Biological studies of a European fruit fly, *Euphranta connexa* (Diptera: Tephritidae), a candidate biological control agent for invasive swallow-worts

By

Alicia M. Leroux

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Department of Entomology

University of Manitoba

Winnipeg, Manitoba

ABSTRACT

The biology of *Euphranta connexa* was investigated to assess its potential as a biological control agent for introduction in North America against the invasive weeds *Vincetoxicum rossicum* (Kleopow) and *V. nigrum* (L.) (Apocynaceae). A range of temperatures suitable for development was determined for the pupal and egg stages of *E. connexa*. The pupa is the overwintering stage of *E. connexa* and does not exhibit a diapause, but undergoes a cold induced quiescence. The relationship of temperature to pupal developmental was investigated and did not differ among insects from sites over a range of altitude of 1300 m, indicating *E. connexa* may not be locally adapted. When adult female *E. connexa* emerged from pupae they had not developed eggs but 10 to 15 days later they had a full complement of developed eggs. Mating of *E. connexa* did not affect the egg load of females but egg load was reduced in females that had spent longer as pupae in cold conditions. There was evidence that females could resorb previously developed eggs. In surveys for larval and larval-pupal parasitoids of *E. connexa* infested seedpods of *V. hirundinaria* from three countries, there were a total of 1599 parasitoids from eight Hymenoptera families,and most parasitoids were Braconidae. Prior to the survey, the fruit midge, *Contarinia asclepiadis* (Diptera: Cecidomyiidae) was considered rare, but it was found in seedpods from all collection sites in Switzerland and infested about 9.2% of seedpods at two localities in 2013. Of 13 parasitoid morphospecies found in the survey, two braconid and one ichneumonid morphospecies were frequent and were parasitoids of *E. connexa*, three platygastrid morphospecies were probably parasitoids of *C. asclepiadis*, and three morphospecies were probably hyperparasitoids. The thesis research will allow

development of effective methods for studying impact and host range of *E. connexa*, studies that are required to assess the potential as a biological control agent against *V. rossicum* and *V. nigrum*.

ACKNOWLEDGEMENTS

I would like to start by thanking Dr. Holliday for his time and patience. His support and drive to see his students improve and succeed have helped me through both my undergraduate and graduate careers.

Andre Gassman provided a healthy work place balanced with pragmatism and humor that was needed and welcomed through the duration of my thesis. Other members of CABI I would like to thank are Hariet Hinz for her constructive feedback and Ulli Kuhlmann for his help in developing collaboration between CABI and the University of Manitoba that has made my thesis possible.

The entire committee provided me with freedom that was difficult at times but provided me with a chance to establish myself in my field.

I would like to thank the many students at CABI for providing assistance over the years that helped aid me in my thesis: Maja Penic, Meaghan Bennett, Janine Brooke, Ariel Firebaugh, Emily Palmer, Clesson Higashi, Cornelia Closa, Sara Abdallah, and Léna Durocher-Granger. Special thanks go to Alyson Carter, Letitia de Ros, Sean Pablo, and Matt Wahlberg for all the support and understanding.

Thank you to my many office mates over the years that were always accepting, offering support and keeping quiet about the collection of coffee mugs always present around my space.

My parents were a constant and necessary support and without them, finishing this degree would have been difficult, if not impossible. They helped maintain my life in Canada while trying to understand my field.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

International trade over the past 200 years has increased, as has the rate of biological invasions (Ding et al. 2008; Mack 2000). Once introduced and naturalized, invading organisms have the potential to become serious problems (Pimentel et al. 2005). Of the total exotic flora in North America, 2% have become persistent and cause serious negative effects (Doorduin and Vrieling 2011). The definition of invasive species has not reached a consensus in the literature (Colautti and MacIsaac 2004; Valéry et al. 2008). This thesis will use the term invasive to mean non-native naturalized organisms that spread from the site of introduction (Richardson et al. 2000) and cause negative impact environmentally and/or economically (Inderjit 2005). Often control options are limited for invasive plants, especially in sensitive or natural areas (Cronk and Fuller 1995; DiTomaso 2000). Classical weed biological control is a method based on the introduction of specialized natural enemies, including animals and microbial pathogens, from the native range of an invasive plant with the aim of providing persistent control (van Driesche et al. 2008).

In the past twenty to thirty years, populations of the invasive plants *Vincetoxicum rossicum* (Kleopow) Barbar (Apocynaceae) and *V. nigrum* (L.) (Apocynaceae) have increased to levels of concern, mainly in the north east of North America (Sheeley 1992; Sheeley and Raynal 1996; Tewksbury et al. 2002). Chemical and mechanical methods of control are neither effective nor practical; therefore, research was initiated to determine potential biological control agents in 2006 (Weed and Gassmann 2007). To date six species of herbivorous insects have been investigated (Weed 2010; Weed and Gassmann 2007). Currently, research on the potential control agent *Euphranta connexa* (Diptera: Tephritidae) as a new host association biological control agent has shown that *E. connexa*

can complete development on *V. rossicum* and *V. nigrum* (Gassmann et al. 2009; Weed 2010). To comply with the requirements of a petition for the release of an arthropod biological control agent in the United States (USDA-APHIS 1998) or Canada (De Clerck-Floate et al. 2006) more research is needed on the biology and host specificity of *E. connexa*.

The thesis will explore the potential of *E. connexa* for biological control of *Vincetoxicum* spp. The thesis is written in paper style in which the research is described in three research chapters, in the style of scientific papers covering: i) the effects of temperature on the pupal and first instar larval development of *E. connexa*, ii) the effect mating, age, and oviposition stimuli have on the egg load of *E. connexa*, and iii) a survey of parasitoids emerging from seedpods of *Vincetoxicum hirundinaria*. Before the research chapters will be a review of the biological control procedures, the literature of *Vincetoxicum* spp. and *E. connexa*, and the current status of the *Vincetoxicum* biological control program.

CHAPTER 2: LITERATURE REVIEW

The following is a review of the impacts and management methods of invasive plants with a focus on biological control, the steps required for the introduction of an arthropod biological control agent into the United States (USDA-APHIS 1998) or Canada (De Clerck-Floate et al. 2006), and a review and discussion of the literature on the biology of *Vincetoxicum* spp. and *E. connexa*, in the context of its use for biological control of *V. rossicum* and *V. nigrum*. The following will focus mainly on invasive plants in natural and semi-natural systems because of the more frequent use of biological control in these areas.

Negative effects of invasive plants

The establishment of large populations of invasive plants in ecosystems can have far-reaching negative effects (DiTomaso 2000; Pimentel 2001; Pyšek and Richardson 2010; Simberloff 2005; Wagner and van Driesche 2010). The alteration of ecological and physical properties of the native ecosystem by the establishment of invasive plant monocultures is detrimental (Wagner and van Driesche 2010) through displacement of native flora and fauna, which causes a decrease in species diversity, and occasionally leads to species extinctions and shifting food webs (Cronk and Fuller 1995; Wagner and van Driesche 2010). Belcher and Wilson (1989), Stinson et al. (2007), and Lawlor (2000) have reported decreases in species diversity as a result of the introduction and spread of *Euphorbia esula* L. (Euphorbiaceae), *Alliaria petiolata* (Bieb.) (Brassicaceae), and *Vincetoxicum rossicum*, respectively. In forest understories, regeneration is negatively affected by *Alliaria petiolata* (Stinson et al. 2006). There are also concerns over hybridization between invasive plants and closely related native species potentially

creating new invasive taxa (Thompson 1991). One such case involved *Spartina alterniflora* Loisel (Poaceae), introduced in Britain, hybridizing with the native *S. maritime* Fernald (Poaceae), producing a sterile hybrid that underwent chromosome doubling to form a highly plastic aggressive invasive plant, *S. anglica* Hubb (Thompson 1991). *Spartina alterniflora* also hybridized with *S. foliosa* Trinius (Poaceae) in the San Francisco Bay area creating a new species with higher fitness than its parental lineages (Mooney and Cleland 2001). Alteration of the environment does not only occur through population shifts or hybridization, but also through changes in physical properties such as decreases in pH of soil by *Tamarix* spp. (Tamaricaceae) (Sexton et al. 2006), increasing nitrogen in barren ecosystems by *Myrica faya* Ation (Myricaceae) (Adler et al. 1998), and increased fire frequency in areas invaded by *Bromus tectorum* L. (Poaceae) (Young and Longland 1996).

Invasive plants in agriculture systems can be detrimental to production decreasing the monetary value of the systems. For example, *Euphorbia esula* can cause direct losses to producers through a 50 to 70% reduction in the cattle carrying capacity of land (Alley et al. 1984). Also, because of chemical constituents of *E. esula*, cattle and horses can become ill (Selleck et al. 1962) and in rare cases death can occur (Kronberg et al. 1993). These effects cause an estimated loss to cattle producers in North Dakota of US$7 million dollars annually (Messersmith and Lym 1983).

The presence of invasive plants can also cause economic problems by decreasing the value of the land in natural areas, as is the case with *Melaleuca quinquenervia* (Cav) Blake (Myrtaceae) which impairs the use of natural areas for recration and tourism and is estimated to cost between 418 million dollars and 1.25 billion dollars (Serbesoff-King 2003). Over 55 years the negative effects of *Tamarix* spp., through losses to irrigation

water, municipal water, hydropower, and flood control, are estimated to have cost from 3.8 to 11.2 billion dollars (Zavaleta 2000). In rangelands, there is an estimated monetary loss from weeds of 2 billion dollars annually in the United States (DiTomaso 2000). Even with these few examples of the direct and indirect costs of invasive weeds, it is clear invasive plants have a large negative impact fiscally and ecologically.

Management of invasive plants

To alleviate the negative effects of invasive plants control measures are warranted. Preventative control through predictive measures and modeling anticipates the establishment and spread of invasive weeds (Kolar and Lodge 2001; Rejmánek 2000). Preventive control aims to assess which species are most likely to become invasive, assess the most probable avenues of introduction, and alter these pathways to prevent invasions (Kolar and Lodge 2001; Rejmánek 2000; van Driesche et al. 2008). Other preventative methods include the education of shippers and regulators about potential invasive weeds and how their importation can be prevented (DiTomaso 2000). If early detection and monitoring are successful, establishment of invasive weeds can be prevented, but appropriate training and vigilance is required (Zamora D.L. and Thill 1999). Once weeds are established, the most dominant method for weed control in agricultural cropping systems and rangelands is the use of herbicides (DiTomaso 2000; Harker and O'Donovan 2013). Chemical control in natural areas can be costly and difficult to apply on non-uniform terrain (Cronk and Fuller 1995). Mechanical control methods such as mowing, hand-pulling, and tilling can be effective management techniques, although they are not easily carried out in most natural and rangeland areas (DiTomaso 2000). Cultural control methods, which include grazing, burning regimes, and re-vegetation, are also used in natural areas and can be effective (Kirby et al. 2003;

Landgraf et al. 1984; Selleck et al. 1962; Walker et al. 1994). Finally, because invasive plants are able to spread without the assistance of humans it is beneficial to use biological control methods (van Driesche et al. 2008).

Classical biological control of invasive plants by insects

Weed biological control exploits predator/prey relationships to control the target weed or invasive plant with an ecologically appropriate natural enemy (van Driesche et al. 2008). Extirpation of the invasive plant from the area is seldom the goal of weed biological control, rather the aim is to reduce the invasive plant`s dominance to allow for coexistence of native flora, and to slow the spread of the invasive plant (van Driesche et al. 2008). Classical biological control for invasive plants is focused on the identification and testing of host-specific natural enemies that can be released to establish a population of agents that persists and provide effective control (Harris 1979; Thomas and Reid 2007). Biological control is based on the enemy release hypothesis, which states plants that are introduced into a new ecosystem become invasive due to a lack of specialist natural enemies and reduced attack by generalists (Colautti et al. 2004; Keane and Crawley 2002). Biological control concerned with the utilization of an insect not evolutionarily associated with the invasive weed is termed new host association biological control (Hokkanen and Pimentel 1989). This method of control attempts to exploit the lack of co-evolved defensive systems in the new host (Hokkanen and Pimentel 1984; 1989).

Benefits of classical biological control

The definition of the success of a biological control program will depend on the goal of the program itself (van Klinken and Raghu 2006). Over 50% of the programs aimed at weed suppression are considered successful (van Wilgen et al. 2013).

Benefits of biological control in natural systems can be seen when the weed population is decreased to the point where native plants can co-exist with the invasive species (Blossey et al. 1994; Denslow and D'Antonio 2005; Schooler et al. 2006). For example, it has recently been shown that *Centaurea diffusa* Lamarck (Asteraceae), diffuse knapweed, has been successfully controlled by *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) in the province of British Columbia (Myers et al. 2009). The control of St. John's Wort, *Hypericum perforatum* L., in New Zealand and North America is another example of a successful control, in this case, by two chrysomelid beetles *Chrysolina hyperici* L. and *C. quadrigemina* L. (Groenteman et al. 2011). Within aquatic ecosystems, the biological control of *Salvinia molesta* D. S. Mitchell (Salviniaceae) has been successful through the release of *Cyrtobagous singularis* Hustache (Coleoptera: Curculionidae) (Room et al. 1981).

Agricultural and natural areas have benefited from the effective control of *Euphorbia esula* through the use of six species of *Aphthona* (Coleoptera: Chrysomelidae) (Bourchier and Van Hezewijk 2013). The use of these weed biological control insects to decrease populations will benefit agricultural producers allowing the reclamation of these lands and therefore, reduce illness to cattle (Bangsund et al. 1999).

Not only are there ecological benefits of biological control, but also economic benefits where the value of the benefits greatly exceeds the cost of research and implementation for a program (McFayden 2008). In McConnachie et al. (2003) the

economic benefit obtained by the successful establishment of *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) on *Azolla filiculoides* Lamarck (Azollaceae) was estimated to be a net present value of US$206 million dollars. Prior to the success of *S. rufinasus* aquatic systems with *A. filiculoides* were rendered unuseable (McConnachie et al. 2003). De Groote et al. (2003) calculated a benefit of an estimated US$260 million dollars for the successful control of *Eichhornia crassipes* (Martius) (Pontederiaceae) by *Sameodes albiguttalis* (Warren) (Lepidoptera: Pyralidae), *Neochetina eichhorniae* Warner, and *Neochetina bruchi* Hustache (Coleoptera: Curculionidae). The control of *E. crassipes* resulted in revenues from the restoration of the fishing industry in Southern Benin (De Groote et al. 2013).

Risks of classical biological control

The introduction of a non-native control agent into a new environment is never free of risks (Howarth 1983). However, the risks associated with insect releases could outweigh the risks of not introducing a biological control (Howarth 1983). Most biological control programs do not result in negative non-target effects (Waterhouse 1998) with over 400 species of biological control agents being released globally, only a few have demonstrated direct negative effects (van Wilgen et al. 2013). Direct negative effects on non-targets occur when introduced weed biological control agents develop or reproduce on non-target plants in the area where they were introduced. One example is the seed-feeding weevil *Rhinocyllus conicus* Frölich (Coleoptera: Curculionidae) which was introduced from Europe into North America in 1968 for the control of invasive *Carduus* spp. (Asteraceae), but has led to a decrease in viable seed heads on the native North American *Carduus canescens* (Asteraceae) and *C. undulatum* (Asteraceae) (Louda 2000). *Rhinocyllus conicus* has also been found on six thistle species in California with no

evidence of a direct impact on their populations (Herr 2000). The control agent, *Cactoblastis cactorum* (Lepidoptera: Pyralidae), was released to control native cacti in the Caribbean, but in 1989 *C. cactcorum* was found feeding on native *Opuntia* sp. (Cactaceae) in Florida (Pemberton 1995). Since, *C. cactorum* has spread across to the northern states and down into the Isla Mujeres islands of Mexico threatening native cacti of these regions (Rose et al. 2011).

Introduced weed biological control agents can cause indirect negative effects as well; for instance, the biological control program for knapweed *Centaurea maculosa* (Lam.) (Asteraceae) included the introduction of the seed-feeders *Urophora affinis* (Frauenfeld) (Diptera: Tephritidae) and *U. quadrifasciata* (Meigen) (Ortega et al. 2004; Pearson and Callaway 2006). *Peromyscus maniculatus* (Wagner) (Cricetidae), the deer mouse, are able to detect the presence of *Urophora* spp., a highly nutritional resource, in the galls of *C. maculosa*, and consume them (Pearson and Callaway 2006). As a result, the population of *P. maniculatus* in knapweed-infested areas has increased, also increasing the levels of Hantavirus, which is carried by *P. maniculatus* (Pearson and Callaway 2006). The negative indirect effects seen through the introduction of *Urophora* spp. would have been difficult to predict beforehand (Pearson and Callaway 2006). Unreasonable expectations of the exploration of indirect effects on weed biological control practitioners to predict such indirect effects could prevent implementation of weed biological control programs that could prevent the significant ecological damage resulting from unchecked invasive plants.

Elements required for release of a classical biological control agent

There are several steps in implementing a biological control project prior to release approval. The major steps include the selection of a target weed, the development of a list of potential biological control agents based on a literature review and field surveys, and an assessment of selected agents (De Clerck-Floate et al. 2006; USDA-APHIS 1998). In Canada, the Canadian Food Inspection Agency (CFIA) is responsible for approval of the importation of biological control agents for the control of weeds (De Clerck-Floate et al. 2006) and in the United States, the United States Department of Agriculture Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ) (USDA-APHIS 1998). Specifically, in Canada, for potential control agents to be imported a petition for release must be compiled. The three major sections of the petition require information on the target weed, list of potential control agents, and an assessment of the control agent proposed for importation. This latter section requires information in 10 areas. The elements of the three major sections and subsections are briefly outlined below.

Selection of a target weed

Although biological control of weeds has proved to be a self-sustaining, environmentally safe and cost effective method (McFayden 2008), it is often considered as a last resort method when other control options are ineffective, environmentally detrimental, or too expensive (Hoffmann and Moran 1998). The information in this section requires details on the nature of the weed problem and its environmental and economic impacts, as well as all relevant taxonomic, molecular, biological, and geographic data that are needed for the importation and release of arthropod biological control agents in Canada (De Clerck-Floate et al. 2006). Economic impact and environmental impact data will provide useful

information on weighing the risks and benefits of the program once an agent's assessment is completed. Impact information may also aid in obtaining financial support for a biological control programme.

Establishing a list of potential biological control agents

The first step involves a literature review to identify all natural enemies known from the target plant and related species, their geographical distributions, and known host ranges (Barratt 2012; De Clerck-Floate et al. 2006; USDA-APHIS 1998). The second step is to survey for identified potential biological control agents and possibly yet unknown agents from the target weed, old associations, and from species related to the target plant, new associations (De Bach 1964; Hokkanen and Pimentel 1984). Once identification has been confirmed for all specimens collected, a priority list is prepared according to the known narrowest host range, potential greater impact on the target plant and compatibility with other potential agents collected (De Clerck-Floate et al. 2006). Compatibility of insects is a concern when there are introductions of multiple agent species (Ehler and Hall 1982) with the risk to introduce a less effective but highly competitive species. This is the reason why biological control agents with different food niches are given priority, but documented interference is rare (Denoth et al. 2002).

Potential agent assessment

Assessment of a potential biological control agent is based on the following:

<u>Taxonomy</u>

Proper characterization of an agent is critical for host range testing and possible future releases of a correctly identified species (Huber 1998; Noyes 1994). Further, of increasing importance is the molecular identification of both the agent and the target weed to identify potential cryptic species, hybridization, or the genetic variability of an agent or

target plant population (Gaskin et al. 2011). Molecular tools can also be used to identify source populations and the release history of a biological control agent (Mlonyeni et al. 2011). Molecular identification can show the genetic status of the population studied for release and potential endosymbionts (Gaskin et al. 2011). It is of course imperative that voucher specimens are preserved and their location be identified for further traditional and molecular taxonomic study.

Current and expected geographic distribution

Knowledge of the current native geographic distribution of a potential biological control agent will contribute to assessing its potential distribution in the release area (De Clerck-Floate et al. 2006; USDA-APHIS 1998). This can be assessed with information regarding climatic thresholds and the dispersal ability of the species (De Clerck-Floate et al. 2006; USDA-APHIS 1998).

Agent population source

The source of the agent population must be correctly identified and recorded, to allow for recollection and re-release of the studied population. Knowing the source population allows for molecular taxonomic work with post release studies to evaluate shifts in the genetic structure of a released population and to assess potential hybridization (Gaskin et al. 2011).

Host range

This section details the species of plants on which the control agent has been found, with the information derived from records in the literature and field surveys. Information on the fundamental and ecological host range is detailed in the host specificity testing section.

Life history

Information in this section should provide a detailed description of the biology of the agent and of the interactions between the agent and its host plant(s) in the native range. Information on the reproductive biology and life history of the agent allows the formulation of biologically appropriate methods for host range testing. For instance, the knowledge of time of egg maturation and period of egg-laying is essential to develop appropriate oviposition tests. Information on the fecundity of an agent, voltinism, mortality factors, dispersal ability, and interactions with the host will contribute to assessments of the overall potential impact on the target plant in the area of introduction.

Natural enemies

It is important to avoid releasing natural enemies, in particular parasitoids and diseases that are associated with the potential biological control agent in its native area. An agent's natural enemies, their congeners, and natural enemies related at higher taxonomic levels, or with similar ecology, could reduce agent effectiveness in the area of introduction (Edwards et al. 1996). Therefore, an assessment of the known parasitoids and pathogens in the native range of the potential agent is also required (De Clerck-Floate et al. 2006; USDA-APHIS 1998).

Insect handling

Good insect handling and rearing practices are essential for maintaining healthy colonies and to ensure the colony is free from parasitoids and pathogens. Field collected insects meant for field releases need to be reared through a generation in quarantine to ensure they are free from parasitoids or pathogens.

Host specificity

Assessing the host range of a potential biological control agent is the most critical step in a pre-release weed biological control program (Barratt 2012; De Clerck-Floate et al. 2006; USDA-APHIS 1998) and serves as a predictor of potential risk to non-target plants (Harris 1971; Harris and Zwolfer 1968; Wapshere 1974). Host specificity is the driving factor in the selection of a biological control agent while selecting the most efficacious agent is the "holy grail" of weed biological control (McFadyen 1998). Recognizing that there is always potential risk, the main goal of the selection of the agents is to ensure that the benefit will justify the risk.

First a list of plants must be established to assess the host specificity tests. The test plant list is usually developed on the basis of phylogenetic relationships between the target weed and other plant species. This approach termed the centrifugal phylogenetic method, (Wapshere 1974) assumes plant species that are more closely related to the target weed species are at greater risk of attack than are more distantly related species. Emphasis is given to species from the same genus as the target weed, including synonyms; species from the same tribe as the target weed; and plant species in different tribes but same family as the target weed. The inclusion of closely related plant species identified as economically, crop or ornamental plants, or environmentally important, especially threatened, endangered species of concern is prioritized (USDA-APHIS 1998). The test plant list also considers the biology, phenology, architecture, habitat, geographical distribution and availability of plant species, along with the known host plants of the potential candidate agent and related, particularly congeneric, species (De Clerck-Floate et al. 2006; USDA-APHIS 1998).

The next step is to assess the fundamental and ecological host range of the potential biological control agent. The fundamental host range defines the absolute limits of a species host range and is tested in no choice conditions (Van Klinken 1999). It is therefore a broader concept than the physiological host range which acknowledges only simple physiological requirements. Schaffner (2001) suggests distinguishing between the fundamental host ranges of different stages during the life cycle of the insect. The ecological host range is the list of plant species which a species uses under natural conditions (Schaffner 2001) and includes the need for appropriate behavioral stimuli for host recognition and host acceptance for all hosts that are used by an agent in no-choice conditions tests (Hill 1999; Sheppard et al. 2005).

In no choice tests, the agent is confined with a plant of a single species in a small cage (Hill 1999). If a plant is not accepted or attacked in no-choice testing, it is no longer considered to be a host and there is no further testing (Hill 1999). If a test plant is accepted or attacked under no-choice conditions, further testing with choice and multiple choice experiments are used to enable searching and location behaviours to occur, and allow the assessment of whether positive test result from no-choice testing indicates that a host is within the fundamental host range but outside the ecological host range of an agent, or whether the host is within the ecological host range (Barratt 2012; Wapshere 1989).

Impacts once control agents are released

In this section potential beneficial environmental and economic impacts of the biological control agents should be detailed to enable the reviewers of the petition to make a sound scientific judgement on the release of an agent versus not releasing the agent (De Clerck-Floate et al. 2006).

Post release monitoring

Post release monitoring is needed to assess the beneficial or detrimental impacts of the biological control agent (De Clerck-Floate et al. 2006; USDA-APHIS 1998). This ideally includes: information on agent establishment and distribution, frequency of attack on target plants and non-target plants if any, changes in target plant fitness, changes in target population density and spread, changes in non-target plant fitness or populations if any, changes in species diversity or land use.

The *Vincetoxicum* spp. and *Euphranta connexa* study system

The first section will detail what is known of the taxonomy, distribution, and life history of the three *Vincetoxicum* spp. of relevance to this thesis. Following this will be a section on the effects of the invasive plants, *V. nigrum* and *V. rossicum* in North America.

Taxonomy of *Vincetoxicum* spp.

Vincetoxicum spp. are in the family Apocynaceae, within the subfamily Asclepiadaceae (Endress and Bruyns 2000; Liede-Schumann et al. 2012; Nazar et al. 2013). Currently, the *Vincetoxicum* Wolf genus is merged with *Tylophora* based on chloroplast and nuclear molecular markers (Liede-Schumann et al. 2012). Prior to this there were three divergent opinions on the placement of *Vincetoxicum* (Endress and Bruyns 2000; Hooker 1885; Markgraf 1972). Based on morphological features *Vincetoxicum* was merged with *Cynanchum* (Gilbert et al. 1995; Hooker 1885), but based on morphology and chemical characteristics *Vincetoxicum* was separated from *Cynanchum* (Markgraf 1972). Liede (1996) showed *Vincetoxicum* was closely related to *Tylophora* based on secondary metabolite composition.

