why these parasitoids would not spread along with their hosts is unclear, but could have been due, for example, to a low relative capacity to adapt to different climatic conditions. The parasitoid species found to attack the swede midge in Europe by this study could be examples of generalist species already present in Northern and Western Europe that included the swede midge in their host repertoire following its spread and increase in abundance. There have been cases where attack by species indigenous to the invaded area can provide substantial control of a gall midge pest. *Mayetiola destructor* Say (Diptera: Cecidomyiidae), for example, which is invasive in the United States, is attacked by several Nearctic species of parasitoids which are considered very important for the suppression of pest populations (Bullock et al. 2004; Clausen 1978). The species responsible for the most mortality, however, are generalist ectoparasitoids (Clausen 1978). The fact that the life history of swede midge may provide refuge from attack by ectoparasitoids (see above) may help to explain why substantial control of this pest's populations by natural enemies in many areas of Northern and Western Europe seems to be absent. Mirroring the situation in North America, the swede midge is sometimes a serious pest in Holland, Northern France, Belgium, Norway, and Switzerland, and management efforts currently rely on insecticidal control. To test the hypothesis that the swede midge is invasive in Northern and Western Europe, surveys are needed in areas of Europe where the swede midge is not reported as a pest, ostensibly because of effective control by natural enemies. Candidate

areas include southern Europe (Italy, Greece, Spain) and Eastern Europe and Western Asia (Ukraine, Belarus, Russia, Turkey).

The above discussion raises an important point previously raised by Waage (1990). Foreign exploration for parasitoids to use as classical biological agents should not only include outbreak populations, which could be in that very state due to lack of effective control by natural enemies (Waage 1990). Various studies have found evidence for different natural enemy complexes acting on pest populations of different densities; the low-density complexes may be best suited for biological control of the target pest (Price 1973; Waage 1990). However, this raises practical difficulties which were also encountered in this study. If an insect is not a pest in a particular region, there are unlikely to be contacts that have sufficient knowledge to guide the collector to locations where the pest is present at the correct time. Because of limited travel budgets, collectors are hesitant to risk an unsuccessful collecting trip by traveling to distant locations without prior knowledge of where the pest occurs or when is the best time to collect. However, if strong candidates for use as classical biological control agents are indeed being missed by failing to sample from these areas, it would be prudent for researchers to include them in the collection plans and accept the associated risk.

As stated previously, further research is still needed to assess whether *S. myles* and *M. chalybeus* do play any significant role in regulating swede midge populations in Europe, and whether they are more host-specific than the current evidence shows. If it is found that neither *S. myles* nor *M. chalybeus* would be a suitable candidate for classical biological control of the swede midge in North America, other options will have to be considered to manage this damaging pest. Life table studies assessing the importance of

other mortality factors in both Europe and North America could help to identify possible management methods. For example, Axelsen (1992) found that ground predators such as carabid beetles were an important mortality factor for *Dasineura brassicae* Winnertz (Diptera: Cecidomyiidae) when larvae left their feeding sites to pupate in the soil. In 2010 and 2011, we occasionally observed syrphid larvae (Diptera: Syrphidae, unknown species) feeding on swede midge larvae inside infested growing tips; the importance of these and other generalist predators in causing mortality in swede midge populations needs to be assessed. Research is also needed to follow up on the potential for nematodes to be used for swede midge control (Corlay et al. 2007). Chen et al. (2011) suggested that transgenic crucifer plants expressing *Bacillus thuringiensis* var. *israeliensis* insecticidal proteins could be developed. However it is accomplished, integration of biological control methods into current management practices for the swede midge is needed to increase the success of swede midge management and lessen current dependence on insecticidal control.

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