Vincetoxicum rossicum (Kleopow) has been referred to with the incorrect specific epithets, *Cynanchum medium* R. Br., 1809 and *Vincetoxicum medium* (R. Br.) Decne.,

1844, which are synonyms of *V. hirundinaria* (DiTommaso et al. 2005b; Sheeley 1992). The synonyms for *V. rossicum* are *C. rossicum* Kleo., 1929, and *Antitoxicum rossicum* (Kleo.) Pobed., 1952 (DiTommaso et al. 2005b; Sheeley 1992). The common name for *V. rossicum* is pale swallow-wort (DiTommaso et al. 2005b).

Vincetoxicum nigrum (L.) has two synonyms, *Cynanchum nigrum* (L.) Pers., 1805 and *C. louiseae* Kartez and Gandhi, 1994 (DiTommaso et al. 2005b; Sheeley and Raynal 1996). Commonly used vernacular names for *V. nigrum* are black dog-strangling vine, black swallow-wort, and Louise's swallow-wort (US) (DiTommaso et al. 2005b).

Vincetoxicum hirundinaria (L.) has five synonyms within the literature: *V. officinale* Moench, 1794, *C. vincetoxicum* (L.) Pers., 1805, *C. medium* R. Br., 1809, *V. album* (Mill.) Aschers., 1864, and *A. officinale* (Moench) Pobed., 1952 (Sheeley and Raynal 1996).

For this thesis, the target weeds will be classified as: family Apocynaceae, subfamily Asclepiadoidaea, tribe Asclepiadeae, *V. nigrum*, *V. rossicum* (Endress and Bruyns 2000), and *V. hirundinaria* (Liede-Schumann et al. 2012).

Distribution and habitat requirements of *Vincetoxicum* spp. in Europe

The natural range of *V. rossicum* is the Ukraine and southwestern Russia (Markgraf 1972) where it grows along forested slopes. Outside of this range *V. rossicum* is now considered an invasive plant in Norway (Bjureke 2007) and small populations have been found in Germany (Markgraf 1972). The elevation over which *V. rossicum* can be found is not well documented; however, the plants have been found at elevations of 148 m to 173 m in research localities in the Ukraine (Weed unpublished data).

The native range of *V. nigrum* includes Italy, France, Spain, and Portugal (Markgraf 1972). One small naturalised population of *V. nigrum* has been found in

Bulgaria (Petrova 2010). *Vincetoxicum nigrum* can be found growing in dry stony soils in a range of habitats from open to forested slopes (DiTommaso et al. 2005b; Douglass et al. 2009). Documentation of the elevation of sites is sparse; plants have been recorded in field localities growing at elevations ranging from 68 m to 322 m (Weed unpublished data), and from 0 m to 500 m (Fournier 1977).

Vincetoxicum hirundinaria is Palearctic and has been recorded from Scandinavia at elevation from 0 m to about 30 m (Leimu 2004), Spain (600 m) (Pagola-Carte and Peydro 2012), Switzerland (520 - 1800 m) (Table 2, Chapter 3), Germany (272 – 386 m) (Weed unpublished data), Ukraine (30 - 234 m) (Weed unpublished data), and France (0 – 1800 m) (Fournier 1977). *Vincetoxicum hirundinaria* occurs naturally in calcareous soils on exposed slopes (Ågren et al. 2008; Leimu and Syrjänen 2002; Solbreck 2000; Solbreck and Ives 2007), along forest edges (Weed et al. 2011b) and in shaded habitats (Ågren et al. 2008) in small clumps or patches (Solbreck 2000). In Spain *V. hirundinaria* and *V. nigrum* have been found growing together (René Sforza, personal communication) and in France populations of *V. hirundinaria* and *V. nigrum* occur in close proximity (Maguire et al. 2011). The ranges of *V. nigrum* and *V. rossicum* overlap in Ukraine (Maguire et al. 2011) and localities where each plant species have been found growing are in close proximity (Weed unpublished data).

Distribution and habitat requirements of *Vincetoxicum* spp. in North America

In North America there are three species of *Vincetoxicum*. *Vincetoxicum hirundinaria* has been reported to be in North American since 1908 (Robinson and Fernald 1908) and is yet to be of concern; it is restricted to southern Ontario, western New York state, and Michigan and shows no evidence of increased population growth and spread (Sheeley and Raynal 1996). In contrast, *V. nigrum* and *V. rossicum* have been classified as noxious

weeds (DiTommaso et al. 2005a; Sheeley and Raynal 1996). Detailed records of the expansion of *V. rossicum* and *V. nigrum* are ambiguous due to nomenclature disagreements in the past 150 years. It is hypothesized that both species were introduced accidentally through botanical gardens, nurseries, and estate gardens (DiTommaso et al. 2005b; Sheeley and Raynal 1996).

The presence of *V. rossicum* in North America was first recorded in 1885 by James Fletcher in Victoria, British Columbia, Canada (Sheeley 1992; Sheeley and Raynal 1996; USDA and NRCS 2011) although it has yet to become established in natural areas in the province. In eastern Canada, *V. rossicum* was first found around 1889 in Toronto, Ontario, Canada, under the synonym *C. medium* (Moore 1959). Introductions of *V. rossicum* could have occurred through deliberate plantings of *V. rossicum* from 1940 to 1942 at the Ottawa Central Experimental Farm, Ottawa, Canada for exploration of its use as a rubber alternative (DiTommaso et al. 2005b). In the United States, *V. rossicum* was first collected in 1891 in New York State (Douglass et al. 2009). Pringle (1973) recommended defining *V. rossicum* as a weed because of its vigorous growth pattern. Since its introduction, *V. rossicum* populations have expanded as far north as the Outaouais region (Quebec, Canada), south to Green County, Pennsylvania (USA), and east to Rockingham (New Hampshire, USA).

Vincetoxicum nigrum was found in North America in 1854, about 30 years earlier than *V. rossicum* (DiTommaso et al. 2005b), and first appeared in Gray's Manual in 1868 (Sheeley and Raynal 1996). From 1854 onward, *V. nigrum* was reported in 18 states: Connecticut, Illinois, Indiana, Kansas, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Nebraska, New Hampshire, New Jersey, New York, Ohio, Pennsylvannia, Rhode Island, and Vermont (USDA and NRCS 2011). DiTommaso et al.

(2005b) assume sightings in California, Kansas, and Kentucky are misidentifications. The broader distribution of *V. nigrum* compared to *V. rossicum* is thought to be the result of the species' plasticity, allowing adaptation to climatic conditions outside those found in its native range (Douglass et al. 2009).

Populations of *V. rossicum* and *V. nigrum* can be found in transportation corridors, alluvial forests, limestone quarries, old fields, pastures, dry grasslands, no-till agricultural systems, coastal shores, and river banks (DiTommaso et al. 2005b; Douglass et al. 2009; Weston et al. 2005). Forest understories in North America are more likely to be inhabited by *V. rossicum* than by *V. nigrum* (Averill et al. 2011; Lumer and Yost 1995). Populations of *V. rossicum* are more dense in the shaded understory than in sunny habitats (DiTommaso et al. 2005b).

Vincetoxicum rossicum inhabits a range of soil textures, often associated with limestone or glacial till (Christensen 1998; DiTommaso et al. 2005b). Both *V. rossicum* and *V. nigrum* can establish in a range of pH (DiTommaso et al. 2005a; Lawlor 2000; Magidow et al. 2013) and also inhabit areas of hydrologic extremes (DiTommaso et al. 2005b). The temperatures experienced by *V. rossicum* and *V. nigrum* in North America do not differ greatly from those found in their native ranges (DiTommaso et al. 2005b).

Biology of *Vincetoxicum* spp.

Vincetoxicum spp. are long-lived herbaceous perennials (Leimu and Mutikainen 2005; Solbreck and Sillén-Tullberg 1990). Literature pertaining to the reproductive phenology of all three *Vincetoxicum* spp. in the areas of interest is summarized in Table 1.

Stems of *Vincetoxicum* spp. can be erect during development, but then twine around surrounding vegetation and abiotic structures. Vines can be from 40 to 200 cm long (DiTommaso et al. 2005a). Sexual reproduction of *Vincetoxicum* spp. is through

self- and cross-pollination by Diptera, Lepidoptera, and Hymenoptera (Leimu 2004; Lumer and Yost 1995; Muola et al. 2011). New plants can form from the root buds of *Vincetoxicum* spp. (Leimu 2004; McKague and Cappuccino 2005) and are able to produce mature seeds in their first year, and plants germinating from seed may not mature until after one season (Averill et al. 2011).

All three species have five-petaled star-shaped flowers 5–8 mm in diameter, produced in the axils of the leaves. *Vincetoxicum rossicum* has pale maroon flowers, *V. nigrum* has dark purple flowers and *V. hirundinaria* has white flowers. There are also five corona, which are most distinctive in *V. rossicum*. In *V. hirundinaria* the number of flowers ranges from zero to more than 100 per plant (Muola et al. 2010a). In European populations of *V. hirundinaria*, less than 10% of flowers produce mature seedpods (Leimu and Syrjänen 2002; Timonin and Savitskii 1997). Flower production, which affects fruit production, is directly affected by sun exposure, soil depth, and population density (Ågren et al. 2008). Fruit production and seed set are negatively affected by shade (DiTommaso et al. 2005b; Sheeley 1992). In moist years, seedpods of *V. hirundinaria* are larger with more seeds (Solbreck and Kugelberg 1972; Solbreck and Sillén-Tullberg 1986b). In *V. rossicum* 40 – 77% of seeds are polyembryonic (von Hausner 1976) and in *V. nigrum*, 45 – 75% of the seed produced are polyembryonic (Douglass et al. 2009). The seeds of *Vincetoxicum* spp. are wind dispersed with the aid of tufts of threadlike hairs, termed comas, attached to the apical end of the seed, and germinate either the same year or the next spring (DiTommaso et al. 2005b). DiTommaso et al. (2005a) found most *V. rossicum* seeds weigh from 2.3 to 7.7 mg. One fruit contains from 5 to 13 seeds in *V. rossicum*, 7 to 12 seeds in *V. nigrum* (Averill et al. 2011), and on average 20 seeds per seedpod in *V. hirundinaria* (Muola et al. 2010a). Larger seeds may have increased

germination (DiTommaso et al. 2005a), although Cappuccino et al. (2002) found no correlation of seed mass and germination success in *V. rossicum*. *Vincetoxicum rossicum* seed set and above ground biomass are greater in high density patches of conspecifics, as opposed to low density patches (Cappuccino 2004).

Many authors report that *Vincetoxicum* spp. have chemical constituents that can cause negative effects and potentially deter unspecialized insects from consuming these species (Douglass et al. 2011). Pailer and Stricher (1965) were the first to find alkaloids in *V. hirundinaria*. In *V. hirundinaria*, monoamine alkaloid phenylethylenamine (Lavault et al. 1999), (-)-antofine, antofine oxide (Capo and Saa 1989; Muola et al. 2010b), pregnane glycosides; cynatratoside E, cynatratoside C, hirundicoside B, hirundicoside C, hirundicoside D (Lavault et al. 1999), chlorogenic acid, and catechin derivatives (Muola et al. 2010b) have been found. Tissues of both *V. rossicum* and *V. nigrum* contain (-)-antofine, which can be an antimicrobial and antibacterial agent (Cappuccino 2004; Douglass et al. 2011), and is highly phytotoxic, inhibiting the growth of other plants (Gibson et al. 2011). Under experimental conditions the tissue exudates of *V. rossicum* and *V. nigrum* exhibited allelopathic properties, decreasing the rhizome growth of *Apocynum cannabinum* L., *Asclepias tuberosa* L., and *A. syriaca* L. (Gibson et al. 2011).

Effects of *Vincetoxicum* spp. in North America

Large dense monocultures of *V. rossicum* and *V. nigrum* cause negative effects through the displacement of native flora and fauna (DiTommaso et al. 2005b). *Asplenium scolopendrium* L. var. *americanum* (Fern.) Kartesz & Gandhi (Aspleniaceae) and *Agalinis acuta* Pennell (Orobanchaceae) are rare and endangered plants in the United States facing displacement by *V. rossicum* (Douglass et al. 2009; Lawlor 2000). There are only three sites remaining for *Astragalus robbinsii* (Oakes) A. Gray; in one of these sites

V. nigrum has established, threatening the continuance of *A. robbinsii* (DiTommaso et al. 2005b).

North American host plants for *Danaus plexippus* L. (Lepidoptera: Saturniidae), the monarch butterfly, include *Apocynum cannabinum, A. tuberosa*, and *A. syriaca*, and are found within dense stands of *V. rossicum* and *V. nigrum*, (Casagrande and Dacey 2007; Ernst and Cappuccino 2005; Gibson et al. 2011). Several authors have found negative effects of, *V. rossicum* and *V. nigrum*, on North American *D. plexippus* individuals. Female *D. plexippus* accept *V. rossicum* and *V. nigrum* for oviposition, but larvae cannot metabolize the chemicals in *Vincetoxicum* spp. and they soon die (Casagrande and Dacey 2007; DiTommaso and Losey 2003). It is likely that *Vincetoxicum* spp. are competing with the larval food plants of *D. plexippus.*

There is potential for greater negative effects on native flora and fauna if *Vincetoxicum* spp. are not controlled (Averill et al. 2008; DiTommaso et al. 2005b; Lawlor and Raynal 2002; McKague and Cappuccino 2005). Chemical control methods can be effective, but are not a long-term solution (Averill et al. 2008; Lawlor and Raynal 2002). Mechanical control through mowing is labour intensive and often not effective because the entire rhizome of the plant must be removed (Lawlor 2000; Lawlor and Raynal 2002). No native herbivores are known that effectively reduce populations of *Vincetoxicum* spp. in North America (Milbrath 2010), presumably native herbivores are deterred by the toxins the plants contain. Cattle will consume *Vincetoxicum spp.* and decrease *V. rossicum* populations, but the effect is of short duration: once the cattle are removed *V. rossicum* populations increase (Lawlor 2000). It was because of a lack of native enemies (Ernst and Cappuccino 2005; Milbrath 2010) and a lack of other effective control methods (Averill et al. 2008; DiTommaso et al. 2005b; Douglass et al. 2009;

Lawlor and Raynal 2002; McKague and Cappuccino 2005) that a program was initiated to seek potential biological control agents in Europe.

Euphranta connexa

Taxonomy of *Euphranta connexa*

The taxonomy of *E. connexa* is well established: the species is in the family Tephritidae and within the genus and subgenus *Euphranta*. *Euphranta connexa* (Fabricus) is the accepted species name, there are five synonyms in the literature**:** *Cephalia caloptera* Bigot, *Ortalis zetterstedti* Fallen, *Musca connexa* Fabricus, *Tephritis dorsalis* Macquart, and *Trypeta alcinoe* Walker, (Norrbom 1993a). There are also two misspellings of *Euphranta connexa* as *Euphranta conexa* Foote (Norrbom 1993a) and *Euphrantha conexa* F. (Gheorghiu 1984).

The majority of *Euphranta* species are Oriental – Australian. *Euphranta* is represented by two species in Europe, *E. connexa* and *E. toxoneura* (Loew). *Euphranta toxoneura* feeds on *Salix* spp. and is common in Europe (White and Elson-Harris 1992). In the western hemisphere there are two species, *Euphranta mexicana* Norrbom in Mexico (Norrbom 1993a) and *Euphranta canadensis* (Loew), in North America (White and Elson-Harris 1992). Both of these species feed on *Ribes* spp. (Norrbom 1993b; White and Elson-Harris 1992). *Euphranta connexa* is monphagous on *V. hirundinaria* (Solbreck 2000).

Life history of *Euphranta connexa*

Euphranta connexa is distributed from southern Scandinavia (Leimu 2004; Solbreck 2000) to the Ukraine and from Switzerland (Weed et al. 2011b), to Northern Spain (Pagola-Carte and Peydro 2012). What is known about the life history for *E. connexa* is mainly from studies in Scandinavia (Solbreck 2000; Solbreck and Ives 2007) with one

study from Spain (Pagola-Carte and Peydro 2012). The emergence of adult *E. connexa* from overwintering pupae begins in late June to early July (Solbreck 2000). The longevity of adults is not known. However, adults can be found in the field until late August to September (Solbreck 2000). In the field, this species is thought to be univoltine (Solbreck 2000), although in the laboratory it has been found on occasion that adults will emerge from pupae within the same season, without overwintering (Pagola-Carte and Peydro 2012). Most tephritids emerge from pupae without developed eggs (Fletcher and Prokopy 1991), and it is thought *E. connexa* does too. Solbreck (2000) dissected 16 females from the field and found 2 to 60 mature eggs in the ovaries.

Mating of *E. connexa* is preceded by an elaborate dance of specialized wing movements of hamation, supination, and evanation (Sivinski et al. 2000), as well as a "kiss" (Figure 12a). The "kiss" involves labellum upon labellum contact in which it is hypothesised nutritional compounds are being transferred to the female from the male (Solbreck 2000). If the male is successful in courtship he will mount the female and transfer sperm. Females choose oviposition sites by walking up the stem of *V. hirundinaria* plants searching for seedpods and will oviposit in seedpods which are just initiated all the way up to 70 mm with immature seeds and a fleshy wall (Solbreck 2000). In Scandinavia, *E. connexa* have been found to infest an average of 74% (range 11.2 – 99.5%) of the population of *V. hirundinaria* seedpods (Solbreck and Ives 2007) and in infested seedpods, *E. connexa* consumes most of the seeds in the seedpod (Solbreck and Sillén-Tullberg 1986b). Eggs, approximately 0.2 mm in width by 0.9 mm in length (Janzon 1982), are deposited on the inside wall of the seedpod, leaving behind an oviposition scar (Solbreck 2000). After oviposition, the female drags her abdomen over the seedpod leaving a shiny film suspected to be an oviposition deterrent pheromone

(Fletcher and Prokopy 1991; Solbreck 2000). However, it is not uncommon for seedpods to have more eggs than a seedpod can support; on average one larva per attacked seedpod completes development (Solbreck 2000). Solbreck and Ives (2007) hypothesized that large larvae consume eggs and smaller larvae, or that less fit individuals die due to a lack of resources. The duration of egg development before first instar larvae hatch is unknown. The yellowish cream coloured larvae are monophagous on the seeds of *V. hirundinaria* over the duration of three larval instars (Janzon 1982; Solbreck 2000). The mature larvae rasp a hole in the fruit wall, leave the fruit and pupate in the soil (Solbreck 2000).

Natural enemies affecting *Euphranta connexa*

Adults of *E. connexa* are consumed by several spider species (Solbreck 2000). On one occasion a *Vespula* spp. has been recorded consuming *E. connexa* (Solbreck 2000). The following larval parasitoids have been reared from infested seedpods of *V. hirundinaria*: *Scambus brevicornis* (Gravenhorst) (Hymenoptera: Ichneumonidae) (Seguy 1934), *Bracon picticornis* (Wesmael) (Hymenoptera: Braconidae) (Janzon 1982), *Pteromalus helenomus* (Graham) (Hymenoptera: Pteromalidae) (Graham 1969), *Stenomalina muscarum* Mayr (Hymenoptera: Pteromalidae), *Eurytoma curculionum* (Mazr.) (Hymenoptera: Eurytomidae), *Macroneura vescularis* (Retzius) (Hymenoptera: Euplemidae), *Aphanogmus* sp. (Hymenoptera: Ceraphronidae) (Solbreck 2000), *Rhysipolis meditator* (Haliday) (Hymenoptera: Braconidae), and *Rhysipolis decorator* (Haliday) (Hymenoptera: Braconidae) (Medvedev et al. 1995). In Scandinavia, *Scambus brevicornis* is the most common parasitoid, emerging from up to 30% of seedpods (Solbreck 2000) and can make up 90% of the parasitoids (Solbreck and Ives 2007). The second most common parasitoid is *B. picticornis* (Solbreck 2000). The occasionally encountered parasitoids found by Solbreck (2000) could be parasitoids of *E.*

*connexa,*could be from the rare *Contarinia asclepiadis* Giraud (Diptera: Cecidomyiidae), or could be hyperparasitoids. One species of larval-pupal parasitoid, *Psyttalia concolour* (Szépligeti) (Hymenoptera: Braconidae), has been found (Fischer and Koponen 1999). In Sweden, parasitoid interactions with *E. connexa* appear not to regulate or change the population of *E. connexa* and parasitism is independent of *E. connexa* density (Solbreck and Ives 2007).

Host plant interactions of *Vincetoxicum hirundinaria* and *Euphranta connexa*

In the wild, the life history of *E. connexa* is tightly linked with its host plant *V. hirundinaria* (Solbreck 2000) and *E. connexa* are dependent on the density of the fruits of *V. hirundinaria* (Solbreck and Ives 2007; Solbreck and Sillén-Tullberg 1986b). If there is no fruit produced in one year, local extinction of *E. connexa* occurs, however, re-colonization occurs the next year that fruit are produced (Ågren et al. 2008; Solbreck and Ives 2007; Solbreck and Sillén-Tullberg 1986b). From this it is inferred that *E. connexa* can move over large distances (Solbreck 2000). Sites of *V. hirundinaria* with high damage levels by *E. connexa* have higher concentrations of catechin derivatives and antofine (Muola et al. 2010b).

Euphranta connexa directly impacts the *V. hirundinaria* by decreasing the seed output of *V. hirundinaria* (Leimu and Lehtilä 2006). The population growth of *V. hirundinaria* is reduced more by seed predation by *E. connexa* than by herbivory from *Abrostola asclepias* Denis and Schiffermüller (Lepidoptera: Noctuidae) and as the predation of seeds increased, the effect of *A. asclepias* decreased (Leimu and Lehtilä 2006). The growth and survival of individual plants were monitored for two growing seasons and no effect on fitness was found, however, it was recognized that short-term studies of a perennial plant may not show a fitness effect (Leimu and Lehtilä 2006).

Current status of the *Vincetoxicum* spp. biological control program

Vincetoxicum nigrum and *V. rossicum* were classified as invasive plants in the United States and Canada (USDA and NRCS 2011) because they both cause negative environmental damage and economic losses for agricultural production systems (Lawlor 2000). Effective, environmentally friendly, and economically sustainable options were limited (Averill et al. 2008; Lawlor 2000), therefore biological control was sought as a viable sustainable option. Background research into the potential insect biological control agents highlighted eight insects of interest as potential agents (Tewksbury et al. 2002). In 2006 field surveys were conducted in search of these insects and to find potential new agents (Weed et al. 2011b). Insects discussed as potential control agents in Tewksbury et al. (2002) that did not make the final list of potential agents from 2006 onwards were removed because they were found to be polyphagous. Since 2006, the biological control program on *V. nigrum* and *V. rossicum* has focused its investigations of potential control agents on *Chrysolina asclepiadis asclepiadis* (Villa) (Coleoptera: Chrysomelidae), *Chrysochus* (*Eumolpus*) *asclepiadeus* Pallas (Coleoptera: Chrysomelidae), *A. asclepiadis*, *Hypena opulenta* (Christoph) (Lepidoptera: Erebidae), and *E. connexa* (Bourchier et al. 2013).

The Technical Advisory Group (TAG), a committee of representatives from all land management federal agencies of the USDA-APHIS, approved a test plant list for host specificity for potential biological control agents of *V. nigrum and V. rossicum* in 2007 (Milbrath and Biazzo 2007). Depending on the agent undergoing testing the number of species on the host specificity list will change as the last category under TAG consists of species that are fed on by insects closely related to the agent (De Clerck-Floate et al. 2006; USDA-APHIS 1998).

The larvae and adults found from Switzerland of the univoltine *C. a. asclepiadis* cause damage to the leaves of *V. hirundinaria* (Weed and Casagrande 2011). *Chrysolina a. asclepiadis* is polyphagous on a broad range of species (Gassmann et al. 2009; Weed et al. 2011a) and as a result, testing of this insect as a potential control agent ceased in 2008 (Gassmann et al. 2009).

Chrysochus asclepiadeus can be found on *V. hirundinaria*, *V. nigrum*, and *V. rossicum* and occurs in southern Switzerland, southern France, and the Donetsk region of Ukraine (Bourchier et al. 2013; Weed and Gassmann 2007; Weed et al. 2011b). Adults feed on the leaves and larvae feed externally on the roots (Weed et al. 2001b). This insect can take one to three years to complete development (Weed et al. 2011b).Testing has shown that *C. asclepiadeus* is capable of completing development on the target weeds (Weed and Gassmann 2007) as well as on North American species in the genus *Asclepias* (Gassmann et al. 2012). *Chrysochus asclepiadeus* can lay eggs on *Asclepias* spp. under no-choice conditions (Gassmann et al. 2012). Under single choice and multiple choice experiments adult feeding and oviposition has also been documented on *Asclepias* spp. (Gassmann et al. 2009; Gassmann et al. 2011). Consequently, research has been interrupted. Discussions are planned on the relevance of conducting open field tests (personal communication, Andre Gassmann).

Abrostola asclepiadis larvae are leaf feeders (Forare 1995). The adults were collected from *V. hirundinaria* plants in Switzerland and Germany, and from *V. hirundinaria* and either *V. rossicum* or *V. scandens* in the Ukraine (Weed and Gassmann 2007). In Italy, *A. asclepiadis* is bi-voltine and in higher latitudes, univoltine (Bourchier et al. 2013). No choice testing indicated larvae of *A. asclepiadis* can complete development on *V. rossicum* and *V. nigrum* with little to no potential risk to non-target

test plants (Casagrande et al. 2011; Weed and Gassmann 2007). Defoliation of target plants was extensive; however, no impact on individual plant performance was found after one year (Casagrande et al. 2011; Weed et al. 2011a). It is hypothesised that this is the result of testing under optimal conditions for the plants and if tested in the field with repeated years of defoliation there would be an impact (Weed et al. 2011a). Researchers are comparing voltinism of *A. asclepiadis* populations from Finland and from Ukraine. Depending on the results, a decision will be made regarding the petition and under what conditions a release will be made.

Hypena opulenta is an overlapping multivoltine species and was found on *V. rossicum* in Ukraine and can complete development on *V. nigrum* (Weed and Gassmann 2007; Weed et al. 2011b). Development of eight larvae from egg to pupae results in 100% defoliation of *V. rossicum* (Weed and Casagrande 2010). There was no impact of one generation of larval development on *V. nigrum*, but it is predicted an impact could be seen in the field as *H. opulenta* is multivoltine providing sustained pressure on the plant species over the summer (Casagrande et al. 2011; Weed and Casagrande 2010). Host specificity testing of *H. opulanta* on 82 test plants showed it is specific to *Vincetoxicum* spp. and therefore there are no predictable non-target impacts (Hazlehurst et al. 2012). The Canadian Biological Control Review Committee-CFIA granted a release permit for *H. opulenta* in 2013. Five hundred larvae reared from a tested population of *H. opulenta* from Ukraine have been released in Ottawa, Canada (Robert Bourchier, personal communication).

For its potential to limit seed production *E. connexa* has been prioritized as a potential control agent on *V. nigrum* and *V. rossicum*. Weed et al. (2011b) have shown that *E. connexa* females accept *V. rossicum* and *V. nigrum* for oviposition under no choice

conditions, and offspring can complete development on these plants with pupae from *V. nigrum* being significantly heavier than from *V. rossicum* (Weed et al., 2011b). Pupae from *V. hirundinaria* tend to weigh less than those from *V. nigrum*, but the difference was not significant (Weed et al. 2011b).

Research needed to assess *Euphranta connexa*

Several types of information are needed to assess the potential of *E. connexa* as a control agent of *V. rossicum* and *V. nigrum* in North America. The information would be included in a petition to CFIA and the USDA-APHIS-PPQ for release of *E, connexa* in North America.

Information on the geographic and climatic distribution of *E. connexa* in its native range will help in assessing its potential distribution in North America. Predictions of the distribution of *E. connexa* can be enhanced with data on thermal thresholds. Information on habitat requirements such as soil moisture, soil pH levels, soil type, can also enhance predictions of the distribution of *E. connexa* at a local scale.

It is unknown whether there are populations of *E. connexa* in Europe that naturally utilize *V. rossicum* or *V. nigrum*. Solbeck (2000) describes the population dynamics of *V. hirundinaria* resulting in periodic shortages of oviposition sites for *E. connexa*, which could result in the utilization of another host plant if available. Investigation of host utilization of *E. connexa* in areas of coexistence of *Vincetoxicum* spp. may reveal *E. connexa* populations that are already adapted to the target weed species. If such a population exists, they might be more suitable sources for agents to introduce.

There is little in the literature on the reproductive biology of *E. connexa* and the only data available are from field collected females (Solbreck 2000). Information on

bottom-up effects of *V. hirundinaria*, *V. nigrum* and *V. rossicum* on egg load, time and pattern of egg development of *E. connexa* would contribute to assessments of the demographic potential of the fly on each of the three hosts.

More information on the natural enemies and other mortality factors of *E. connexa* is needed to eliminate the risk of an accidental introduction of a natural enemy of *E. connexa*, if the species is introduced into North America for biological control of *Vincetoxicum* spp.

The research needs require the understanding of whether *E. connexa* accepts the target plants, *V. nigrum* and *V. rossicum*, in a natural setting; whether the resource limitation/competition population dynamics of *E. connexa* that prevail with *V. hirundinaria* will change, and if so how, with new hosts; and the fitness implications for any new hosts attacked by *E. connexa*.

Assessing the safety of *E. connexa* is however a pre-requisite for its use as a potential biological control agent. It is therefore crucial to determine its fundamental and ecological host range. Assessing the safety of *E. connexa* and impact on the target plants require a reliable means of synchronizing oviposition by *E. connexa* females and feeding by *E. connexa* larvae with appropriate fruiting stages of target and non-target plants. Information on determinants of adult emergence (thermal biology of the pupae), development time for egg maturation and larval development, and fecundity are essential for this purpose, and also constitute components of the information on life history that is required for a petition for release. Therefore, the thesis research focussed on:

i) investigating the timing of *E. connexa* emergence from the overwintering pupal stage;

ii) determination of time of eggs to hatch;

iii) determining the effects of mating, oviposition, and date of emergence on the fecundity of *E. connexa*;

iv) determining the time from female emergence until the mature egg development, and;

v) conducting a survey of the parasitoids that attack *E. connexa* larvae.

Table 1. The time of flowering, fruit production and mature/dehiscing seedpods of *Vincetoxicum hirundinaria*, *V. rossicum*, and *V. nigrum* in different geographical regions. Note that timing of flowering, seedpod production, and senescence of *Vincetoxicum* spp. vary according to weather, microclimate and growing location and so dates are approximate.

	Growth stage	Geographic origin of data	May	June	July	August	September	Citations
Vincetoxicum hirundinaria	Flowering	CH						Leroux unpublished data
		FI, SE						(Leimu 2004; Muola et al. 2010a; Solbreck and Sillén-Tullberg 1986b)
Vincetoxicum rossicum		US						(Sheeley 1992; St Denis and Cappuccino 2004)
Vincetoxicum nigrum		US						(Douglass et al. 2009)
Vincetoxicum hirundinaria	Fruiting	CH						Leroux unpublished data
		FI, SE						(Leimu 2004; Solbreck and Sillén-Tullberg 1986b)
Vincetoxicum rossicum		US						(Averill et al. 2011; Douglass et al. 2009; Sheeley 1992; St Denis and Cappuccino 2004)
Vincetoxicum nigrum		US						(Averill et al. 2011; Douglass et al. 2009; Lumer and Yost 1995)
Vincetoxicum hirundinaria	Mature seedpods/ Dehisce	CH						Leroux unpublished data
		FI, SE						(Muola et al. 2011)
Vincetoxicum rossicum		US						(Lawlor 2000)
Vincetoxicum nigrum		US						(Lumer and Yost 1995)

Note: CH is Switzerland, US is the United States, FI is Finland, and SE is Sweden.

CHAPTER 3: EFFECTS OF TEMPERATURE ON PUPAL AND EGG DEVELOPMENT IN *EUPHRANTA CONNEXA* (DIPTERA: TEPHRITIDAE) AND IMPACTS FOR BIOLOGICAL CONTROL

Abstract

Vincetoxicum rossicum (Kleopow) Barbar and *V. nigrum* (L.) (Apocynaceae) are invasive perennial weeds in North America. Research into biological control agents has identified *E. connexa* as a potential control agent. Information regarding the developmental time and rate is lacking in the literature and is needed for studies that require synchronizing the emergence of *E. connexa* with vulnerable stages of target and non-target plant species. Pupae from field collections made in 2010 and 2011 were held over winter, then placed in constant temperatures between 9.4 and 35 °C and time of adult emergence was recorded. Adult emergence occurred at constant temperatures between 11.8 and 27.3 °C and there was no difference in the time of adult emergence between the sexes or among sites. Based on fitting a truncated normal model to the data, the lower limit of pupal development, was TH_{08} 4.2 °C and development was most rapid at 25.8 °C. It was concluded that in this experiment insects did not undergo an overwintering diapause. In a second experiment, newly laid eggs were exposed to constant temperatures of 11.8 to 30 °C. Egg hatch occurred at all temperatures, and the estimated lower limit of egg development was 9.3 °C. This study found the suitable temperature range of pupal development and the lower and maximum rate of development, allowing biological control practitioners to easily manipulate adult emergence for further testing. There was also no difference in the time to develop among sites in Switzerland, indicating climate matching for this species in biological control is of less importance.

Keywords: developmental rate, biology, pupal development, egg development, *Vincetoxicum*.

Introduction

Vincetoxicum hirundinaria (L.) (Apocynaceae), *V. rossicum* (Kleopow) Barbar (Apocynaceae) and *V. nigrum* (L.) (Apocynaceae) are perennial vines native to Europe. Two species, *V. nigrum* and *V. rossicum*, are invasive in north eastern North America (DiTommaso et al. 2005a; Lawlor 2000), where they are naturalised and can form extensive monocultures of up to 1000 stems/m^2 (Sheeley 1992) that negatively affect natural and agricultural areas (Casagrande and Dacey 2007; DiTommaso and Losey 2003; Lawlor 2000). *Vincetoxicum* spp. contain alkaloids (Eibler et al. 1995) that are poisonous to animals (Solbreck and Sillén-Tullberg 1990). In North America, chemical and cultural methods do not provide adequate control of *Vincetoxicum* species (Averill et al. 2008; Lawlor and Raynal 2002), and native herbivores have negligible effect (Milbrath 2010; Tewksbury et al. 2002). A biological control program is being considered as natural enemies do not appear to have been introduced with the plant (Tewksbury et al. 2002). Exploratory research identified *Euphranta connexa* (Fabricius) (Diptera: Tephritidae) as a potential biological control agent (Weed 2010).

Euphranta connexa is a univoltine tephritid fruit fly with a western palearctic distribution (Carroll et al. 2002). The adults can be found in the field from June to August on *V. hirundinaria* where they can be seen feeding, mating, mate guarding, fighting for territory, and ovipositing (Solbreck 2000). The timing over which the seasonal activities occur differs depending on the geographic region (Solbreck 2000). In Scandinavia the production of flowers and fruits of the host plant, *V. hirundinaria*, occurs from June to

August (Leimu and Syrjänen 2002). Mating is preceeded by an elaborate dance between the male and female, involving a series of pronation and supination movements by the male. The male will then contact the female through a proboscis to proboscis "kiss", and if the male is accepted, mating commences. Mating in the field lasted 5 to 10 s (Pagola-Carte and Peydro 2012; Solbreck 2000). In the laboratory, the copulatory dance lasts approximately 50 s and mating can last up to about 60 s (personnal observation).

Female *E. connexa* oviposit inside the wall of immature seedpods of *V. hirundinaria* that are 2–70 mm in length (Solbreck 2000). Solbreck (2000) reports that females lay one egg per oviposition hole. In southern Sweden, depending on the year, from 11.2 to 99.5% of seedpods of *V. hirundinaria* are attacked by *E. connexa* (Solbreck and Ives 2007). Both the peak abundance of adult flies and the peak oviposition period are in July (Solbreck 2000). The larvae of *E. connexa* feed on the seeds of *V. hirundinaria* and develop through three instars before exiting through a hole rasped in the fruit wall (Janzon 1982; Solbreck 2000). On average one prepupa emerges from an infested fruit, regardless of the number of eggs laid in a seedpod (Solbreck 2000). The prepupae fall and burrow into the ground where they pupate and overwinter until the following spring (Solbreck 2000).

The initial stage of weed biological control research often requires investigation of the basic biology of potential agents to develop appropriate rearing methods, testing protocols, and release strategies. For host range and impact studies of *E. connexa* it is also necessary to synchronize the oviposition period of the insect and test plant. Specifically, laboratory mated gravid female *E. connexa* must be available at the time that immature fruits are present on plants for testing. Furthermore, it is important to be able to predict the time of egg hatch given knowledge of the time of oviposition. As well, understanding

of the thermal requirements of specific populations of the potential control agents aids in laboratory rearing and in climatic matching of source populations with areas of intended release (McClay 1996). Therefore, the goals of this study were to evaluate the thermal requirements for the development of *E. connexa* from pupation until adult emergence and for the development of eggs from oviposition until the hatching of first instar larvae.

Materials and methods

Adult development

Mature fruits of *V. hirundinaria* were collected in late July/August from sites where *E. connexa* was known in Switzerland. Five sites were sampled in 2010 and nine sites in 2011, from different geographic regions and elevations (Table 2). To collect *E. connexa*, fruits were placed on a fabric screen of 30 squares/cm^2 at the base of a plastic cylinder (15.5 cm x 11 cm diameter) with a screen lid. This cylinder was placed on top of a second cylinder of similar dimensions. The second cylinder had an open top and a solid base with a thin layer of slightly moist vermiculite. The prepupae emerging from the fruits could move through the holes in the fabric screen, fall on to the vermiculite, and pupate. Pupae were collected from the vermiculite approximately every second day. For storage in the laboratory and for overwintering, batches of 50 pupae were grouped by site and placed into 9 cm x 2 cm Petri dishes. In both years the Petri dishes with pupae were maintained in the laboratory where temperatures were ambient without air-conditioning. The pupae were moved into an unlit, unheated, underground storage facility at CABI on 30 September 2010 and 27 September 2011. On 4 February 2011 and 2 February 2012 the pupae were moved to environmental chambers and held at 2 ± 1 °C until the commencement of the experiment. From September 2011 until January 2012,

temperatures in the unlit, unheated, underground storage facility were recorded hourly using HOBO® data loggers (Onset Computer Corporation).

The 2011 experiment began on 15 April 2011, and the 2012 experiment began on 8 May 2012. On these days pupae were placed individually into vials (6 cm x 2 cm diameter) in a temperature controlled room at 2 to 5 °C. Each vial contained a 2 cm layer of moist vermiculite; a pupa, and subsequently an additional 2 cm layer of vermiculite to cover the pupa. Lids of the vials were punctured to create small holes for ventilation. In 2011, the pupae from each locality (Table 2) were equally allocated across temperature treatments for a total of 66 vials of individual pupae randomly assigned to each treatment. Treatments were constant temperatures of 9.4, 14.7, 19.7, 24.8, 30 and 35 °C (Table 2). In 2012, ten vials of individual pupae from each of the nine sites were randomly assigned to each of the constant temperature treatments of 11.8, 17.8, 22.4, 27.4, and 29.7 °C. All experiments were carried out in environmental chambers. In both years, the temperatures in the environmental chambers were monitored with HOBO® data loggers, and the photoperiod was set at 16:8 h light:dark. The vials were checked daily for emergence of *E. connexa* or parasitoids. During these inspections moisture conditions were monitored and water added when required. The date of emergence and sex of *E. connexa* was recorded.

The percent emergence of *E. connexa* adults at each temperature in each year was calculated from:

$$Emergence\ of\ E.connexa\ (\%) = \frac{100 \text{ x } Number\ of\ E.connexa\ emerged}{(Total\ number\ of\ pupae - Number\ of\ parasitoids\ emerged)}$$

Days to emergence was calculated as days from the placement in the temperature treatment until the adult *E. connexa* emerged. Taylor's (1981) truncated normal model was the basis for modelling the relationship between temperature and rate of development:

$$R_t = R_m e^{\left[-\frac{1}{2}\left(\frac{T-T_m}{T_\sigma}\right)^2\right]}$$

Where rate of development R_t = 1/ (days to emergence) for a temperature T °C, R_m = maximum rate of development, which occurs at temperature T_m. The T_σ represents the spread of the normal distribution. The model fit was by non-linear regression (Systat 2009) using the method of Quasi-Newton estimation to minimize least squares.

To examine the possibility that development occurred prior to the start of the 2011 and 2012 experiments, the model of Taylor (1981) was inverted, producing a reciprocal truncated normal model to predict the days to emergence. The number of days to emergence for both years was modelled together incorporating a notation, D_p, representing differences between years in amount of development completed before constant temperature treatments began.

$$Days = \left\{\frac{1}{R_m e^{\left[-\frac{1}{2}\left(\frac{T-T_m}{T_\sigma}\right)^2\right]}}\right\} - (D_p * Yearcode)$$

Where Yearcode was 0 for 2011 and 1 for 2012. Pupae from different locations were randomly allocated to temperature treatments in each of the two years, and so the term, D_p, can be used to represent the average difference between years in pupal development before the experiment, and thus allow common estimates of the parameters T_m, T_σ and TH_{08} to be obtained from the pooled data for two years.

To estimate the lower threshold of development the formula from Lamb (1992) was used:

$$TH_{08} = T_m - 2.23T_\sigma$$

Where TH_{08} is the estimated temperature at which 8% of the maximum developmental rate is predicted to occur and approximates the developmental threshold that would be obtained if a linear degree-day model had been fitted (Lamb 1992). The temperature experienced by pupae in constant temperatures was estimated by averaging the HOBO® data for each environmental chamber for the duration of the experiment.

To estimate the temperatures experienced at sites similar in altitude to those where *E. connexa* were collected, monthly average, monthly mean minimum and monthly mean maximum temperatures were obtained for three Swiss weather stations (MeteoSwiss 2012a; MeteoSwiss 2012b; MeteoSwiss 2012c). Blatten was chosen to approximate Ausserberg and Kandersteg temperatures. Piotta was chosen to represent S. Antonio, Anzonico, and Pratto temperatures. The Bern weather station was chosen to represent the temperatures around Soyhières, Vermes (a,b), and Moutiér. Daily average temperatures for CABI were chosen from Courtmelon for 2010 and 2011 during August and September of the 2011 and 2012 experiments. HOBO® data recorded the daily average temperature in the underground storage facility from 6 September, 2011 to 31 January, 2012, the overwintering period of the pupae for the 2012 experiment. In addition, as a result of a specific request, MeteoSwiss (the Swiss Federal Office of Meteorology and Climatology) provided data for Delémont on deviation from the long-term average temperatures for the years of the experiments.

Egg development

From 16 June to 22 June, 2012, male and female adult *E. connexa* that were newly emerged from overwintered pupae originating from the 2011 field collections, were placed in a plastic cylinder for mating and mature egg development to occur. The cylinder

was 15 cm x 10.5 cm diameter and had a 2 cm diameter hole cut into the plastic lid with a cylindrical sponge inserted to allow for ventilation. In the bottom of the cylinder, a 6 cm x 2 cm diameter water-filled vial was secured to the side and fitted with a paper towel wick. Three dishes, one each of granulated sugar, dried skim milk powder, and instant yeast were placed on the bottom of the cylinder. The cylinders were maintained in environmental chambers at 20 to 22.5 °C, 16:8 h light:dark.

In the laboratory, on 2 July 2012, potted fruiting *V. hirundinaria* plants were covered with fabric screen and the mated pairs of *E. connexa* were placed inside. After 16 h, *V. hirundinaria* fruits were collected and dissected to collect *E. connexa* eggs. The eggs were randomly allocated to Petri dishes (5 cm x 2 cm) and placed in environmental chambers held at 11.8, 16.6, 17.2, 20.2, 22.2, 27.3, or 30 °C. For each temperature, 20 eggs were placed on moist filter paper in Petri dishes and placed in an open container floating in a closed water bath that maintained relative humidity at 100%. Temperatures were assessed using data loggers HOBO® in each environmental chamber. Eggs were checked daily for hatching larvae.

Percent larval hatch was calculated for each temperature. The days to hatch was calculated from the day of laying until the day of larval emergence, and the reciprocal was taken as an estimate of rate of development. The relationship of the rate of egg development to temperature was modelled using the unmodified truncated normal model (Taylor 1981). The lower limit of development was estimated using the formula of Lamb (1992). HOBO® data were averaged for the duration of the experiment to estimate temperatures experienced by the eggs during development.

Results

Adult development

Adults did not emerge at 9.7, 29.7, 30.85, and 35.1 °C, and emergence was low at 27.1 °C (Figure 1). Between 11.8 and 24.8 °C, percentage emergence was relatively high. Within this temperature range, in each year, percentage emergence was unaffected by temperature, however the percentage emergence was lower in 2011 than in 2012.

To test whether development data of females and males could be amalgamated, the developmental rate was graphically evaluated separately for *E. connexa* males and females at each temperature (Figure 2). Except for the 27.1 °C treatment, which had poor survival, error bars for males and females overlapped, therefore, the data were combined for analysis.

To evaluate if there were differences among sites the development rate at each site was plotted against temperature (Figure 3). Even though Ausserberg graphically showed a faster developmental rate than the other sites in 2011, the addition of a site term to the non-linear regression did not improve fit, and so, the results from all sites were pooled for each temperature replicate for 2011 and 2012.

To evaluate the effect of temperature on the developmental rate of *E. connexa* data was analysed using Taylor (1981). In 2011, the parameter estimates divided by their asymptotic standard error exceeded the critical value of *t* and the upper and lower Wald confidence intervals did not include zero (Table 3), so the model appeared to be a good fit. In fitting the model to the 2012 data, the parameter estimates were significant (Table 3), but the T_m was well above the highest temperature at which development was seen in the experiment. Because of this there can be little confidence in this value. The number of surviving individuals at 27.1 °C was low and the number of days to develop at this

temperature was highly variable, contributing to the poor fit of this data point to the model depicted in Figure 4. For parameter estimates of the 2011 and 2012 models, the Wald confidence intervals did not overlap for R_m and T_m, so the models for the two years were not identical.

To assess the difference in days to development the reciprocal truncated normal model was used and was found to be significant (Table 4) and explained 99.6% of the among-temperature variation in the number of days to emergence. However, the residuals among temperatures were also significant. Graphical investigation showed little discrepancy between the observed values and those predicted by the model, with the exception of adults emerging from 27.1 °C, where survival was low (Figure 5). The parameter estimates for this model are significant, as the Wald confidence intervals do not include zero (Table 3). The T_m estimate in this model is a good estimate of the temperature at which the maximum rate of development occurred during the experimental period; this value does not include a temperature outside the observed development. By accepting that T_m is a good parameter estimate, then T_σ correctly represents the spread of temperatures for which development occurred (Table 3). The fitted model accounts for different amounts of development occurring before the experiment in the two years, but if some development occurred prior to the commencement of the experiment in both years, the R_m parameter is likely an overestimation of the true value.

The estimate of TH_{08}, the lower threshold for development, was slightly lower for the reciprocal model than for the individual truncated normal models (Table 3). During the experimental period, development was extremely slow at 11.8 °C, the lowest temperature at which adult emergence occurred (Table 3).

The constant, D_p, in the reciprocal model, indicates there was more pupal development prior to the start of the 2012 experiment than before the 2011 experiment. From the date of collection (Table 3) until entry into the underground storage facility at the end of September, larvae, and pupae experienced the room temperature in the laboratory. For the 2011 experiment the 10th percentile of larvae had pupated by 16 August 2010, 8 August 2011 for the 2012 experiment. The 50th percentile of larvae had pupated by 25 August 2010, for the 2011 experiment and 12 August 2011 for the 2012 experiment. For the 2011 experiment, the 90th percentile had been reached by 30 August 2010, while for the 2012 experiment this value was reached on 20 August, 2011.

Long-term average monthly temperatures from three weather stations were strongly related to elevation, with the highest sites being cooler in all months (Table 5). The temperatures from the Courtemelon weather station indicate that during the time the pupae were left in the laboratory, August to October, the temperatures were higher during the 2012 experiment (Figure 6). For the duration the pupae were kept in the underground storage facility for the 2012 experiment the temperatures did not go below the lower limit of development until December. Also, once the temperature in the underground storage facility went below the lower limit of development it fluctuated from 2 to 6.7 °C (Figure 6). In addition, for Delémont, the long-term monthly averages for August and September of 2010 were close to the long-term averages, but in August and September 2011, during the 2012 experiment, temperatures were 2.5 and 2.9 °C above the long-term averages.

Egg development

Larval hatch occurred at all experimental temperatures. The percentage hatch was low at 27.3 °C (Figure 7), but at other temperatures was higher and ranged between 50 and 75%.

The effect of temperature on the developmental rate of *E. connexa* eggs conformed to the truncated normal model by Taylor (1981), the fit of which was highly significant and explained 95.2% of the among-temperature variation (Table 6). The model was a good fit for all the data points except 17.2 °C (Figure 8). The parameter estimates were significant based on the Wald confidence intervals, although the T_m is about 1 °C above the experimentally evaluated temperatures (Table 7) and should be interpreted with caution. The estimated lower limit for development was 9.3 °C.

Discussion

Adult development

In this study, the development to adult emergence of pupal *E. connexa* occurred between 11.8 and 27.1 °C. However, the favourable range for pupal development most likely does not extend as high as 27.1°C as emergence at this temperature was very low. The mortality of all *E. connexa* at 29.7 °C and above suggests this temperature is above the upper lethal limit, defined as the temperature above which mortality results from temperature-related cellular imbalances in reaction rates and accumulation of metabolites (Hill et al. 2004). The low emergence at 27.1 °C could also be the result of cumulative metabolic stress or be a consequence of dehydration of pupae. Early emerging individuals developed faster than predicted by the models and so it is likely that only the fastest were able to complete development before cumulative stresses became lethal.

Adults did not emerge below 11.8 °C. By April, the lower elevation field sites attain average daily maximum air temperatures above 11.8 °C and the daily mean air temperatures are above 11.8 °C in all sites by June (Table 5). Until snow melts the temperature rise in pupal locations in the soil may lag behind air temperature; pupae would experience temperatures well above 11.8 °C in summer. Because of this, testing

sustained temperatures below 11.8 °C may have little relevance to adult emergence in the field. As the TH_{08} value, 4.2 °C, predicts development should have occurred at the lowest experimental temperature of 9.4 °C, it is likely that the lack of emergence at this temperature was due to one or a combination of factors such as dehydration, bacterial or fungal growth, or exhaustion of the stored energy supply within the puparium.

The TH_{08} values can be used to compare my results from the truncated normal model with developmental thresholds from linear models. The developmental threshold obtained for *E. connexa* (4.2 °C) is much lower than that for pupae of other tephritid flies that have been studied. Pupae of *Ceratitis capitata* (Wiedemann) have lower limits of development from 10.4 to 11.2 °C (Duyck and Quilici 2002; Ricalde et al. 2012). In two other *Ceratitis* spp., thresholds for pupal development are 9.2 °C, for *C. catoirii* Guérin-Méneville, and 11 °C, for *C. rosa* Karsch (Duyck and Quilici 2002). Judd et al. (1991) found similar lower developmental thresholds of 9.3 °C for the pepper maggot, *Zonosemata electa* (Say) (Diptera: Tephritidae). Finally, *Bactrocera correcta* (Bezzi) was found to have a theoretical lower limit of development at 9.5 °C (Liu and Ye 2009). *Euphranta connexa* is found in cooler climatic regions (Solbreck 2000) whereas *B. correcta* is native to tropical Asia (White and Elson-Harris 1992), *C. capitata* originated from East Africa (Baliraine et al. 2004), *C. rosa* is found in east and southern Africa (De Meyer et al. 2008), *C. catoirii* is endemic to Mascarenes, Mauritius (Duyck and Quilici 2002), and *Z. electa* is most abundant along the east coast of the United States and in Southern Ontario, Canada. With the exception of *Z. electa*, the places of origin are in warmer climatic regions, and therefore insects can develop successfully with higher thresholds for development.

Females of the apple maggot *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) and the Caribean fruit fly, *Anastrepha suspense* (Loew), emerge prior to males at the beginning of the reproductive season (Allen and Fluke 1933; Glass 1960; Sivinski and Calkins 1990). *Euphranta connexa* in this study did not show a significant sexual dimorphic rate of development except at 27.1 °C, where males developed more rapidly than females. As it is likely that developmental rates at this temperature were distorted by differential mortality of fast and slow developers, it is possible that sexual dimorphic pupal tolerance of the often lethal stresses at this temperature account for the observed earlier emergence of males.

The variation in developmental rate from year to year indicates that, under the experimental conditions used, pupal development occurred before the start of the experiment. Although D_p's nominal units are days, the differences in development after pupation occurred during a time of variable temperatures in the laboratory and before temperatures in the underground storage facility were constantly below TH_{08} (4.2 °C). Therefore, the value of 13 days gives an order of magnitude estimate of the difference in development, but is not a precise estimate, and allows for common estimates of the parameters T_m, T_σ and TH_{08}.

The difference in development between the years may be attributed to the date of collection (Table 2) and date of arrival of insects in the laboratory. Plant materials with larvae were collected about one week earlier for the 2012 than the 2011 experiment. As a result, in the 2012 experiment the larvae and pupae were exposed to laboratory temperatures that were constant and warmer than field site temperatures, about 10 days earlier than for larvae in the 2011 experiment. Larvae for the 2012 experiment pupated earlier than those for the 2011 experiment, with the 10, 50, and 90th percentile pupation

dates respectively 8, 13 and 10 days earlier for the 2012 experiment. The difference in pupation dates probably accounts for most of the difference in the number of nominal days to development, and corresponds well to the D_p value of 13. Differences in summer and fall temperatures in the two years may also have contributed. Temperatures near the research station were about normal for August and September preceding the 2011 experiment and approximately 2.5 to 2.9 °C above normal for the same period before 2012 experiment (data from MeteoSwiss). Since laboratory temperatures are likely to be influenced by the external environment, the pupae collected for the 2012 experiment would have experienced higher temperatures. The higher late summer temperatures for the 2012 experiment might have also resulted in higher temperatures in the underground storage facility than in the previous year, resulting in a longer period of pupal development before winter in the 2012 experiment than in the 2011 experiment. The earlier collection date, pupation date, and the possibly warmer temperatures in the laboratory and underground storage facility would result in pupal development being more advanced prior to the 2012 experiment.

The literature on the biology and geography of *E. connexa* and *V. hirundinaria* mostly covers Scandinavian populations (Ågren et al. 2008; Leimu 2004; Leimu and Lehtilä 2006; Solbreck 2000; Solbreck and Ives 2007; Solbreck and Sillén-Tullberg 1986b) with one publication from Northern Spain (Pagola-Carte and Peydro 2012). The seasonality of *E. connexa* in Northern Spain, Scandinavian, and my study were similar (Pagola-Carte and Peydro 2012; Solbreck 2000). The population in Northern Spain is at about 600 m (Pagola-Carte and Peydro 2012) and my sites in Switzerland were at relatively high altitudes. Localities of *E. connexa* collections are near sea level in the

northern part of the range and at higher elevations in more southerly latitudes; from this it can be inferred that *E. connexa* is adapted to cooler temperatures.

Geographically there were no significant differences in developmental time among sites even though they differed in altitude by over 1000 m, and differed climatically: on average, temperature declines by 5.5 °C for each 1000 m gain in altitude (Korner 2007). This highlights that the time to development for *E. connexa* is not affected by altitude. The narrow range of variation of developmental rate and time in days to emergence of adults among sites can also indicate there is no local adaptation.

Two possible strategies of overwintering for the univoltine *E. connexa* are i) diapause or ii) cold-induced quiescence. In the literature *E. connexa* is univoltine (Solbreck 2000), and if diapause was the method of overwintering would likely undergo obligatory diapause (Gullan and Cranston 2010). Interestingly, in the 2010 and 2011 field season, *E. connexa* adults emerged from some pupae in the laboratory prior to placement in the underground storage facility. As well, Pagola-Carte and Peydró (2012) report that a male emerged on 21 January from a puparium held at room temperature since larval collection in July. My finding that winter is not spent at a constant point of development is consistent with an absence of obligatory diapause. Thus, as assumes Solbreck and Ives (2007), *E. connexa* does not diapause but goes through a cold-induced quiescence where development is decreased in response to unfavourable conditions and resumes when conditions are favourable (Gullan and Cranston 2010). A strategy to decrease risk, such as diapause, is the result of environmental uncertainty (Hopper 1999). If diapause had evolved in *E. connexa*, selection would favour seedpod production prior to or long after the emergence of emerging adults of overwintered *E. connexa.* The overwintering strategy of *E. connexa* can be considered bet-hedging as it is likely to spread the risk for

ovipositing females the following year. The production of fruits and the presence of *E. connexa* in the field from June to August suggest *E. connexa* is not regularly in a situation of uncertainty. If females oviposit over an extended period, there would be variation in emergence date of their offspring, and this amounts to spreading of risk, with respect to the risks of no mates, no suitable seedpods, and unsuitable weather. Populations of *V. hirundinaria* which are more genetically variable are more locally adapted to avoid predation by *E. connexa* (Klaske et al. 2012). More genetic variation in *V. hirundinaria* could provide enough certainty that there would be seedpods available whenever in the summer *E. connexa* emerges, thus not requiring *E. connexa* to have precise synchronization through diapause. Also, *E. connexa* are strong fliers able to re-colonize sites with local extinctions (Solbreck and Ives 2007), and this would also decrease the risk to *E. connexa*.

Egg Development

Larvae hatched at all the experimental temperatures tested, but percent hatch was lowest at 27.4 °C. It is not clear what caused the low rate of hatch at this temperature. As the eggs from different females were randomly distributed among the different temperature treatments, it is unlikely to be a consequence of low fertility of eggs of one or a few females. Also, the temperature fluctuations for this environmental chamber were about 0.5 °C. Percentage larval hatch in my study was similar to that of *C. capitata* (Ricalde et al. 2012) and generally higher than the 10-71% hatch of larvae of *B. zonata* (Duyck et al. 2004).

The range of temperatures suitable for hatching of eggs of *E. connexa* is similar to that for *Ceratitis* spp., 15 to 30 °C (Duyck and Quilici 2002). In my study, 35 °C was not

tested, but emergence did not occur for *Ceratitis* spp. at this temperature (Duyck and Quilici 2002) and it appears that the upper limit of development for *E.connexa* is less than 35 °C, similar to that of *Ceratitis* spp. In my study the theoretical lower limit for egg development was much higher than for the pupal stage, and similar to that seen in *Ceratitis* spp. of 11.6 °C (Duyck and Quilici 2002). The TH_{08} is likely different between pupae and egg stages as the pupae experience cooler overwintering temperatures but the eggs develop inside the seedpod during the warmest parts of the season.

Practical implications for biological control

Euphranta connexa is currently being studied for its potential as a biological control agent on the invasive weeds *V. rossicum* and *V. nigrum* in North America. Prior to this study, synchronisation of the emergence of *E. connexa* with a range of host and test plants was extremely difficult. This study found a suitable temperature range for pupal development, the threshold below which no development occurs, the temperature at which development rate is maximum, and that overwintering is in a state of quiescence. With this information, insects can be acquired from the field, held over the winter at temperatures below the lower limit of development and emergence can either be staggered by placement at different temperatures or pupae can be placed directly at a temperature that will generate emergence of *E. connexa* when required. To improve the developmental rate model, the experiment could be repeated except that immediately after pupation the pupae should be placed either directly into experimental temperatures or held below 4.2 °C until subsequent exposures to experimental temperatures. In the context of rearing and synchronizing insects for the purposes of biological control studies, the data presented here are sufficient. No difference was found in the time to develop

from pupa to adult between females and males, thus, males and females do not need to be treated differently to achieve emergence. Failures of biological control agents have been attributed to poor climatic matches (Clausen 1978) and the introduction of insects from similar climates is often suggested (McClay 1996; Messenger and van den Bosch 1971; Stiling 1993). The absence of differences in thermal responses of pupal development among sites from a range of altitudes implies climate matching is of little importance if *E. connexa* is to be introduced as a control agent against *V. rossicum* and *V. nigrum*. This also implies that *E. connexa* is not locally adapted to the *V. hirundinaria* populations in Switzerland which could increase its success if introduced as a biological control agent into North America on *V. nigrum* and *V. rossicum.*

Table 2. The location of collection sites in Switzerland, dates of collection, and number of pupae used in each experiment for *Euphranta connexa* in 2011 and 2012.

	GPS coordinates			2011 Experiment		2012 Experiment	
Locality	Longitude	Latitude	Elevation (m)	Collection date	Total pupae in experiment	Collection date	Total pupae in experiment
Ausserberg	46° 20'21.1"N	7°52'43.9"E	1300	10 August 2010	60	1 August 2011	50
Kandersteg	46° 30'22.0"N	7°43'50.1"E	1700-1800	21 August 2010	60	11 August 2011	50
S. Antonio	46°10'12.6"N	9°03'43.6"E	860	11 August 2010	132	30 July 2011	50
Anzonico	46°26'13.5"N	8°51'09.8"E	750 – 1000	11 August 2010	72	30 July 2011	50
Prato	46° 29'28.7"N	8° 45'13.9"E	890	12 August 2010	72	29 July 2011	50
Moutiér	47° 16'58.6"N	7° 23'14.6"E	600 - 900	.	.	5 August 2011	50
Soyhières	47° 24'30.7"N	7° 22'53.5"E	560	.	.	5 August 2011	50
Vermes (a)	47° 19'48.5"N	7° 28'21.5"E	570-700	.	.	5 August 2011	50
Vermes (b)	47° 19'50.6"N	7° 26'45.8"E	520	.	.	5 August 2011	50

Table 3. Parameter estimates for models of temperature dependent rate of development of *Euphranta connexa* pupae, in 2011 and 2012 using the truncated normal model. In the reciprocal truncated normal model the term *Dp* was used to estimate the nominal number of days difference in development completed before the 2011 and 2012 experiments; allowing for the R_m, T_m, T_σ, and TH_{08} to be estimated over both years.

Year	Parameter[A]	Estimate	ASE[B]	Parameter/ASE	Wald 95% Confidence Interval	
					Lower	Upper
Truncated normal model (2011)	R_m	0.027304	0.000637	42.8347	0.026044	0.028565
	T_m	24.567042	0.963503	25.4976	22.662263	26.471822
	T_σ	8.237855	0.903034	9.1224	6.452618	10.023091
	TH_{08}	6.200000				
Truncated normal model (2012)	R_m	0.048697	0.004056	12.0074	0.040711	0.056683
	T_m	31.664724	1.948826	16.2481	27.827092	35.502356
	T_σ	11.682127	0.915528	12.7600	9.879267	13.484987
	TH_{08}	5.610000				
Reciprocal truncated normal model	R_m	0.025968	0.000735	35.3157	0.024523	0.027414
	T_m	25.832960	0.633907	40.7519	24.586763	27.079157
	T_σ	9.704463	0.353176	27.4777	9.010156	10.398771
	D_P	13.214437	0.913021	14.4733	11.419531	15.009343
	TH_{08}	4.190000				

[A] R_m is the maximum rate of development expressed as 1/days; T_m, the temperature at which the maximum rate of development occurs, T_σ, the spread of the normal distribution, and TH_{08}, the lower threshold for development, are measured in ^{o}C, and *Dp* is a measurement of nominal days.

[B] Asymptotic standard error

Table 4. Variance partitioning for reciprocal truncated model of temperature dependent rate of development of *Euphranta connexa* pupae.

Source	Sum of squares	df	Mean Squares	*F*-Ratio	*p*-Value
Among temperatures	250623.60	6	41770.60	564.64	< 0.0000
Regression model	249537.87	4	62384.47	114.92	0.0086
Residual among temperatures	1085.73	2	542.87	7.34	0.0007
Within temperature	29443.08	398	73.98		
Total	280066.68	404			

Table 5. The monthly average of daily mean, minimum, and maximum temperatures (°C) for weather stations at similar altitudes to field sites at which *Euphranta connexa* was collected.

	Blatten (1535 m altitude)			Piotta (990 m altitude)			Bern (552 m altitude)		
Month	Mean	Minima	Maxima	Mean	Minima	Maxima	Mean	Minima	Maxima
August	12.4	5.8	19.7	16.6	11.8	22.2	17.7	12.3	23.7
September	8.9	2.9	16.2	12.8	8.7	17.8	13.7	8.9	19.1
October	4.6	-0.3	12	8.3	4.7	12.9	9.3	5.4	13.8
March	-1	-6.4	5.6	3.7	-0.4	8.5	4.7	0.2	9.5
April	2.6	-2.8	8.6	7	2.6	11.7	8.1	3	13.4
May	7.6	1.4	13.8	11.4	6.8	16.4	12.7	7.4	18.2
June	13.8	3.8	17.7	15.1	9.9	20.7	16	10.5	21.6
July	5.9	13.2	20.5	12.1	18.3	23.1	12.5	17.4	24.3

Note: Weather data are long-term averages from 1981-2010 from MeteoSuisse at 2 m above the ground. The minima and maxima are the averages of the daily minima and maxima for the month.

Table 6. Variance partitioning for truncated normal model of temperature dependence of rate of development of eggs of *Euphranta connexa*.

Source	Sum of squares	*df*	Mean Squares	*F*-Ratio	*p*-Value
Among temperatures	0.565655	6	0.0943		
Regression model	0.538660	3	0.1796	19.95	0.0174
Residual among temperatures	0.026995	3	0.0090	0.45	0.7183
Within temperature	0.060032	75	0.0008		
Total	0.625687	81			

Table 7. Parameter estimates for truncated normal model of temperature dependent rate of development of eggs of *Euphranta connexa*.

Parameter[A]	Estimate	ASE[B]	Parameter/ASE	Wald 95% Confidence Interval	
				Lower	Upper
R_m	0.284	0.012	24.315	0.261	0.307
T_m	31.164	1.598	19.505	27.983	34.344
T_σ	9.803	1.069	9.174	7.676	11.930
TH_{08}	9.30				

[A] R_m is the maximum rate of development expressed as 1/days; T_m, the temperature at which the maximum rate of development occurs, T_σ, the spread of the normal distribution, and TH_{08}, the lower threshold for development, are measured in °C, and D_P is a measurement of nominal days.

[B] Asymptotic standard error

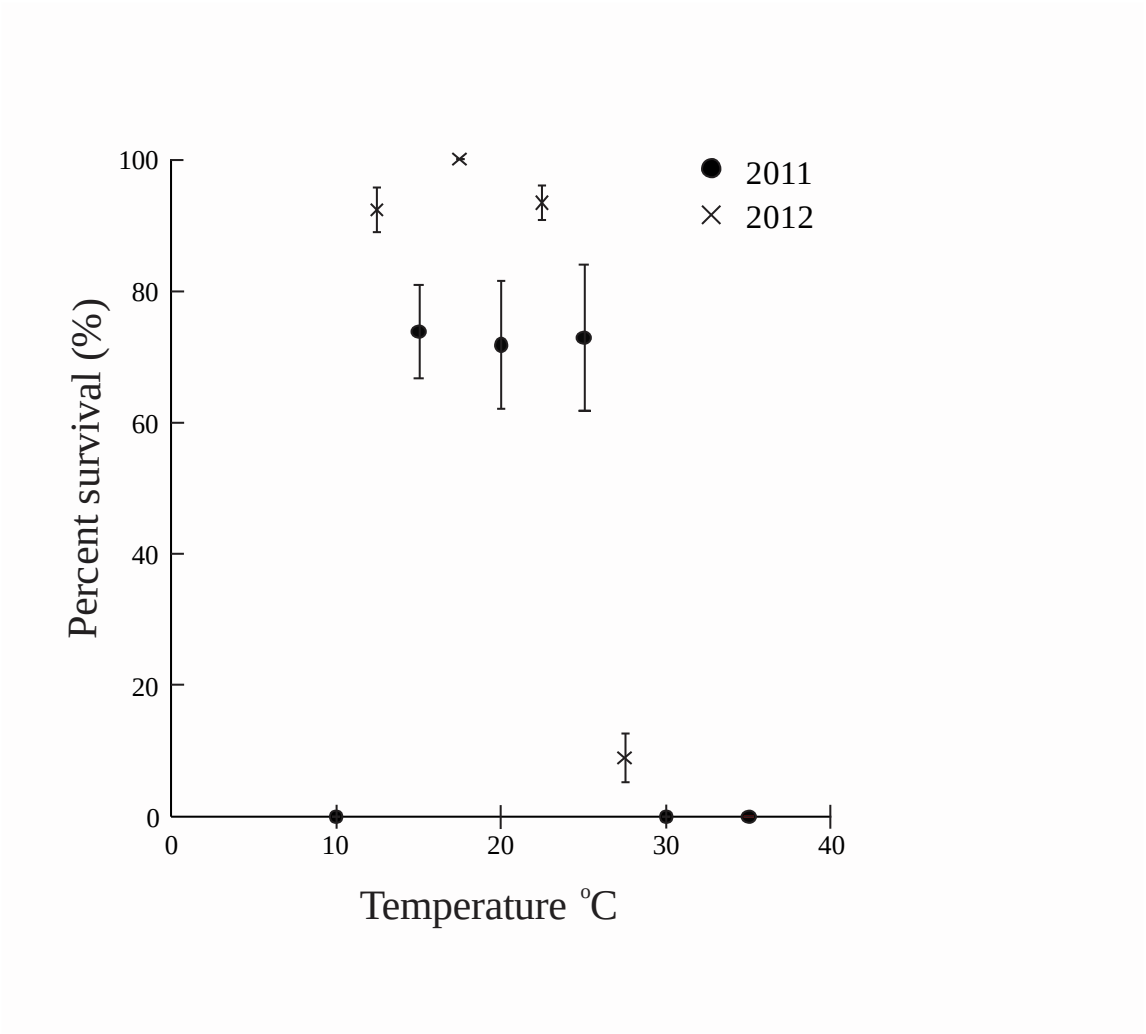

Figure 1. The percent survival (mean ± SEM) to adult emergence of *Euphranta connexa* pupae pooled over sites and sexes at eleven constant temperatures in the 2011 and 2012 experiments.

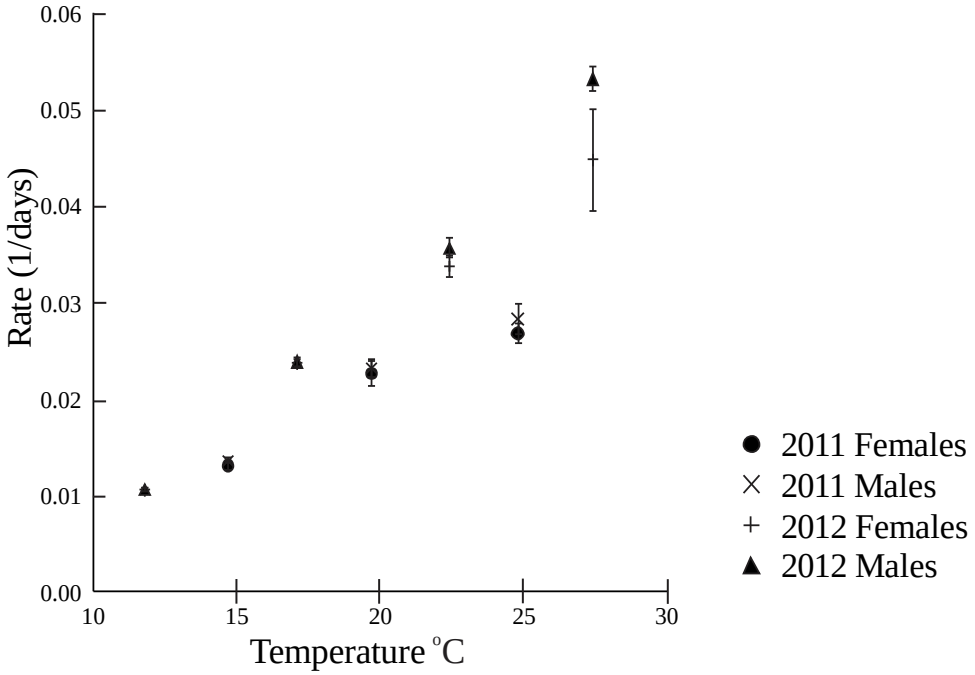

Figure 2. Rates of development (mean ± SEM) of female and male pupae of *Euphranta connexa* at seven constant temperatures in the 2011 and 2012 experiments.

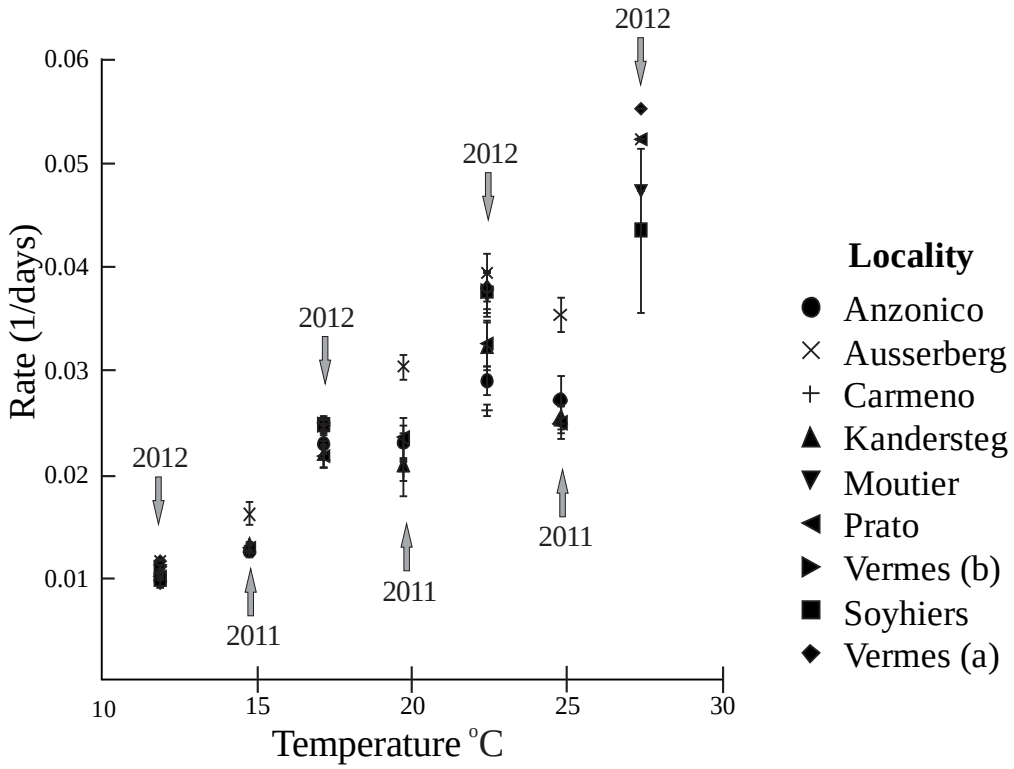

Figure 3. Rates of development (mean ± SEM) of pupae in relation to temperature for *Euphranta connexa* from different sites within Switzerland.

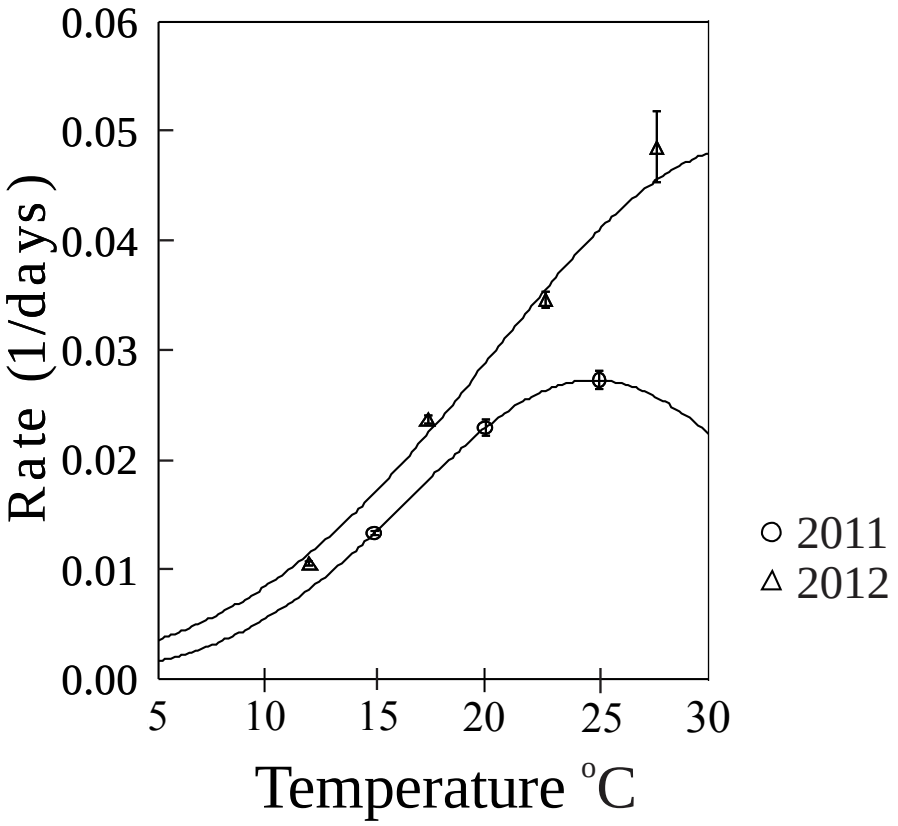

Figure 4. Rate (mean ± SEM) of pupal development at three constant temperatures in 2011 and four constant temperatures in 2012, and fitted lines from truncated normal models for the 2011 and 2012 experiments.

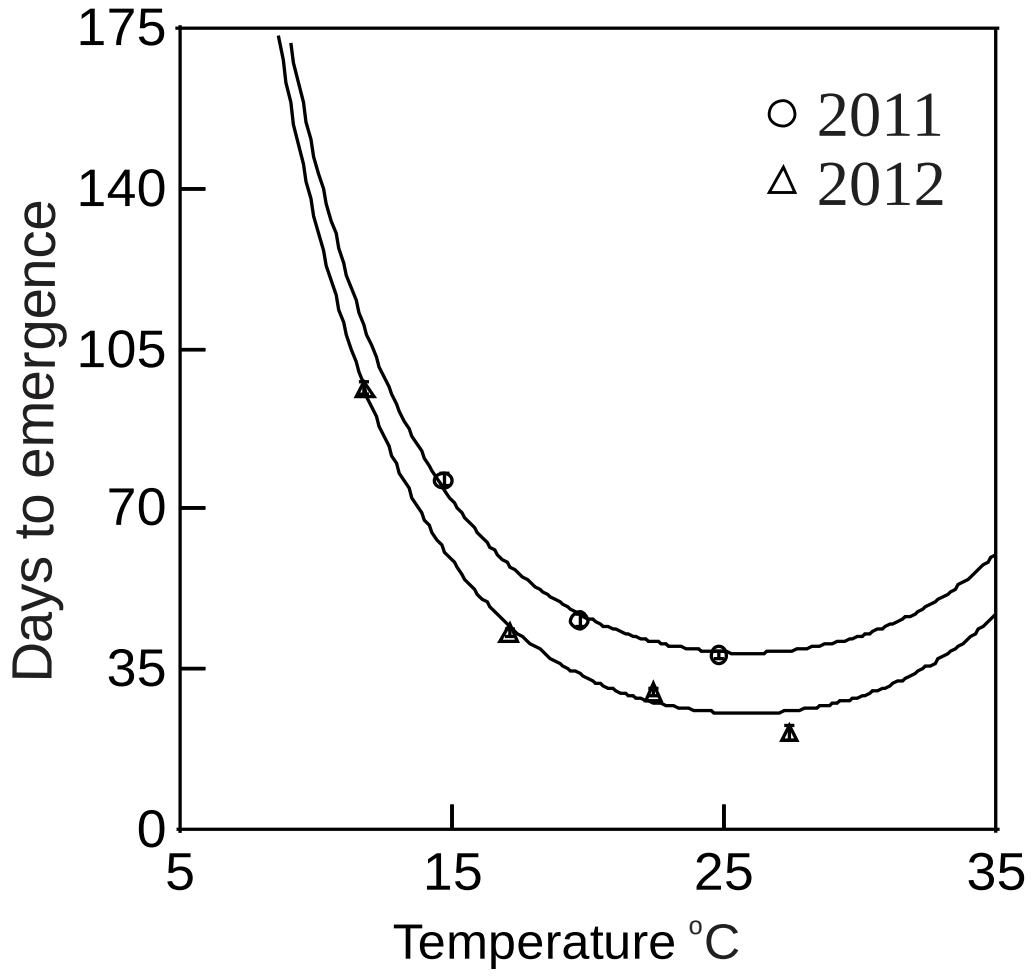

Figure 5. Mean (± SEM) days to adult emergence of pupae of *Euphranta connexa* at three constant temperatures in 2011 and four constant temperatures in 2012, and fitted lines from reciprocal truncated normal model for both years, with a factor D_p (13 nominal days) representing the difference in prior development between the two years.

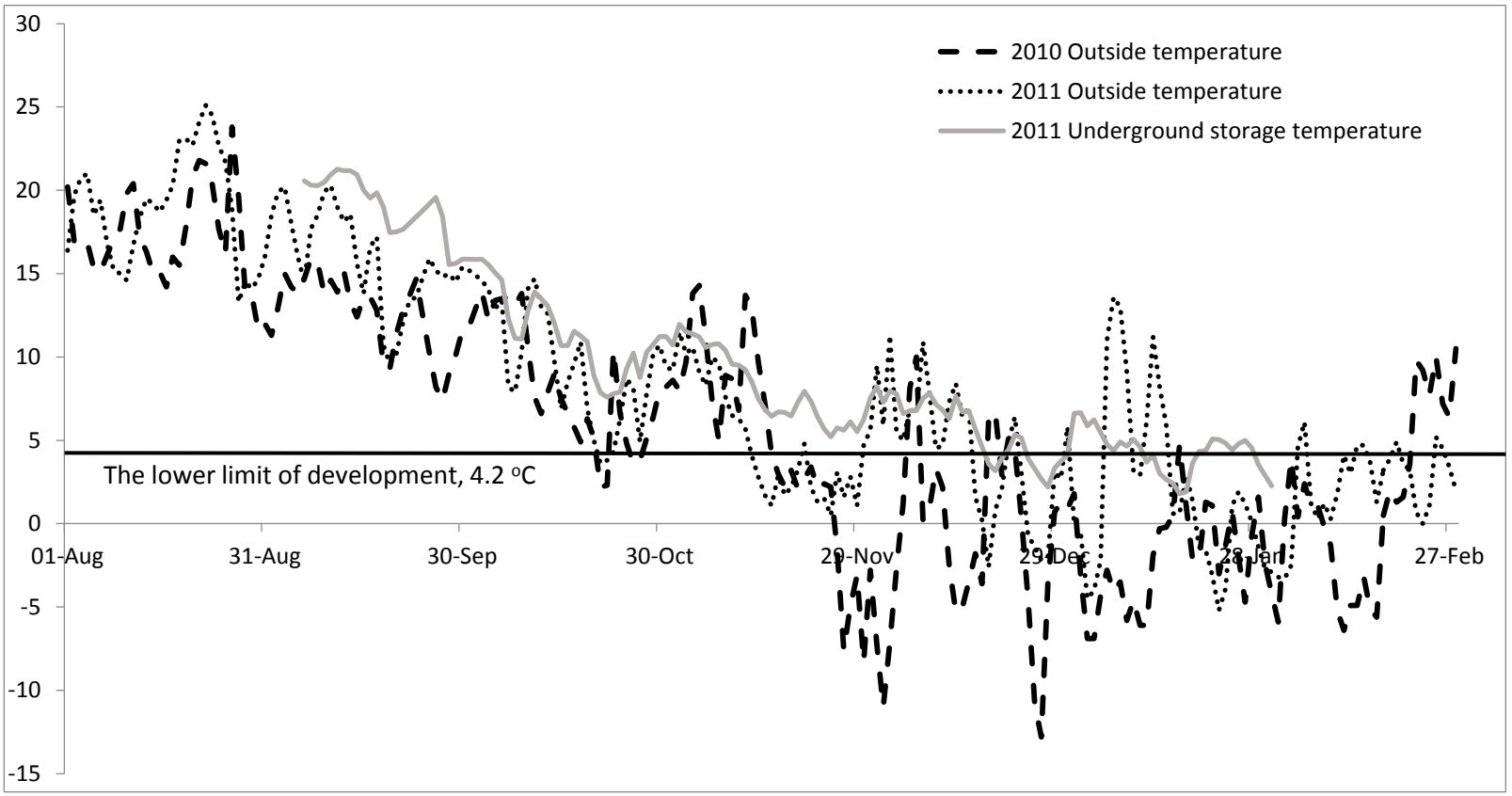

Figure 6. The average daily temperature (°C) from Courtemelon (435 m elevation), Switzerland from 1 August, 2010 to 28 February, 2011 and from 1 August, 2011 to 28 Februrary, 2012, and the average daily temperatures recorded in the underground storage facility at CABI from 6 September, 2011 to 31 January, 2012.

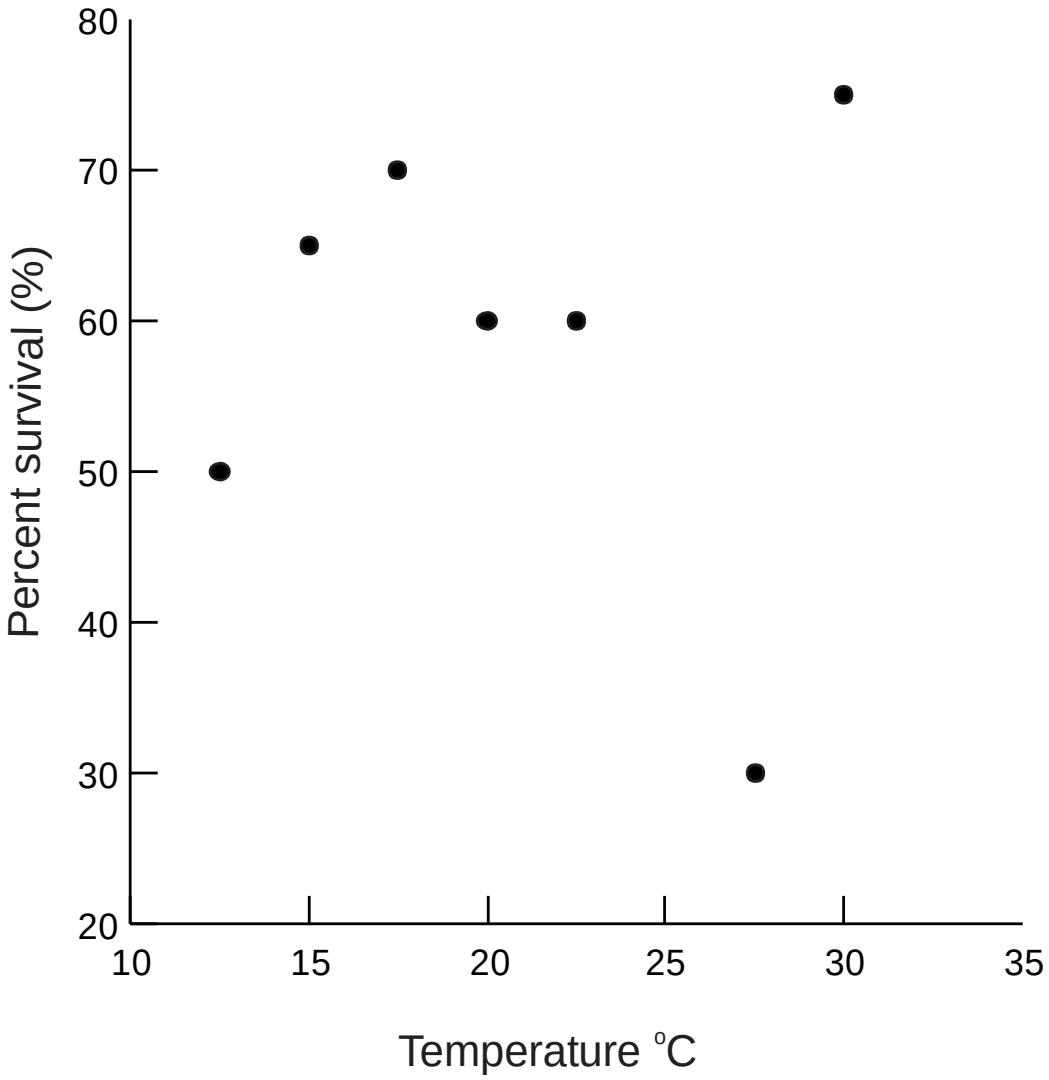

Figure 7. The percent of *Euphranta connexa* eggs surviving to eclosion at seven constant temperatures with 20 eggs at each temperature.

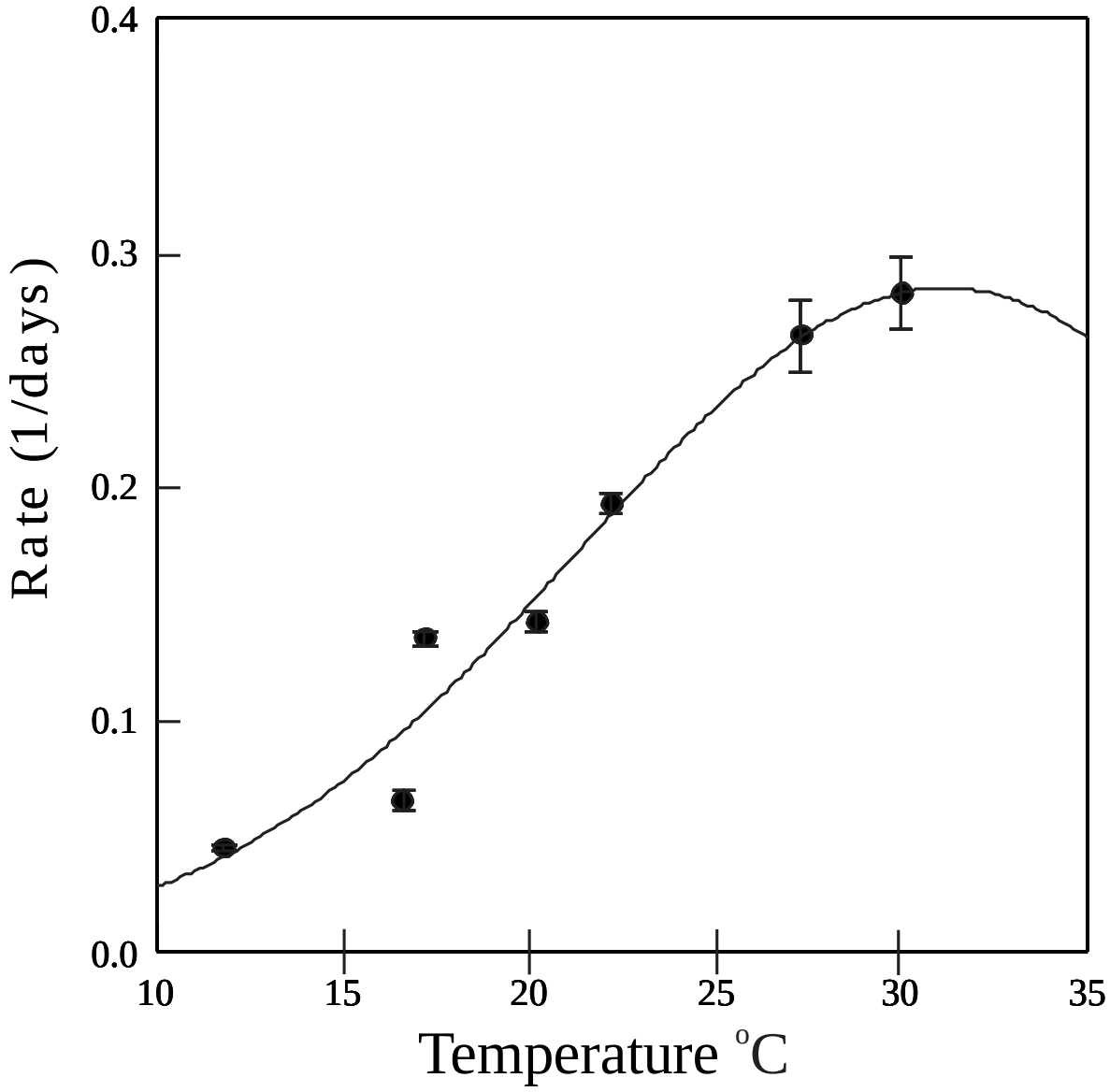

Figure 8. Rate (mean ± SEM) of egg development of *Euphranta connexa* at seven constant temperatures and fitted truncated normal model.

CHAPTER 4: THE EFFECT OF AGE, MATING, AND OVIPOSITION STIMULI ON THE EGG LOAD OF *EUPHRANTA CONNEXA* (DIPTERA: TEPHRITIDAE)

Abstract

Before the introduction of insect agents for biological control of weeds, sufficient evidence must be provided to demonstrate minimal risk to native plants in the area of introduction. To provide these results, host range tests ranging from no-choice to multiple-choice experiments must be conducted. For the potential control agent *Euphranta connexa* (Fabricius) (Diptera: Tephritidae) for swallowworts (*Vincetoxicum* spp.) (Apocynaceae) in North America, basic biological data required for the development of appropriate testing procedures were lacking. The goals of this study were to establish the time required for females to develop mature eggs within their ovaries, the age at which the maximum egg load is attained, and the effects of mating and oviposition on egg load. In this study, females at emergence contained no eggs and the peak egg load of 47 eggs was approached by day 8. There was no effect of mating on egg load. However, the number of eggs in the ovaries of mated females 15 days after emergence was greater than in mated females 30 days after emergence that had been offered a seedpod for oviposition on day 15. The reduction in the number of eggs by day 30 was greater than the number of eggs laid in the seedpod, suggesting that some eggs were resorbed. Also, the number of eggs in ovaries declined in females in which emergence was delayed. The data acquired in this study show females reach asymptotic egg load by day 8. As a result it is recommended that, in tests of oviposition host range, females that are from 8 to 15 days post emergence be used.

Keywords: egg load, mating, oosorption, *Vincetoxicum*.

Introduction

One of the major steps in a program for biological control is to assess the potential host range and risk to non-targets in the area of introduction (Blossey 1995; Briese 2005; Sheppard 2003; Sheppard et al. 2005; Simberloff 1996; Wapshere 1985). Testing the host range of a potential biological control agent can allow prediction of direct impacts in the non-native environment (Blossey 1995). The potential control agent is tested under no-choice conditions, choice, and multiple choice conditions (Blossey 1995) to determine whether it will attack plants of species other than the target weed species in the area to which it may be introduced (Blossey 1995). For a larval herbivore that feeds in concealment and in which host choice is made by the ovipositing female, testing before the female can lay eggs could produce false negative results. A series of false negatives for a potential control agent would underestimate risk of non-target attack in the area of release and so it is important to determine when females are capable of oviposition and when the maximum egg load is attained. The seed feeding fly *Euphranta connexa* (Fabricus) (Diptera: Tephritidae) oviposits in seedpods of its host, and is a potential control agent for *Vincetoxicum rossicum* (Kleopow) Barbar and *V. nigrum* (L.) in North America. The reproductive period of non-target plants might be different from that of the target plants or from that of the European field host making it crucial in host range testing to know when female *E. connexa* have eggs available to lay.

Euphranta connexa is distributed over a range of latitudes in the western Palearctic region (Carroll et al. 2002) but much of the published information on the biology and population ecology of *E. connexa* is from Scandinavia (Ågren et al. 2008; Janzon 1982; Solbreck 2000; Solbreck and Ives 2007; Solbreck and Sillén-Tullberg 1986a; 1986b). Depending on climatic conditions, *E. connexa* adults emerge from overwintering puparia around the middle of June and can be found in the field until

the end of August; the fly is univoltine (Solbreck 2000). After emergence, the females and males can be found on *V. hirundinaria* (L.), its only known natural host plant species (Solbreck 2000). The mating of *E. connexa* follows an elaborate mating dance, as described in the literature review. Following egg maturation, a female searches for a suitable seedpod of *V. hirundinaria* and oviposits an egg underneath the pericarp of the seedpod. Suitable seedpods range from 2 to 70 mm in length (Solbreck 2000). It is thought that the female applies an oviposition deterrent pheromone to the seedpod, after she has oviposited (Solbreck 2000). After hatching, larvae develop through three instars within the seedpod and when feeding is complete, a prepupa emerges from the seedpod, and pupates in the soil (Janzon 1982; Solbreck 2000). The female usually lays one egg per seedpod, although in years when seedpods are few, there may be many eggs per seedpod; the average number of prepupae emerging from attacked seedpods is one (Solbreck 2000; Solbreck and Sillén-Tullberg 1986b). The pupa is the overwintering stage and adults emerge from pupae the following spring (Solbreck 2000).

Despite this information on the basic biology of *E. connexa*, data are currently lacking on the time required following emergence for females to develop mature oocytes. The objective of this study was to determine the temporal pattern of egg development and to investigate whether egg load was influenced by mating or oviposition as mating can either increase egg load (Senger et al. 2007; 2008; Zhao and Zhu 2011) or not (Neilson 1975; Silva et al. 2012).

Materials and methods

The collection of fruits of *V. hirundinaria* to acquire insects for these experiments was described in Chapter 3. For all the fecundity experiments, Swiss-collected adult *E. connexa* that had emerged from pupae originating from field-collected larvae from

2010 and 2011 were placed in clear plastic cylinders,10 cm in diameter and 5.5 cm in height. In the lid of the container there was a hole, 2 cm in diameter, which was closed with a cylindrical sponge. On the bottom of each cylinder were four Petri dish bases 0.6 by 2.2 cm diameter, containing powered skim milk (Rapilait, MIGROS), water, granulated sugar, and instant yeast (Patissier, MIGROS). The cylinders were maintained at a constant temperature of 20 °C at 16:8 h light:dark with food and water being replenished when required.

Egg development

In 2011, 39 adult, virgin, and previously unfed *E. connexa* females from overwintered pupae were individually placed in plastic cylinders as above. Each female was randomly assigned a number from 1 to 20. This random number corresponded to the number of days the female was kept alive before her ovaries were dissected. Dissections of ovaries were carried out on fresh specimens and the number of mature eggs, hereafter referred to as egg load, was recorded. Mature eggs were defined as eggs ≥ ca. 0.9 mm long (Janzon 1982) without differentiation of the nurse cells and ova, and with a reflective chorion shell.

In the 2011 experiment, the number of days until a female *E. connexa* produced her full complement of eggs was estimated by fitting the logistic curve (Snedecor and Cochran 1967):

$$T = \frac{M}{1 + (b(\rho^{age}))}$$

Where *T* is the total number of mature eggs in the ovaries, *M* is the asymptotic maximum number of mature eggs in the ovaries, *b* affects the midpoint of the rise between zero and *M*, and ρ is the slope parameter. Preliminary analysis showed that *b* and ρ were highly negatively correlated, and that as a result parameter estimates were

not stable and not always significant. To deal with this, the value of *b* was fixed at 20. Fixing *b* at 20 did not diminish the goodness of fit of the equation to the data, but did provide stable significant parameter estimates that allowed the asymptotic maximum number of eggs, and the time at which this maximum was approached, to be estimated. The curve was fitted using a least squares non-linear regression method (Systat 2009). The parameter estimates for this model were considered significant if the Wald confidence intervals did not include zero.

Effects of age, mating and oviposition on egg load and total eggs

In 2012, the time of emergence of adults was varied by placing pupae at 17, 20, and 25 °C in spring. To evaluate the effect of mating and oviposition on egg load, newly emerged females were randomly assigned to one of three treatments:

1. One female held without mating in a cylinder for 15 days
2. One female and male held together in a cylinder for 15 days
3. One female and male held together in a cylinder for 15 days, on day 15 the female was offered an isolated seedpod of *Vincetoxicum hirundinaria* or *V. nigrum* for 24 h, the pod was then removed and the female and male left in the cylinder until day 30.

On the last day of each treatment, the females were dissected and the number of mature eggs in the ovaries recorded. In treatment 3, with seedpod exposure to females on day 15, the seedpods were dissected the day after exposure and the number of eggs within the seedpod was recorded.

In the 2012 experiment, descriptive statistics were calculated for the number of eggs in the ovaries for treatments 1, 2, and 3, and for the number of eggs found in the seedpods for treatment 3. The effect of treatment on egg load and on total egg number (egg load plus eggs in seedpods) was investigated by analysis of variance (ANOVA)

followed by planned contrasts of treatments 1 vs. 2 and 2 vs. 3. To assess if there was an effect of day of emergence (days since 1 June) on the total number of eggs, analysis of variance (ANOVA) was conducted on the date of emergence, treatment, and their interaction.

Results

Egg development

In 2011, most females did not produce eggs before 5 days of age. After 5 days, the majority of females had 20 eggs or more (Figure 9). The modelled average maximum number of eggs, *M*, was 47.08 ± 3.89, and was significant based on the Wald test and associated confidence intervals (Table 8). The slope parameter, ρ, was also significant. By 8 days post emergence, the line of the regression was within the range of the 95% confidence limits of the asymptote (Figure 9, Table 8). The time for females of *E. connexa* to produce 99.8% of a full complement of eggs was about 15 days at 20 °C.

Effects of age, mating and oviposition on egg load and total eggs

Average counts of eggs in the three treatments of the 2012 experiment are shown in Table 9: egg load was noticeably lower in 30 day mated females that had access to an oviposition site than in females 15 days after emergence.

The number of mature eggs in ovaries at the end of the treatment was significantly affected by treatment ($F = 28.2$, df = 2, 143, $P < 0.001$). Whether females had mated or not did not affect the number of eggs in ovaries 15 days after emergence ($F = 3.2$, df = 1, 143, $P = 0.07$); however, the number of eggs in ovaries of mated females 15 days after emergence, was significantly higher than that of mated females dissected 30 days after emergence where the females had the opportunity to oviposit on day 15 ($F = 31.1$, df = 1,143, $P < 0.001$).

Even when the number of eggs found in the seedpod was added to the egg load, the effect of treatment remained significant (F = 10.6, df = 2, 143, $P < 0.001$): mated females dissected 30 days after emergence, having the opportunity to oviposit on day 15, had significantly lower total number of eggs than mated females dissected 15 days after emergence (F = 9.08, df = 1,143, $P < 0.005$). As before, whether females had mated or not did not affect the total number of eggs 15 days after emergence (F = 2.7, df = 1, 143, P = 0.10). If the lower number of eggs in ovaries of females in treatment 3 on day 30 was solely the result of eggs being laid in the seedpod, then the analysis of total eggs should indicate a non-significant treatment effect. However, this was not so. Of the average of about 51 eggs females contained in treatment 2 (Table 9), about 23 eggs could not be accounted for as laid or retained in the ovaries on day 30 in females of treatment 3.

When day of emergence (days since 1 June) was added to the model to investigate the effect on total eggs, treatment remained highly significant (F = 12.6, df = 2, 140, $P < 0.001$), and day of emergence was also significant (F = 12.1, df = 1,140, $P < 0.001$). The effect of day of emergence did not differ significantly among treatments (F = 1.2, df = 2, 140, P = 0.3). Pooled over treatment, for each day delay in emergence after 1 June, there was a reduction of 0.43 total eggs (Figure 10).

Discussion

Egg development

This study confirmed that, at the time female *E. connexa* emerge from puparia, they contain no mature eggs (Solbreck 2000). In my study full egg load was attained at about 8 days. The value of 8 is based on the first day that the number of eggs predicted by the model is within the confidence interval for its asymptote. The range of egg loads in this experiment was similar to that found by Solbreck (2000), who

dissected field collected individuals and recorded that they contained from 2 to 60 eggs with an average of 29 eggs. There were several individuals in this study with larger egg loads than found in Solbreck (2000). However, this could be expected since the insects in Solbreck (2000) were field collected and of unknown age: they could have been too young to have developed all their eggs or may have already laid some. In the 2011 experiment, the females were not given the opportunity to oviposit.

The time required for the production of mature eggs of *E. connexa* is similar to that for other frugivorous tephritids where, upon emergence, activities of importance are foraging and dispersal, followed by oviposition (Fletcher and Prokopy 1991). In the closely related species *Euphranta canadensis* (Loew) the first eggs are laid around the 6^{th} day (Christenson and Foote 1960; Severin 1917), although, eggs could have been matured prior to this. In the tephritid *Dacus dorsalis* Hendel, the pre-oviposition period lasts 8-12 days or about 5 days if fed a high protein diet (Christenson and Foote 1960; Severin 1917).

Effects of age, mating and oviposition on egg load and total eggs

In this study, there was no effect of mating on egg production and the egg load obtained was similar to the 2011 experiment and to the field data from Solbreck (2000). Solbreck and Ives (2007) constructed a life table of *E. connexa* and estimated the "realized fecundity". However, estimates from this study are not comparable to the egg load because realized fecundity was obtained by dividing the number of eggs laid in seedpods by the previous year's number of large larvae. Besides Solbreck (2000) and Solbreck and Ives (2007), there are no other studies of the time to egg production, egg load, or fecundity of *E. connexa*.

Literature shows tephritids have diverse, flexible and complex reproductive responses. In *Ceratitis capitata* (Wiedmann), mating was found to increase the

number of eggs oviposited compared to the number laid by virgin females (Blay and Yuval 1999), but Chapman et al. (1998) found no effect of mating on egg load. Among species of the same genus there can be marked differences in response to mating. Senger et al. (2008) found mated and crowded females of *Rhagoletis indifferens* Curran have nearly triple the amount of eggs in their ovaries compared with isolated virgin females. In *Rhagoletis cerasi* L. there was also an increase in egg load with mating (Katsoyannos et al. 1987). However, in *R. pomonella* (Walsh) virgin and mated females laid similar numbers of eggs (Neilson 1975). It can be suggested that there is no significant cost of mating for female *E. connexa* and those tephritids in which there is no marked difference in fecundity or egg load between mating and non-mating individuals. The effect of mating on fecundity or egg load may be species-specific and likely involves more factors, (crowding, nutrition, or host plant presence) than examined in this study.

In my study, those mated females offered a seedpod on day 15 had significantly lower numbers of eggs when dissected on day 30 than those mated females dissected on day 15. The reduction in the number of eggs was not explained by the number of eggs laid in the seedpod. Thus, it seems likely that eggs were resorbed. This result differed from that found by Alonso-Pimentel et al. (1998) where in the constant presence of a host the fecundity of female *Rhagoletis juglandis* Cresson increased. It is possible that females deprived of a host for too long may cease developing eggs; my study provides evidence that oosorption occurs. In the field, if a female *E. connexa* is deprived of oviposition sites she may migrate to another site (Solbreck and Sillén-Tullberg 1986b). In tephritids, *Dacus jarvisi* (Tryon), *D. cucumis* French, and *D. cacuminatus* (Hering), but not in *D. tryoni*, oocyte development slowed or ceased in the absence of suitable places to oviposit (Fitt 1986). This

phenomenon, called `ecological castration`, has also been found to occur in parasitic Hymenoptera (Flanders 1942; Flanders 1950). Further study of this behaviour in *E. connexa* would be beneficial as it could affect host range testing procedures to assess its potential as a biological control agent.

There is clearly a dynamic equilibrium allowing the allocation of resources to egg production or maintenance, and in unfavorable circumstances there is a reallocation of resources from egg production to maintenance. If in the field the oviposition substrates are sparse females may resorb eggs. Resorption of eggs could also allow the females to re-allocate energy for flight to forage for food, mate, locate fruits for oviposition, or avoid predators (Alonso-Pimentel et al. 1998), indicating an adaptive capacity of *E. connexa* to react to unfavorable environments.

Poor diet is unlikely to be the reason for the decrease in egg load in those females dissected on day 30 after emergence. Nutritional deficiencies causing egg oosorption would be seen on Figure 9 as a decrease in egg load the longer a female remains alive after maximum egg load was attained. In my study the diet used included carbohydrates and proteins similar to other studies on tephritids (Alonso-Pimentel et al. 1998; Blay and Yuval 1999; Senger et al. 2007).

In the 2012 experiment, increased duration of pupal development resulted in reduced fecundity, regardless of the treatment. Longer dormancy has similar trade-offs with fecundity in *Neodiprion sertifer* (Geoffroy) (Hymenoptera: Diprionidae) (Sullivan and Wallace 1967), *N. swainei* Middleton (Lyons 1970), and *R. cerasi* Loew (Diptera: Tephritidae) (Moraiti et al. 2012). The trade-off for prolonged duration of pupal development is a decrease in fecundity for *E. connexa*.

The number of eggs laid by *E. connexa* when offered a seedpod was far fewer than the potential reproductive capacity. This strategy is congruent with the life

history for *E. connexa*. In the field, there is competition among larvae within a seedpod and on average, one prepupa emerges per fruit (Solbreck 2000). If *E. connexa* oviposits one egg in a seedpod and uses a deterrent marking pheromone (Solbreck 2000) it could be expected that a female would not oviposit all her eggs when only one fruit is offered. Adult longevity could also play a role, but the adult life span of *E. connexa* is not very well known. Solbreck (2000) and Solbreck and Sillén (1986b) mention adult flies are present in the field for a long time; however, this does not indicate that the flies are long-lived. If emergence of adults from overwintering is asynchronous, adults will be present in the field over a long duration although individual flies may have short adult lives. From this study it is known that individuals, at least in the laboratory, can live for 30 days at 20 °C. Because of intra-specific larval competition, *E. connexa* may have developed an adaptive strategy of long life span and ovipositing a low number of eggs in a seedpod. This pattern also occurs in *Bactrocera latifrons* Hendel where the number of eggs laid in the presence of an oviposition substrate was one twelfth of the egg load (Wingsanoi and Siri 2012). My study indicates that in unfavorable environments resources are allocated to maximize longevity, thus *E. connexa* females may have the chance to mate, increase feeding, and search out seedpods for oviposition; even if this is at the expense of fecundity.

Conclusions for biological control

This study provided valuable information regarding the time after eclosion at which female *E. connexa* begin producing mature eggs, the age at which the asymptote is reached for egg production, the maximum number of eggs produced, the effects of mating on egg load, and the effects of a prolonged pupal stage on egg load. It is recommended that testing of oviposition choice should occur when females are from 8

to 15 days post emergence. Females that are more than 15 days post emergence should not be used in testing as not enough is known regarding the reabsorption of eggs.

Table 8. Parameter estimates for the logistic model of egg load in relation to days since emergence for unmated adult female *E. connexa* in the 2011 experiment.

Parameter	Estimate	Asymptotic standard error	Parameter/ asymptotic standard error	Wald 95% Confidence Interval	
				Lower	Upper
M	47.08	3.89	12.12	39.21	54.95
ρ	0.54	0.05	9.90	0.43	0.65

Table 9. Mean (± SE) number of mature eggs in the ovaries of female *Euphranta connexa* in three different treatments and number of eggs in seedpods offered in treatment 3 in the 2012 experiment.

Treatment	*n*	Eggs in ovaries	Eggs in seedpod	Total
Unmated female dissected at day 15 (treatment 1)	50	57.8 ± 3.3	—	57.8 ± 3.3
Mated female dissected at day 15 (treatment 2)	52	50.8 ± 2.6	—	50.8 ± 2.6
Mated female allowed to oviposit at 15 days, dissected at day 30 (treatment 3)	44	28.3 ± 2.5	9.25 ± 1.8	37.5 ± 3.5

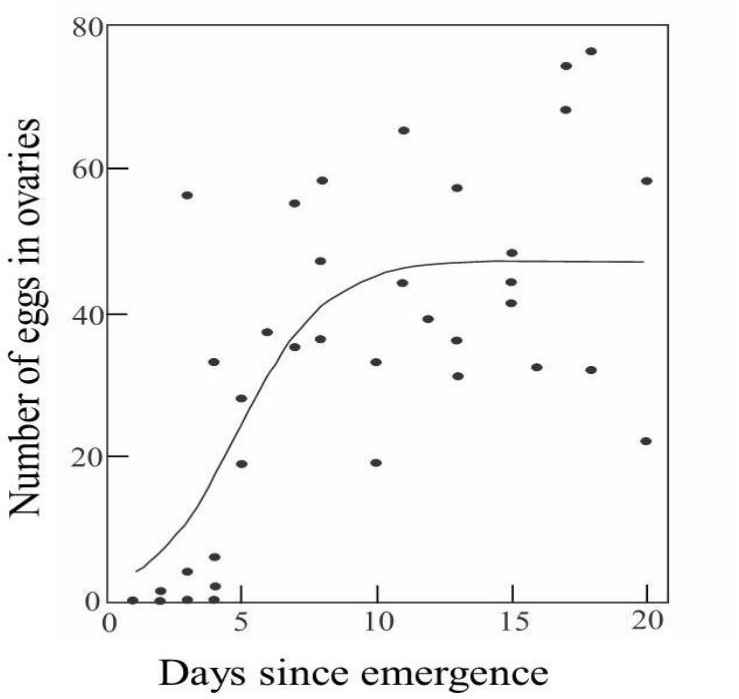

Figure 9. Number of eggs in ovaries of females in relation to time since emergence in the 2011 experiment, and fitted logistic model based on dissections of 39 females ranging from 1 to 20 days since emergence.

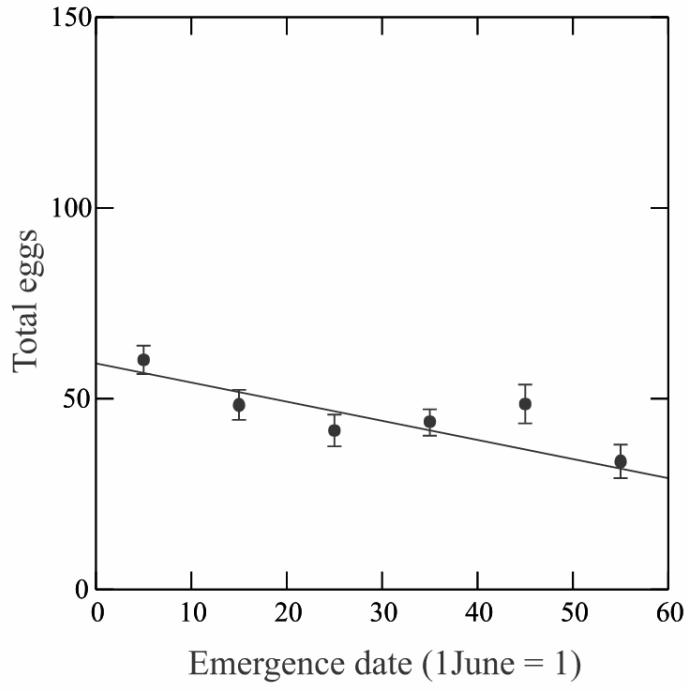

Figure 10. The effect of the date of emergence on the total number of eggs of female *E. connexa*, in the 2012 experiment; means for 10 day intervals are given. Total number of eggs (mean ± SE) was pooled over treatments and includes eggs laid and those in the ovaries at the time of dissections.

CHAPTER 5: PARASITOIDS EMERGING FROM SEEDPODS OF *VINCETOXICUM HIRUNDINARIA* INFESTED WITH *EUPHRANTA CONNEXA* (DIPTERA: TEPHRITIDAE) AND *CONTARINIA ASCLEPIADIS* (DIPTERA: CECIDOMYIIDAE)

Abstract

In preparation for the potential release of a biological control agent, *Euphranta connexa* (Fabricius), for control of *Vincetoxicum rossicum* (L.) and *V. nigrum* (L.), the parasitoids from the seedpods of its natural host *Vincetoxicum hirundinaria* were inventoried. For this purpose, seedpods were collected in late summer of 2010, 2011, 2012, and 2013 in 12 sites in three countries. Adult parasitoids emerging from seedpods and from *E. connexa* pupae were collected, sorted to morphospecies, and identified as far as possible. Three parasitoid morphospecies were identified to species, *Scambus brevicornis* (Gravenhorst) (Hymenoptera: Icheumonidae), *Stenomalina gracilis* (Walker) (Hymenoptera: Pteromalidae), and *Eupelmus fulvipes* Förster (Hymenoptera: Eupelmidae). Six morphospecies were identified to genus: *Opius* (Hymenoptera: Bracondiae), *Dinotrema* sp. (Hymenoptera: Braconidae), *Eurytoma*. (Hymenoptera: Eurytomidae), *Pteromalus* (Hymenoptera: Pteromalidae), *Pseudotorymus* (Hymenoptera: Torymidae), and *Aprostocetus* (Hymenoptera: Eulphidae). One group of morphospecies was identified to the family Braconidae. The most prevalent taxa were those in the family Braconidae, making up 1132 of the 1599 adult parasitoids recovered. Besides *E. connexa*, the gall midge, *Contarinia asclepiadis* (Giraud) (Diptera: Cecidomyiidae) was found in the seedpods of *V. hirundinaria*. Prior to this research *C. ascelpiadis* was considered rare; however its presence was recorded in all sites sampled in Switzerland and in two sites in 2013 an average of 9.2% of the dissected seedpods contained evidence of the midge. Three of the morphospecies identified in the family Platygastridae, Platygastridae sp.1 and 2,

and *Synopeas* sp. (Hymenoptera: Platygastridae), are considered to attack *C. asclepiadis*.

Keywords: parasitoids, *Scambus brevicornis, Stenomalina gracilis, Eupelmus fulvipes,* Braconidae, *Opius concolor, Eurytoma, Pteromalus, Pseudotorymus, Aprostocetus*.

Introduction

Research in preparation for weed biological control emphasizes tests to establish the potential control agents' specificity and the risk to plants (Briese 2005; Wapshere 1974). An aspect that is less frequently studied is the potential for the performance of released control agents to be reduced by parasitism (Goeden and Louda 1976). Either parasitoids introduced with the agent, or ones already in the area of introduction could impair agent effectiveness (McFadyen and Spafford-Jacob 2003). To evaluate the risks from parasitism, the parasitoid guild associated with the potential agent in its native range should be assessed, and studies should be made of the potential parasitoids in the area of introduction (McFadyen and Spafford-Jacob 2003; Paynter et al. 2010). While it is logistically simpler to deal with congeners, other parasitoids, related at the level of higher taxa, or with similar ecology could target the agent in the area of introduction (Edwards et al. 1996).

Of introduced weed biological control agents, dipterous agents have the largest pressure from native parasitoids in the introduced range, where 36% of introduced species have suffered parasitism after introduction (McFadyen and Spafford-Jacob 2003). Of the tephritid weed biological control agents released parasitism has occurred in 17% of species (McFadyen and Spafford-Jacob 2003). This percentage is of particular relevance to the program evaluating the potential of the dipteran *Euphranta connexa* (Fabricus) (Tephritidae) as a new host association biological control agent for the alien invasive weeds, *Vincetoxicum rossicum* (L.) (Apocynaceae) and *V. nigrum* (L.) (Apocynaceae) in North America (Tewksbury et al. 2002).

Euphranta connexa is a European univoltine tephritid that is monophagous on the seedpods of *V. hirundinaria* (Solbreck 2000). *Euphranta connexa* eggs are laid on the inner wall of the seedpod (Solbreck 2000). The larvae develop through three larval instars as they consume the immature seeds of *V. hirundinaria*. The third instar larvae rasp a hole in the side of the seed wall, fall to the ground, and pupate (Solbreck 2000). In general, there is one pupa per seedpod, but there can be many oviposition scars indicating that there could be strong larval competition pressure within the seedpod (Solbreck 2000). Depending on the climate, the emergence of overwintered adults begins in June or July, with a large portion of the eggs being oviposited in July, and third instar larvae being found leaving the fruits in August and September (Solbreck 2000).

The white larvae of the univoltine gregarious *Contarinia asclepiadis* (Giraud) (Diptera: Cecidomyiidae) (Figure 11) are also found within the seedpods of *V. hirundinaria* (Bruun et al. 2012; Widenfalk et al. 2002). The adult female deposits eggs on the inner wall of the seedpod and larvae feed within the seedpod wall forming a pseudo-gall (Widenfalk et al. 2002). Previous observations indicate seedpods attacked by *C. asclepiadis* are often also attacked by *E. connexa* (Bruun et al. 2012; Solbreck 2000). Widenfalk et al. (2002) found female *C. asclepiadis* tend to oviposit in seedpods already attacked by *C. asclepiadis*, leading to multiple instars in one seedpod. *Contarinia asclepiadis* has been collected in Austria, Germany, Netherlands, Czech Republic (Skuhravà 1994), Sweden (Widenfalk et al. 2002), Denmark (Bruun et al. 2012), and Switzerland (Weed et al. 2011b) and is considered a very rare species.

In the literature, there are reports of 11 species of parasitoids reared from collections of *Vincetoxicum hirundinaria* fruits from six families of Hymenoptera

(Table 10). *Scambus brevicornis* (Gravenhorst) (Hymenoptera: Ichneumonidae) is a polyphagous parasitoid identified on *E. connexa* and 90% of the parasitoids in sites in Sweden are of this species (Solbreck 2000). The larval ectoparasitoid, *Bracon picitcornis* (Wesmael) is the second most encountered parasitoid in Sweden (Pagola-Carte and Peydro 2012; Solbreck 2000). *Psyttalia concolor* (Szépligeti) is a larval-pupal parasitoid of many tephritid species and of *E. connexa* (Benelli et al. 2013) and has been introduced into North America from Africa as a biological control agent on the olive fly, *Bactrocera oleae* Rossi (Diptera: Tephritidae) (Daane and Johnson 2010; Wang et al. 2011). *Utestes testicus* (Wesmael) has been found as a parasitoid on *E. connexa* however Wharton and Yoder (2013) suggest exercising caution about the validity of the report. *Rhysipolis decorator* (Haliday) and *R. mediator* (Haliday) (Broad 2009) are parasitoids of Tephritidae, Anthomyiidae, and Lepidoptera (Medvedev et al. 1995). *Eurytoma curculionum* is polyphagous (Boucek 1977; Herting 1973; Herting 1977) and has been recorded as a hyperparasitoid (Herting 1977) and a larval or nymphal parasitoid (Fisher 1965; Gibson et al. 2006b). The literature on *Pteromalus helenomus* consists of two articles, Graham (1969) describing the synonym *Habrocytus helenomus* and from Boucek and Graham (1978) synonymizing *P. helenomus* with *H. helenomus as P. helenomus. Stenomalina muscarum* (Walker) has been found emerging from *E. connexa* larvae in seedpods of *V. hirundinaria* where it may be a primary parasitoid or hyperparasitoid. In other insects it has different life history strategies including primary larval parasitoid, primary pupal parasitoid (Dzhanokmen 1978), and hyperparasitoid (Graham 1969). *Eupelmus* (*Macroneura*) *vesicularis* (Retzius) (Gibson 1995) is frequently recorded as a hyperparasitoid (Herting 1977; Hopkins 1984) and a pupal parasitoid (Henderson and Rutz 1991) and was reported by Solbreck (2000) as emerging from seedpods

infested with *E. connexa* larvae. The genus *Aphanogmus* are hyperparasitoids (Buffington and Polaszek 2009; Streito and Nibouche 1997) but with only genus identification, inferences beyond this are difficult.

Only one parasitoid has been reported on *C. asclepiadis*: a species in the genera *Inostemma* (Hymenoptera: Platygasteridae) (Widenfalk et al. 2002). *Inostemma* spp. are in the subfamily Platygastrinae and are exclusively found on Cecidomyiidae (Masner 1993).

To assess the assemblage of parasitoids on the original host plant of *E. connexa*, *V. hirundinaria* Medik (Apocyaceae), mass collections of seedpods, potentially infested with *Euphranta connexa* (Diptera: Tephritidae) and *Contarinia asclepiadis* were collected from 12 sites across three countries. The primary objective of this research was to establish the parasitoid assemblage of *E. connex*a. The secondary objective was to investigate the distribution, biology, and potential parasitoid assemblage of *C. asclepiadis*.

Materials and methods

In 2010, 2011 and 2012, mature seedpods of *V. hirundinaria* were collected from sites listed in Table 11 and brought back to the laboratory for the collection of endophagous herbivores and the parasitoids that they contained. Except in 2010, seedpods were counted (Table 12). Fruits were placed on a fabric screen of 30 squares/cm^2 at the base of a plastic cylinder (15.5 cm x 11 cm diameter) with a ventilated lid. This cylinder was wedged into a second cylinder of similar dimensions containing a layer of moist vermiculite; the entirety was termed an emergence cylinder. Emerged *E. connexa* prepupae and pupae and adult parasitoids from egg or larval hosts were collected from the cylinder, below the screen lid. The adult larval parasitoids were placed into vials

containing 70% ethanol, labelled with site information, and placed in storage for sorting at a later date.

In 2013, the majority of the collected seedpods were counted and placed in emergence cylinders as before. However, 304 seedpods collected from Anzonico and 560 seedpods from S. Antonio were held individually in plastic vials (6 cm x 2 cm diameter) with ventilated lids. Every two to three days, vials were checked for the emergence of adult egg or larval parasitoids, which were recorded and placed in 70% ethanol. Once emergence was completed for the season, seedpods were dissected to determine the number of dead adult parasitoids and parasitoid pupae, and the presence of *C. asclepiadis* or *E. connexa* as indicated by feeding injury or dead larvae. Dead dried adult parasitoids were momentarily placed into boiling water, removed, and then placed in 70% ethanol. Living parasitoid pupae were moved to plastic vials with 2 cm of moist vermiculite and placed at 2 °C.

Contarinia asclepiadis larvae emerging from seedpods were not normally collected or overwintered. But in 2013, a few *C. asclepiadis* larvae collected from the moist vermiculite were placed in plastic vials containing 4 cm of moist vermiculite. After the midge larvae formed a cocoon, four were dissected out, placed in 99% ethanol and sent for DNA analysis.

In 2010, 2011 and 2012, immature *E. connexa* from emergence cylinders, potentially with larval-pupal parasitoids, were counted (Table 12) and put into Petri dishes, 9 cm x 2 cm, with moist vermiculite, and placed for overwintering in an unlit, unheated, underground storage facility at CABI, Delémont, Switzerland at the end of September. In May of 2011, 2012 and 2013, overwintering pupae collected the previous summer were transferred to temperatures between 12 and 25 °C. From May to August these pupae were monitored for the emergence of *E. connexa* or larval-

pupal parasitoids. Emerged larval-pupal parasitoids from *E. connexa* pupae were recorded and placed in 70% ethanol for identification.

The preserved adult potential egg, larval, or larval pupal parasitoids were removed from the ethanol and separated into morphospecies under a compound microscope and placed back into 70% ethanol. Representatives of each morphospecies from 2010 to 2012 were pointed and sent to the Canadian National Collection in Ottawa, Canada for identification. Once the identifications were received the remaining samples from 2010 to 2012 were sorted. Parasitoids collected in 2013 were compared against those previously identified species and genera. Parasitoids that were not previously identified were keyed as far as possible using Goulet and Huber (1993). Voucher specimens of *Euphranta connexa* and parasitoids will be deposited in the Canadian National Collection, Ottawa, Canada and the Wallis/Roughley Museum of Entomology, University of Manitoba, Canada. Further identification will be attempted by sending material to specialists in Europe.

Results and Discussion

In a review emphasising parasitoids of pest Tephritidae of Latin America and the southern United States Ovruski et al. (2000) found 90% of the literature reports results of methods that detect only larval-pupal parasitoids. The methods used in this study could have collected egg, larval and larval-pupal parasitoids, but not pupal parasitoids. No calculations regarding levels of parasitism were made as sampling for one day over a brief duration of time, does not yield data appropriate for these inferences (van Driesche 1983). Eggs and all instars of larvae were found at the time of collections; however, as noted by Solbreck (2000), most of the third instar larvae leave the seedpods in August. As a result egg parasitoids could be completely missed or under-represented if their attack and emergence occurred before seedpod maturity.

Parasitoids that emerged from first and second instar larvae could also be under-represented relative to parasitoids that emerge from third instars and larval-pupal parasitoids.

Larvae of three species of Diptera emerged from the seedpods of *V. hirundinaria*. Based on larval and adult morphology and biology, two species are *E. connexa* and *C. asclepiadis* and these two species are the only two reported endophagous insects in the seedpods of *V. hirundinaria*. Larvae of *E. connexa* were distinguishable because of their well-developed mouth hooks (Figure 11) which are absent in *C. asclepiadis* larvae (Figure 12). One seedpod from Soyhières contained *C. asclepiadis* larvae, one larval skin, one set of mouth hooks of *E. connexa*, and four unidentified dipterous larvae that were neither *E. connexa* nor *C. asclepiadis*. The unidentified larvae formed pupae in moist vermiculite and adults emerged at the end of August. In the last three years of the study over 22,500 seedpods were collected, and almost 1000 came from Soyhières. The detection of only one seedpod with this third dipterous species makes it extremely improbable that the species hosted any of the parasitoid morphospecies collected, except, possibly, the rarest. The two main Diptera of concern within the seedpods of *V. hirundinara* were *E. connexa* and *C. asclepiadis*.

Euphranta connexa

Over four years of sampling 10,381 pupae were collected from *V. hirundinaria* seedpods for overwintering (Table 12). The largest numbers of pupae, and highest recorded seedpod to pupa ratio was collected from Anzonico in 2011. Localities in the high alps, Ausserberg and Kandersteg, and Soyhières, in the Jura region of Switzerland produced the lowest recorded ratio of seedpods to pupae. Seedpod production in the sites in the Jura region of Switzerland (Moutiér, Soyhières, Vermes

(a), and Vermes (b)) fluctuated greatly; in 2010 and 2013 there were few seedpods available for collection. This is not surprising as Solbreck and Sillén-Tullberg (1986a; 1986b) indicate seedpod production is heavily correlated to abiotic factors such as sunshine and precipitation. Local extinctions of *E. connexa* occur in years when *V. hirundinaria* seedpods are scarce but there is recolonization when seedpods are available again (Solbreck 2000).

Continaria asclepiadis

In all years of study, *C. asclepiadis* was found at all Swiss localities listed in Table 11. Weed et al. (2011b) found one Swiss locality with *C. asclepiadis* in 2008 but the midge was not detected in earlier surveys in four European countries, possibly because surveys did not coincide with *C. asclepiadis* presence in seedpods.

In 2013 seedpod dissections, the ratio of the number of seedpods with *C. asclepiadis*: the number of seedpods with *E. connexa*: the total number of seedpods was 50: 441: 560 for S. Antonio and 29: 239: 304 for Anzonico (note that seedpods with both *C. asclepiadis* and *E. connexa* would be included in both of the first two categories). The total number of larvae of *C. asclepiadis* was not normally determined, but in one seedpod 53 *C. asclepiadis* larvae were found. In southern Sweden (Solbreck 2000; Widenfalk et al. 2002) and Denmark (Bruun et al. 2012) *C. asclepiadis* is rare, but in my study in 2013, *C. asclepiadis* was found in 9.5% and 8.9%, of the dissected seedpods from S. Antonio and Anzonico respectively.

Of the 79 seedpods with *C. asclepiadis* only seven seedpods did not have direct evidence of feeding by *E. connexa* and the association of the presence of the two species was highly significant (Likelihood ratio $\chi^2 = 9.28$, d.f. = 1, P = 0.002). The association of *C. asclepiadis* with seedpods infested with *E. connexa* was probably even stronger than the statistic suggests, as oviposition holes from *E.*

connexa were found in the seven seedpods that did not have feeding damage. The occurrence of *C. aclepiadis* in seedpods containing *E. connexa* was also seen in Denmark by Bruun et al. (2012). The reason for this is not certain. This type of behaviour has been noted before between the agromyzid *Phytobia cambii* (Hendel) and the cecidomyiid *Resseliella dizygomyzae* (Barnes) (Barnes 1933). In terms of biological control, if *C. asclepiadis* is causing damage to the plant it could cause an additive negative impact on *Vincetoxicum* spp.

No larval or adult specimens of *C. asclepiadis* were collected from sampling efforts in 2010 to 2012, although they were present in the samples. In 2013, larvae of *C. asclepiadis* emerged from seedpods of communal collections of *V. hirundinaria* and formed pupae in moist vermiculite (Figure 11). Molecular confirmation of the identity will be made by comparison with sequences deposited into GenBank from Widenfalk et al. (2002). The identification of adult *C. asclepiadis* from overwintering pupae will be attempted by bringing the five pupae up from 2 °C to 20 °C on 1 April, 2014 and any emerged adults will be placed into 70% ethanol, sent for identification, and stored at CABI, Delémont, Switzerland. The results from the molecular and morphological identification will be available after the completion of this thesis, until then the species is presumed to be *C. asclepiadis*.

Recovered parasitoids

Overall there were 1599 parasitoids associated with *V. hirundinaria* from 12 sites spread in three different countries. The collections were sorted into 13 morphospecies, all of which were Hymenoptera. Of the morphospecies, three were identified to species: *Eupelmus fulvipes* Förster (Hymenoptera: Eupelmidae), *Stenomalina gracilis* (Walker) (Hymenoptera: Pteromalidae), and *Scambus brevicornis* (Gravenhorst) (Hymenoptera: Icheumonidae) (Table 13). Further identifications are being pursued

for the remaining unidentified morphospecies. Within Switzerland, Kandersteg had the least number of taxa while S. Antonio supported the most (Table 13). The samples were dominated by two members of the family Braconidae; Braconidae species 1.and *Opius* sp. Braconidae species 1were found in all localities but Kandersteg and *Opius* sp. were found only in Switzerland, but were absent from Soyhières. Detailed information on distribution and number of individual parasitoids can be found in Table 13.

The following sections report on each Hymenoptera taxon found in the survey.

Scambus brevicornis (Hymenoptera: Ichneumonidae)

Perkins (1943) and Broad (2009) synonymize *S. euphrantae* (Schmiedeknecht) with *S. brevicornis*. However, Horstmann (2010) has resurrected *S. euphrantae* as a separate species from that of *S. brevicornis*. For separating *S. brevicornis* and *S. euphrantae* the utilization of molecular techniques are recommended. Until molecular techniques are used, this thesis will consider all specimens to be *S. brevicornis* (Horstmann 2010). It is realized that the literature could be referring to either species. In four summers of collections, *S. brevicornis* (Figure 13) were occasionally collected and are assumed to be larval-larval parasitoids from hosts in the seedpods of *V. hirundinaria* (Table 13). Most of the individuals were collected from Soyhières in 2012. In 2013, there were no parasitoids of this species recorded.

Eggs of *S. brevicornis* are attached to the exterior of *E. connexa*, and Janzon (1982) depicts an egg on a third instar larva. In Sweden *S. brevicornis* is the most prevalent parasitoid, with parasitoids emerging from up to 30% of seedpods (Solbreck 2000). In Switzerland, *S. brevicornis* was not the most prevalent, nor did levels of prevalence reach those found in by Solbreck (2000). Fitton et al. (1978) indicate this species is bivoltine and overwinters as a prepupa. In my rearing of *S. brevicornis* and Solbreck (2000) the adults emerged from seedpods in August. It is possible that *S.*

brevicornis utilizes *E. connexa* as the final host of the season and overwinters as an adult. Alternatively, if August emergence was an artefact of rearing conditions in the laboratory, in the field *S. brevicornis* could overwinter in the prepupal stage.

Scambus brevicornis is a Holarctic species (Glavendekic and Kolarov 1994) and is highly polyphagous, mostly being found on Coleoptera: Curculionidae and Lepidoptera (Elzinga et al. 2007; Fitton et al. 1978; Gultekin 2005; Tóth and Lukas 2005; Wang and Yue 1995). Because *S. brevicornis* is Holarctic there could be parasitism of *E. connexa* if it is introduced into North America. In Solbreck and Ives (2007) the density of *S. brevicornis* depends on the whole range of hosts, and so its importance in North America would depend upon the guild of suitable hosts.

Braconidae species 1

In my study, insects classified as Braconidae species 1 were the most common Hymenoptera (Table 13) (Figure 14). These individuals emerged as adults from seedpods and are assumed to be larval-larval parasitoids. *Bracon* (*Glabrobracon*) *picticornis* Wesmael (Pagola-Carte and Peydro 2012; Papp 2012; Solbreck 2000), *Rhysipolis decorator* (Haliday 1836), and *R. meditator* (Haliday 1836) (Medvedev et al. 1995) are Braconidae that are known to parasitize *E. connexa*. All species are polyphagous, and *R. decorator* is Holarctic (Spencer and Whitfield 1999), whereas *B. picticornis* and *R. meditator* are Palearctic (Medvedev et al. 1995; Papp 2012). The information concerning *Rhysipolis* species on *E. connexa* is a scant mention of their host association by Medvedev et al. (1995). *Rhysipolis* sp. are in the subtribe Rhysipolini which are described as ectoparasitoids of Lepidoptera larvae (Goulet and Huber 1993). *Bracon picticornis* are larval ectoparasitoids that are found throughout the range of *E. connexa* in Sweden (Solbreck 2000; Solbreck and Ives 2007) and occur in Spain on *E. connexa* (Pagola-Carte and Peydro 2012). The specimens from this study are likely *B. picticornis*, due to their frequency in other studies, and since

this species is Palearctic, if *E. connexa* is introduced into North America there is no potential for attack by *B. picticornis*.

Opius species 1 (Hymenoptera: Bracondiae)

Adults of this morphospecies emerged from overwintering pupae, and were the second most numerous parasitoid (Figure 15). Through rearing of these insects it is clear they were larval-pupal parasitoids of *E. connexa*. Two species of *Opius* are identified in the literature as parasitoids of *E. connexa*, *Opius concolor* Szépligeti (Fischer and Koponen 1999), reclassified as *Psyttalia concolor* (Szépligeti 1910), and *Opius testaceus* Wesmael, reclassified as *Utetes testaceus* (Wesmael 1838) (Kandybina 1977). The distributions of *P. concolor* and *U. testaceus* overlap (Fischer 1982; Fischer and Koponen 1999) and both are known to be parasitoids of Tephritidae (Wharton and Yoder 2013). Wharton and Yoder (2013) recommend confirmation of the host record of *Utetes testaceus* from *E. connexa*. With this information it appears the identified *Opius* sp. could be *P. concolor*.

Fourteen species of tephritids have been documented as hosts of *P. concolor* (Benelli et al. 2013). *Psyttalia concolor* is a larval-pupal parasitoid (Benelli et al. 2013) and under laboratory conditions *P. concolor* can attack all larval instars of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Raspi and Canale 2000), but has higher fecundity when attacking third instar larvae (Sime et al. 2006). *Psyttalia concolor* has a long history as a biological control agent against *Bactrocera oleae*, the olive fly, (Ovruski et al. 2000) and in Mediterranean regions is mass produced on *C. capitata* (Wharton and Yoder 2013). The native range of *P. concolor* is Africa (Fischer and Koponen 1999) and it has now been introduced to many countries in Europe (Wharton and Yoder 2013), and more recently has been introduced into California (Yokoyama et al. 2008), and Florida (Wharton and Yoder 2013). If *E. connexa* is introduced into North America and introductions of *P. concolor* result in

establishment and spread, in time, there could be potential for *P. concolor* to utilize *E. connexa* as a host. The impact of *P. concolor* on *E. connexa* may be negligible as over three years of sampling in Switzerland adults of this morphospecies emerged from 4.7% of overwintered *E. connexa* pupae.

Dinotrema sp. (Hymenoptera: Braconidae: Alysiinae)

One individual adult, identified as a *Dinotrema* sp. (Figure 16), came from a seedpod from Moutiér in 2012. *Dintorema* sp. are known to be parasitoids of Phoridae (van Achterberg 1988), Anthomyiidae, and Platypezidae (Fischer et al. 2008). It is not clear where this parasitoid came from; although it may have come from a dipterous host in a seedpod of *V. hirundinaria.* It is also possible that its cocoon or that of its host was a contaminant of the samples and not associated with *V. hirundinaria* seedpods or their contents.

Eurytoma sp. (Hymenoptera: Eurytomidae)

From 2010 to 2013, *Eurytoma* sp. (Figure 17) adults emerged from seedpods attacking larval hosts collected from six sites in Switzerland and one site from the Ukraine (Table 13). Nearly half of the specimens were found in Anzonico in the summer of 2010. Based on dissections of seedpods associated with the morphospecies *Eurytoma* sp. in 2013 it was possible to eliminate *C. asclepidais* as the potential host. It is likely this morphospecies of *Eurytoma* is a hyperparasitoid, potentially of the unidentified Braconidae species 1 (Figure 14). Solbreck (2000) found *Eurytoma curculionum* Mayr from infested seedpods of *Vincetoxicum hirundinaria*. *Eurytoma curculionum* is a known hyperparasitoid of *Bracon intercessor* Nees and is the likely identity of this morphospecies. If so, *E. curculionum* would not pose a threat to *E. connexa* as it is distributed in Europe and is a hyperparasitoid (Boucek 1977; Gibson et al. 2006a).

Stenomalina gracilis (Hymenoptera: Pteromalidae)

Over a four year collection period, adults of *S. gracilis* (Figure 18) emerged from seedpods collected from five of 12 sites. This species was not recovered from high altitude sites, mostly specimenss originated from Vermes (a) and Vermes (b) (Table 4) over 2010 and 2011. Through host associations of *S. gracilis* with Cyclorrhapha, it is hypothesized that *S. gracilis* is a larval parasitoid of *E. connexa*. *Stenomalina gracilis* is found in Europe (Dzhanokmen 1978; Garrido Torres and Nieves-Aldrey 1999), Asia (Xiao and Huang 1999), and Canada (Gibson et al. 2006b) and know to parasitize larvae of species of Coleoptera: Curculionidae (Gibson et al. 2006b), Diptera: Agromyzidae (Garrido Torres and Nieves-Aldrey 1999), Diptera: Chloropidae (Garrido Torres and Nieves-Aldrey 1999), and Diptera: Tephritidae (Vidal 1997), Hymenoptera: Cynipidae (Mitroiu 2001). Of the tephritid fruit flies, *S. gracilis* has been found on *Tephritis formosa* (Loew, 1984) (Vidal 1997) and *Urophora cardui* (L.) (Herting 1978). Solbreck (2000) recorded that *S. muscarum* (L.) emerged from fruits of *V. hirundinaria* containing *E. connexa*. *Stenomalina muscarum* has been misidentified in the literature (Dzhanokmen 1978; Gibson et al. 2006b) and Solbreck`s (2000) specimens should be re-examined to verify their identity. *Stenomalina gracilis* is found in British Columbia, Canada (Gibson et al. 2006b) and is presumed not to be of concern for *E. connexa* as the major problem areas for invasive *Vincetoxicum* sp. are in eastern North America. As well, this study shows *S. gracilis* prevalence on *E. connexa* is negligible.

Pteromalus species 1 (Hymenoptera: Pteromalidae)

Adults of morphospecies *Pteromalus* species 1 (Figure 19) were found from 2010 and 2013 in S. Antonio and Anzonico (Table 13). Most of the specimens were collected in 2013. Dissections of seedpods from 2013 revealed that this species is a hyperparasitoid of the unidentified Braconidae species 1 (Figure 14). Solbreck (2000)

recorded *Pteromalus helenomus* (Graham) from *E. connexa*-infested seedpods of *V. hirundinaria* but his methodology did not allow determination of whether *P. helenomus* was parasitizing *B. picticornis*. There are no known host records for *P. helenomus* (Hedqvist in Solbeck 2000). If this morphospecies is *P. helenomus* there is little concern about negative effects on introduced *E. connexa* in North America as these are then hyperparasitoids, present in low numbers, and so far found only in the Palearctic (Graham 1969; Solbreck 2000).

Pseudotorymus species 1 (Hymenoptera: Torymidae)

Adult *Pseudotorymus* species 1 (Figure 20) emerged in 2010 and 2011 from seedpods from four field sites across Switzerland (Table 13). Records for hosts from this genus include Coleoptera (El Fels et al. 1999), Diptera (Askew et al. 2001; Harris 1970), Hymenoptera (Dubbert et al. 1998), and Macrolepidoptera (El Fels et al. 1999). In the order Diptera, *Pseudotorymus* sp. has been associated with Agromyzidae (Narendran et al. 2005) Cecidomyiidae (Askew et al. 2001; Harris 1970), and Tephritidae (Copeland et al. 2004). With current information, the host of this insect cannot be determined nor whether it is a primary parasitoid or a hyperparasitoid.

Eupelmus fulvipes (Hymenoptera: Eupelmidae)

Eupelmus fulvipes (Figure 21) adults emerged from seedpods from five localities in Switzerland. These parasitoids were predominately found in S. Antonio in 2013 and were not found at the alpine sites of Ausserberg and Kandersteg (Table 13). Dissections of seedpods revealed that this species is a hyperparasitoid of the Braconidae species 1 (Figure 14). Unidentified *Eupelmus* sp. have been considered to be hyperparasitoids of insects in the family Braconidae, specifically within the genus *Bracon* (Herting 1977). This species is rarely collected and is considered to have numerous dubious host records in Europe (Boucek 1977; Fusu 2009). Notwithstanding, *E. fulvipes* hosts are found parasitizing Diptera: Cecidomyiidae

(Simova-Toi and Dobrivojevi 1966), Hymenoptera: Cynipidae (Askew et al. 2006; Fusu 2009), and Lepidoptera: Pieridae (Herting 1975). *Eupelmus fulvipes* has only been found in Europe (Askew et al. 2006; Boucek 1977; Fusu 2009) and is not of concern if *E. connexa* is introduced to North America.

Aprostocetus sp. (Hymenoptera: Eurytomidae)

Adults of *Aprostocetus* (Figure 22) emerged from seedpods collected over four years (Table 13), from seven localities, including Ukraine. Most adults were from Ausserberg and Anzonico (Table 13). Dissections of seedpods in 2013 revealed that *Aprostocetus* morphospecies are potentially external feeders on *E. connexa* larvae or hyperparasitoids of Braconidae species 1. *Aprostocetus* have been found as primary parasitoids of tephritids and as hyperparasitoids of *Bracon* sp. According to Noyes (2013), and references therein, this is a widely distributed genus, the members of which attack an extensive range of hosts.

Platygastridae species 1 and 2 (Hymenoptera: Platygastridae)

Two morphospecies in the family Platygastridae were found only in 2013 from S. Antonio (Figure 23; 24) (Table 13). Adults of both morphospecies emerged from seedpods. Within Platygastridae there are two subfamilies, the Platygastrinae and Sceliotrachelinae (Masner 1993). Members of Sceliotrachelinae are known to parasitize the eggs of Coleoptera and Homoptera (Masner 1993). Species of Platygastrinae, are parasitoids of Cecidomyiidae (Vlug 1995). These individuals were found in communal samples and so, a host association is not certain; however, based on the biology of most members of this family, *C. asclepiadis* is the likely host. The only other known parasitoid on *C. asclepiadis* is an *Inostemma* sp. (Hymeonptera: Platygastridae) (Widenfalk et al. 2002).

Synopeas sp. (Hymenoptera: Platygastridae)

Only two adults were collected of *Synopeas* sp. (Figure 25) from communal seedpod samples from Kandersteg, Switzerland in 2010 (Table 13). As for the previous morphospecies, this record is likely a parasitoid of *C. asclepiadis*.

Recommendations and Conclusions

In general there is a lack of information, not only regarding host associations of parasitoids (Ovruski et al. 2000),but also about the biology of parasitoids of Tephritidae and some of their hosts. This chapter illustrates the lack of information regarding parasitoids of non-pest Tephritidae and the challenges of species identification of members of this parasitoid guild.

To further understand the parasitoid guild of *E. connexa* and *C. asclepiadis* it is recommended that there be a program of structured randomized weekly sampling of seedpods, from the first sign of fruiting to when a majority of the seedpods have dehisced. If half of the seedpods are dissected and the other half are placed individually in plastic vials, definitive host associations, time of attack, and estimates of levels of parasitism of egg and larval parasitoids could be established.

Egg parasitoids were not recognized for *E. connexa*, but may have been missed because of the methods used. Larval parasitoids in Europe appear to be *Stenomalina gracilis*, *Scambus brevicornis*, and Braconidae species 1. Of these *S. brevicornis* also occurs in North America and could reduce *E. connexa* efficacy if population outbreaks of the parasitoid occur. One larval-pupal parasitoid was found and is presumed to be *P. concolor*. This species has been introduced into North America as a biological control agent and could pose a potential problem for the efficacy of *E. connexa* if their ranges came to overlap. *Eupelmus fulvipes*, *Pteromalus* species 1, *Eurytoma* species 1, are hypothesised to be hyperaparasitoids of Braconidae species 1, and to pose no threat to *E. connexa* in Europe or North America. Undeterminable hosts associations

resulted for *Pseudotorymus* species 1, *Aprostocetus* species 1, and *Dinotrema* species 1. All Platygastridae are predicted to be egg or early instar larval parasitoids of *C. asclepiadis*. The precaution of releasing only adults, or of releasing pupae from a parasitoid-free culture, should be sufficient to reduce the risk of European parasitoids inhibiting the agents's population growth and accidental introduction of *C. asclepiadis*.

Acknowledgements

Thanks are due to the taxonomists at the Canadian National Collection for the identification of parasitoid specimens; Gary Gibson, Andrew Bennett, Lubomir Masner, and Henri Goulet. Garry Gibson provided detailed photographs of *Eupelmus fulvipes* and *Stenomalina gracilis*. Also, thank you to Patrick Häfliger and Andrew Mosyakin for collections of seedpods from Spain and Ukraine.

Table 10. A list of the documented parasitoids found associated with seedpods of *Vincetoxicum hirundinaria*.

Family	Valid species name	Synonym in reference	Country and Reference
Ichneumonidae	*Scambus brevicornis* (Gravenhorst)		Sweden (Janzon 1982; Solbreck 2000)
		Pimpla nigriscaposa Th.	France (Seguy 1934)
Braconidae	*Bracon picticornis* (Wesmael)		Spain (Pagola-Carte and Peydro 2012), Sweden (Janzon 1982; Solbreck 2000)
		Exothecus ruficeps (Wesmael)	France (Seguy 1934)
	Psyttalia concolor (Szépligeti)		Finland (Fischer and Koponen 1999)
	Utetes testaceus (Wesmael)	*Opius testaceus* (Wesmael)	Russia (Kandybina 1977) in Stibick (2004)
	Rhysipolis meditator (Haliday)		France (Seguy 1934) Russia (Medvedev et al. 1995)
	Rhysipolis decorator (Haliday)		France (Seguy 1934) Russia (Medvedev et al. 1995)
Eurytomidae	*Eurytoma curculionum* Mayr		Sweden (Solbreck 2000)
Pteromalidae	*Pteromalus helenomus* (Graham)		Sweden (Solbreck 2000)
	Stenomalina muscarum Mayr		Sweden (Solbreck 2000)
Eupelmidae	*Eupelmus vesicularis* (Retzius)	*Macroneura vesicularis* (Retzius)	Sweden (Solbreck 2000)
Ceraphronidae	*Aphanogmus* sp.		Sweden (Solbreck 2000)

Table 11. The localities and dates of collection of infested fruits of *Vincetoxicum hirundinaria* from 2010 to 2013.

Locality	Country	Latitude	Longitude	Elevation (m)	Collection dates			
					2010	2011	2012	2013
Ausserberg	Switzerland	46°20'21.1"N	7°52'43.9"E	1300	10 August	01 August	.	.
Kandersteg	Switzerland	46°30'22.0"N	7°43'50.1"E	1700-1800	21 August	11 August	.	.
S. Antonio	Switzerland	46°10'12.6"N	9°03'43.6"E	860	11 August	30 July	.	25 August
Anzonico	Switzerland	46°26'13.5"N	8°51'09.8"E	750 – 1000	11 August	30 July	16 August	25 August
Prato	Switzerland	46°29'28.7"N	8°45'13.9"E	890	12 August	29 July	.	.
Moutiér	Switzerland	47°16'58.6"N	7°23'14.6"E	600 - 900	.	05 August	21 August	.
Soyhières	Switzerland	47°24'30.7"N	7°22'53.5"E	560	.	05 August	21 August	11 August
Vermes (a)	Switzerland	47°19'48.5"N	7°28'21.5"E	570-700	.	05 August	.	.
Vermes (b)	Switzerland	47°19'50.6"N	7°26'45.8"E	520	.	05 August	.	.
Brig	Switzerland	46°18'51"N	7°59'10"E	NA	.	30 July	.	.
Pyrènèes	Spain	42°40'39.2"N	0°15'01.1"E	1720	7 September	.	.	.
Kiev	Ukraine	NA	NA	NA		.	.	.

NA: not available

Table 12: The number of seedpods of *Vincetoxicum hirundinaria* and the total number of overwintering pupae of *Euphranta connexa* collected between 2010 and 2013 from field sites across Switzerland.

Locality	2010	2011			2012			2013			Overall total # of pupae
	# of pupae	# of seed pods	# of pupae	Ratio pods: pupae	# of seed pods	# of pupae	Ratio pods: pupae	# of seed pods	# of pupae	Ratio pods: pupae	
Ausserberg	380	4126	299	1:0.073	.	.	.	.	.	.	679
Kandersteg	760	2724	307	1:0.113	.	.	.	.	.	.	1067
S. Antonio	130	1729	893	1:0.516	.	.	.	1456	831	1:0.571	1854
Anzonico	200	4023	2722	1:0.677	4737	1484	1:0.313	304	102	1:0.336	4508
Prato	660	335	206	1:0.615	.	.	.	.	.	.	866
Moutiér	5	782	322	1:0.412	254	132	1:0.520	.	.	.	459
Soyhières	.	424	196	1:0.462	522	152	1:0.291	55	7	1:0.127	355
Vermes (a)	.	433	190	1:0.439	.	.	.	.	.	.	190
Vermes (b)	.	625	403	1:0.645	.	.	.	.	.	.	403

#: represents Number.

Note: In 2010 the number of seedpods was not assessed, therefore the ratio number of seedpods to number pupae of *E. connexa* was not established.

Table 13. Morphospecies, taxonomic information and collection data for parasitoids from seedpods of *Vincetoxicum hirundinaria.*

Family	Identified to be	Total	Probable biological role	Locality	Year collected	Number collected
Ichneumonidae	*Scambus brevicornis*	61	larval parasitoid of *E. connexa*	Ausserberg	2010	2
					2011	1
				S. Antonio	2011	4
				Anzonico	2011	7
				Prato	2010	1
					2011	1
				Moutiér	2012	6
				Soyhières	2011	3
					2012	31
				Brig	2011	1
				Pyrenes	2010	4
Braconidae	Braconidae species 1	685	larval parasitoids of *E. connexa*	Ausserberg	2010	6
					2011	12
				Anzonico	2010	14
					2011	121
					2012	3
					2013	26
				S. Antonio	2010	23
					2011	90
					2013	37
				Kiev	2010	16

Family	Identified to be	Total	Attack stage	Locality	Year collected	Number collected
Braconidae	Braconidae species 1			Moutiér	2010	1
					2011	11
					2012	40
				Prato	2010	79
					2011	31
				Vermes (b)	2011	30
				Brig	2011	3
				Pyrenes	2010	1
				Soyhières	2011	32
					2012	28
					2013	24
				Vermes (a)	2011	37
				Anzonico*	2010	20
Braconidae	*Opius* species 1	447	larval-pupal parasitoids of *E. connexa*	Ausserberg	2010	67
					2011	36
				Kandersteg	2010	15
					2011	40
				Anzonico	2010	11
					2011	164
					2012	14
					2013	1
				S. Antonio	2010	11
					2011	21

Family	Identified to be	Total	Attack stage	Locality	Year collected	Number collected
Braconidae	*Opius* species 1			Prato	2010	5
					2011	26
				Prato	2011	1
				Moutiér	2011	21
					2012	1
				Vermes (a)	2011	4
				Vermes (b)	2011	8
	Dinotrema species 1	1	Not determinable	Moutiér	2012	1
Eurytomidae	*Eurytoma* species 1	103	Potentially a hyperparasitoid of Braconidae species 1	Ausserberg	2010	2
				Anzonico	2010	50
					2013	4
				S. Antonio	2010	4
					2013	15
					2011	10
				Prato	2010	2
				Moutiér	2012	1
				Soyhières	2011	4
				Kiev	2010	11
Pteromalidae	*Stenomalina gracilis*	32	Larval parasitoid of *E. connexa*	Anzonico	2010	1
					2011	3
				Prato	2010	1
				Soyhières	2011	2

Family	Identified to be	Total	Attack stage	Locality	Year collected	Number collected
Pteromalidae	*Stenomalina gracilis*			Vermes (a)	2011	7
				Vermes (b)	2011	18
	Pteromalus species 1	20	Hyperparasitoid of Braconidae species 1	S. Antonio	2010	2
					2013	15
				Anzonico	2013	3
Torymidae	*Pseudotorymus* species 1	11	Not determinable	Ausserberg	2011	2
				Anzonico	2011	5
				Moutiér	2010	2
				Vermes(b)	2011	2
Eupelmidae	*Eupelmus fulvipes*	34	Hyperparasitoid of Braconidae species 1	S. Antonio	2011	2
					2013	23
				Anzonico	2013	5
				Prato	2010	3
				Soyhières	2012	1
Eulophidae	*Aprostocetus* species 1	103	Not determinable	Ausserberg	2010	1
					2011	27
				Anzonico	2010	1
					2011	17
					2013	28
					2012	1
				S. Antonio	2013	10
				Prato	2010	1

Family	Identified to be	Total	Attack stage	Locality	Year collected	Number collected
Eulophidae	*Aprostocetus* species 1			Prato	2011	6
				Moutiér	2012	1
				Vermes(a)	2011	1
				Kiev	2010	9
Platygastridae	Platygastridae species 1	10	egg or larval parasitoid of *C. asclepiadis*	S. Antonio	2013	10
	Platygastridae species 2	2	egg or larval parasitoid of *C. asclepiadis*	S. Antonio	2013	2
	Synopeas species 1	2	egg or larval parasitoid of *C. asclepiadis*	Kandersteg	2010	2

Anzonico* indicates the pupae collected were from an area in Ticino Switzerland, close to Anzonico, however the exact location is unknown.

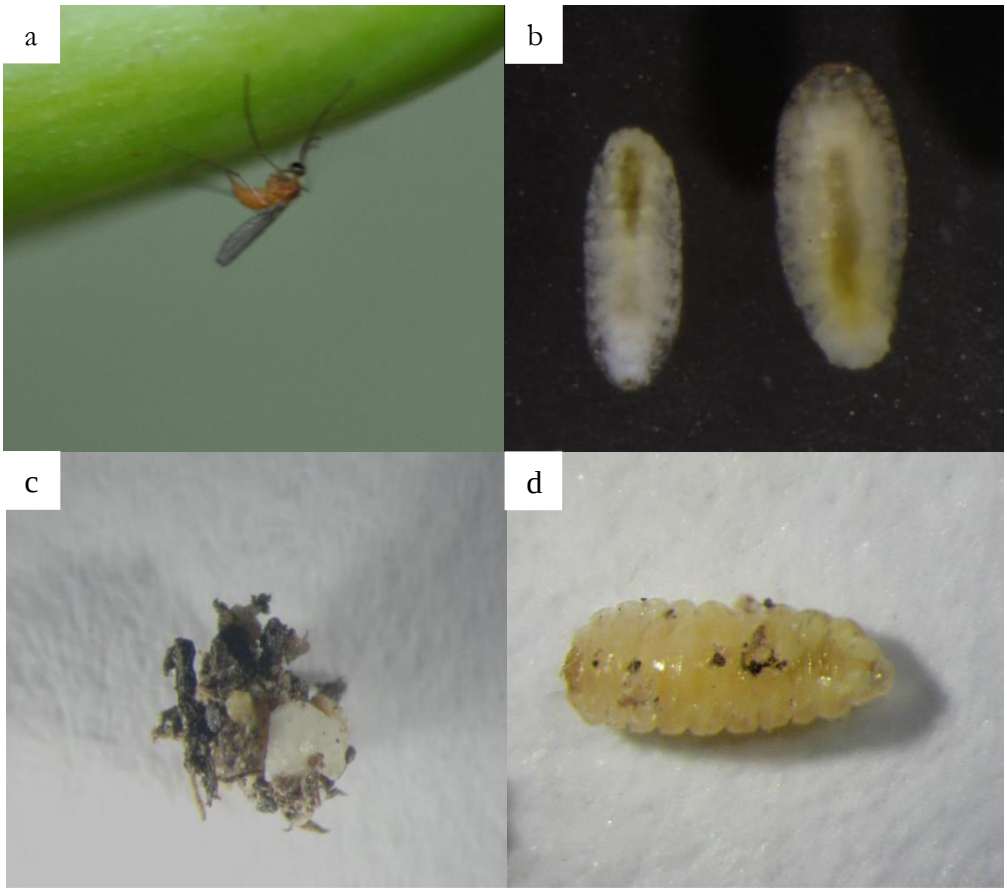

Figure 11. *Continaria asclepiadis* (Diptera: Cecidomyiidae) adult (a), larva (b), pupal caseing (c), and excised pupa (d).

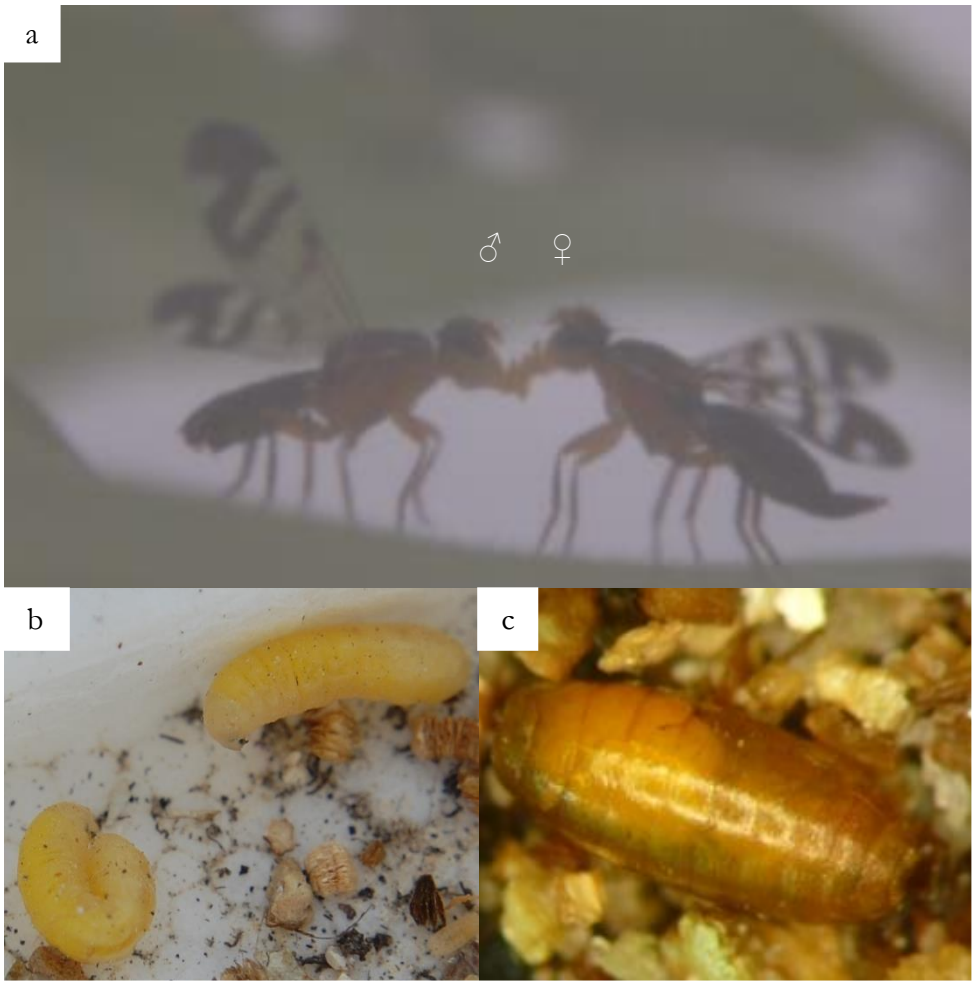

Figure 12. *Euphranta connexa* (Diptera: Tephritidae) adult (a), larvae (b), pupae (c).

Figure 13. *Scambus brevicornis* (Hymenoptera: Ichneumonidae) from seedpods of *Vincetoxicum hirundinaria*.

Figure 14. Braconidae species 1 (Hymenoptera: Braconidae) from seedpods of *Vincetoxicum hirundinaria*.

Figure 15. *Opius* species 1 (Hymenoptera: Braconidae) from pupa of *Euphranta connexa* (Diptera: Tephritidae).

Figure 16. *Dinotrema* species 1 (Hymenoptera: Braconidae) from seedpods of *Vincetoxicum hirundinaria*.

Figure 17. *Eurytoma* species 1 (Hymenoptera: Eurytomidae) from seedpods of *Vincetoxicum hirundinaria*.

Figure 18. *Stenomalina gracilis* (Hymenoptera: Pteromalidae) specimens from seedpods of *Vincetoxicum hirundinaria*.

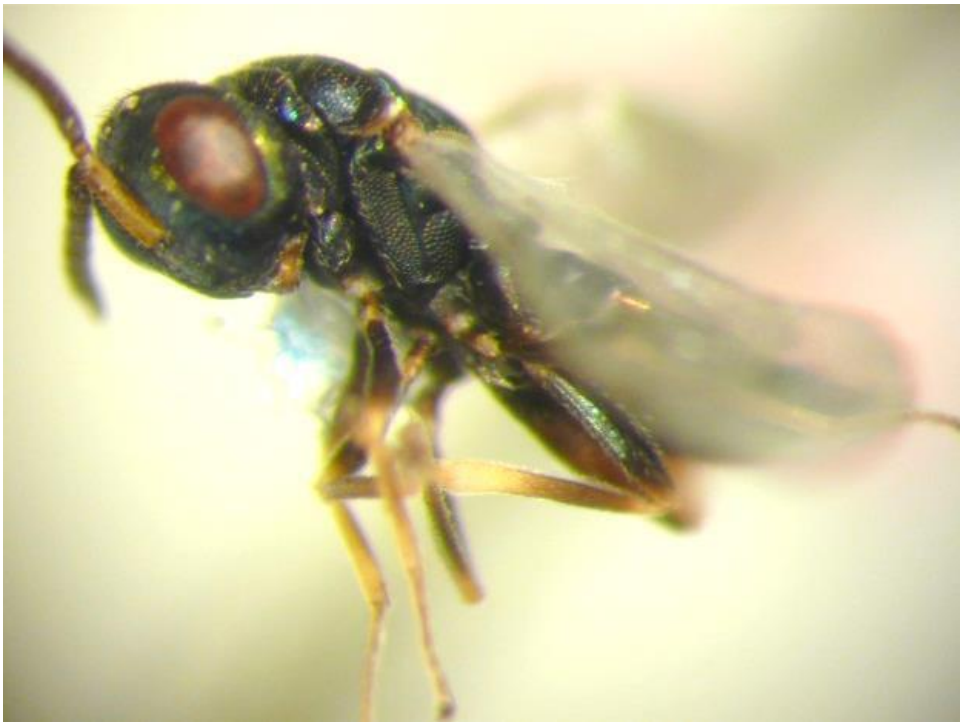

Figure 19. *Pteromalus* species 1 (Hymenoptera: Pteromalidae) from seedpods of *Vincetoxicum hirundinaria*.

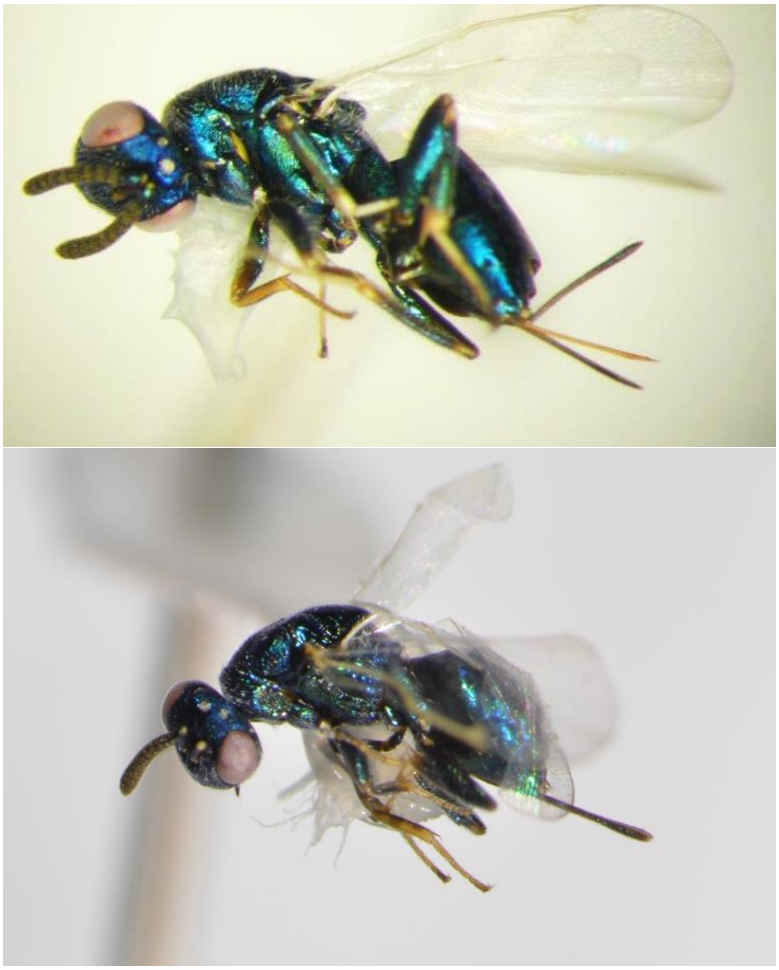

Figure 20. *Pseudotorymus* species 1 (Hymenoptera: Torymidae) from seedpods of *Vincetoxicum hirundinaria*.

Figure 21. *Eupelmus fulvipes* (Hymenoptera: Eupelmidae) from seedpods of *Vincetoxicum hirundinaria* (a: female, b: male).

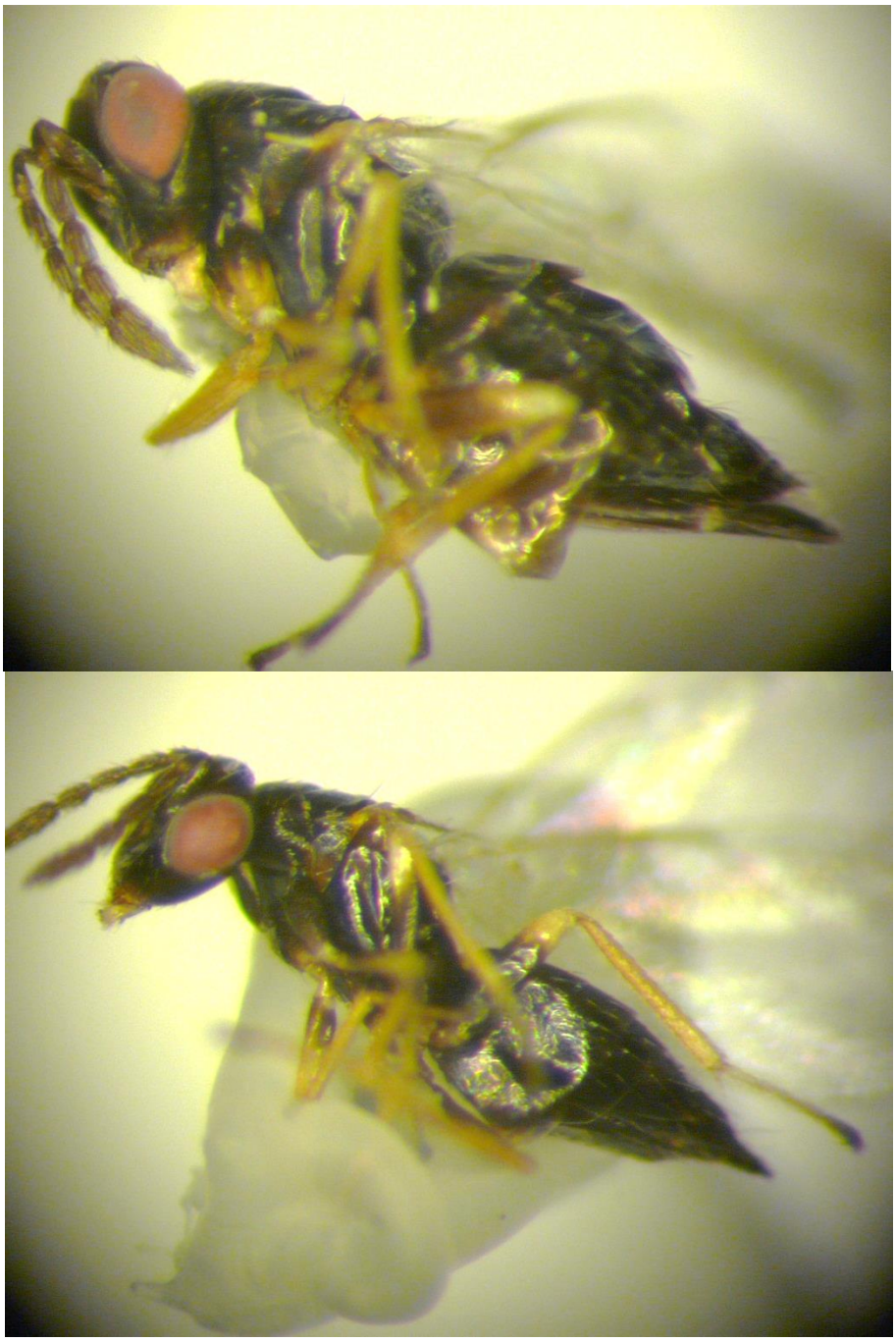

Figure 22. *Aprostocetus* species 1 (Hymenoptera: Eulophidae) from seedpods of *Vincetoxicum hirundinaria*.

Figure 23: Platygastridae species 1 (Hymenoptera: Platygastridae) from seedpods of *Vincetoxicum hirundinaria*.

Figure 24. Platygastridae species 2 (Hymenoptera: Platygastridae) from seedpods of *Vincetoxicum hirundinaria*.

Figure 25. *Synopeas* species 1 (Hymenoptera: Platygastridae) from seedpods of *Vincetoxicum hirundinaria*.

CHAPTER 6: GENERAL DISCUSSION

This thesis describes research in support of assessments of the suitability of *Euphranta connexa* (Fabricus) (Diptera: Tephritidae) as a biological control agent for the invasive weeds *Vincetoxicum nigrum* (L.) (Apocynaceae) and *V. rossicum* (Kleopow) Barbar (Apocynaceae). To date in the biological control program for these *Vincetoxicum* spp., the European foliar feeder, *Hypena opulenta* (Christoph) (Lepidoptera: Noctuidae) has been released in Canada (Rob Bourchier, personal communication). The anticipated role of *E. connexa* in biological control is to decrease the seed output of the two species of *Vincetoxicum*. The research in this thesis mostly contributes basic biological information on *E. connexa* that is essential for detailed evaluation of the potential weed biological control agent.

The first aim was to characterize the thermal requirements for development of *E. connexa* so that effective host range testing methodology can be developed (Chapter 3). This information should allow manipulation of the time to oviposition so that, when harmonized with information on timing of fruit development, it will permit effective impact and host range testing of *E. connexa*. Diapause allows synchronization of insect life cycle events with events occurring in the organisms environment (Hopper 1999). The normal environment of *E.connexa* may not exert selection pressure for diapause: as there are suitable seedpods for oviposition available on *V. hirundinaria* (L.) (Apocynaceae) from June until August. so synchronization of time of oviposition with host stage is not critical. My research supports the view that *E. connexa* does not undergo overwintering diapause (Solbreck and Ives 2007) and that it is likely *E. connexa* pupae overwinter in a cold induced quiescenent state.

My investigations into the thermal developmental requirements of *E. connexa* showed no differences in the requirement of development of *E. connexa* from localities differing in altitudes from 500 to 1800 m above sea level. As elevation increases there is an average decrease of about 5.5 °C for every 1000 m of altitude (Korner 2007). Thus, one might expect local adaptation of *E. connexa* development. One possible reason for a lack of local adaptation is the previously mentioned long duration of availability of suitable seedpods for oviposition. Also, in Scandinavia (Solbreck 2000) and Switzerland (Chapter 5) there are years where seedpod production is sparse in a site. Populations of *V. hirundinaria* are patchy (Solbreck 2000) and *E. connexa* is assumed to be a strong flier and able to move to more suitable sites if seedpods are limited (Solbreck 2000). The selection against local adaptation would also allow for movement between sites, potentially from different altitudes, in response to a local seedpod shortage. This is an advantageous character for an introduced biological agent as it allows for greater dispersal capabilities.

The cold induced quiescence and a lack of local adaptation to altitudes suggests that, if *E. connexa* is to be introduced for the control of invasive *Vincetoxicum* spp. in North America, climatic matching of populations may not be required. In the absence of diapause in *E. connexa* the model for temperature-dependent pupal development was incomplete, as it did not model development from the beginning of the pupal period. To devise a precise model of pupal development, pupae need to be placed at constant temperatures immediately following pupation. Nevertheless, the model from my research is sufficient to provide an estimated time to develop for synchronizing adult emergence with seedpod availability in test plants.

I also investigated the developmental rate of *E. connexa* eggs from oviposition to hatch (Chapter 3). A range of suitable temperatures was identified and a model that allows the prediction of developmental rates within this temperature range was established. Knowing the time required for egg development can provide information on the suitability of a test plant for the development of *E. connexa* eggs. If eggs cannot develop after oviposition, the plant is not a suitable host and can be excluded from the fundamental host range.

My research (Chapter 4) added to the knowledge from Solbreck (2000) that females have no mature eggs at the time of emergence. At 20 ^{o}C, most females had produced some mature eggs five days after emergence, and by 8-15 days after emergence females reached their full complement of eggs. Thus to avoid false negatives, in which females have no eggs to oviposit in host range experiments, females should be held at 20 °C for 8 to 15 days after emergence, before seedpods are offered for host range assessment. The finding that, at 30 days after emergence, the total of the eggs contained or laid by mated females is less than the number of eggs contained 15 days after emergence is important for the development of testing methodology. The evidence that females can resorb eggs is strong. Thus, even though females can live 30 days after emergence in the laboratory, delaying their use in tests of host range is unwise as false negatives may result. I recommend that all host range testing be done when females are about 8 to 15 days post eclosion. The amalgamation of information from the development rate studies with that on the time required before oviposition will aid the biological control program by allowing the development of effective protocols for host range testing and impact studies of *E. connexa*.

The effect on egg load of time since emergence suggests that *E. connexa* females can reallocate resources and so possibly prolong their survival in the absence of suitable oviposition hosts. As previously mentioned, under environmental uncertainty at a site it is thought *E. connexa* can move to more suitable sites. Resource re-allocation from eggs to energy for flight may allow a female to find a suitable patch in which to lay a diminished number of eggs, rather than stay in a patch where oviposition is not possible. In terms of a biological control agent, the ability to find new resource patches is desirable, as the agent can spread without the intervention of humans.

Results from fecundity experiments also show that egg load diminishes with increasing duration of time pupae are left in cold storage. The implications of this for the biological control program is that in experiments conducted at the end of the season females have less egg load, and if left too long may have no eggs to lay. This can also affect the comparison of results of tests conducted early in the season with those from tests conducted at the end of the season.

It follows that the overwintering strategy of cold induced quiescence, the finding that longer duration of cold storage reduces egg load, and the similarity of temperature-dependence of development rate at different altitudes, that altitude could affect egg load. For example, females overwintering in high altitude sites are likely to emerge later in the season after having been exposed longer to colder temperatures. It would be expected that these females would have a smaller egg load and consequently diminished resources available to reallocate to survival or dispersal than females from lower elevations. Thus, it is expected that females from high altitude sites would have a lower fitness compared to those at low altitudes. Further study into the fecundity of females emerging from low and high altitude sites along with characterizing the stability of seedpod production from these

sites could provide insight into the evolutionary landscape of *E. connexa* and resource allocation in females.

One question that has arisen as a result of my studies, but which was not experimentally answered nor a focus of this thesis is, how long do adult *E. connexa* live? Solbreck (2000) indicates that adult flies are long-lived, but provides no evidence for this, other than the presence of adults over several months of the summer. Such prolonged presence could be the result of asynchronous emergence of relatively short-lived adults, rather than the persistence of long-lived adults. My finding that pupae undergo cold induced quiescence, rather than diapause, is in accord with prolonged asynchronous emergence. Sources of variation in emergence date include the date of pupation in the previous year, variable microhabitats in which pupae spend the winter, and altitude-related climatic effects. The effect of emergence date on the egg load of female *E. connexa* and the diminution in fecundity with female age both indicate that females were likely energy-limited as pupae and, in the laboratory, were unable to gain enough energy from feeding to attain maximum potential egg load for prolonged periods. In the laboratory females were provided with sources of carbohydrates and protein, which would have required less energy expenditure to obtain than would foraging for food. Also, early morning and late evening temperatures in the higher altitude sites diminish the time allocated for foraging. My results do not seem to support the hypothesis of long-lived adults; rather the presence of adults for a long period is more likely to be the result of asynchronous emergence of overwintering insects.

My thesis research evaluated the parasitoid assemblage of the known seedpod predators on *V. hirundinaria* to assess the potential for interference with the biological control program (Chapter 5). The most prevalent parasitoids of *E. connexa* were of the

family Braconidae, and consisted of 70% of the total parasitoid individuals found. Two parasitoids could pose a potential risk to the biological control program, if *E. connexa* is introduced into North America. *Scambus brevicornis* and those presumed to be *Psyttalia concolor* (identified as *Opius* species 1) have a Holarctic distribution and so could impair the population growth of *E. connexa* in North America. The other parasitoids found were rare or were hyperparasitoids. Although there is a chance of parasitoid accumulation on *E. connexa* if introduced, it is likely that the known parasitoids would not interfere with biological control by *E. connexa* as mortality by parasitoids in the native range reaches a maximum of only 15% (Solbreck and Ives 2007). However, in Scandinavia the seedpod production is highly variable and influenced by climatic conditions such as sunshine and moisture (Solbreck and Sillén-Tullberg 1986a). In North America, there is no evidence for an annual variability in seedpod production of *V. nigrum* and *V. rossicum* (Averill et al. 2011). Thus, seedpod production is more stable from year to year, reducing the variability in *E. connexa* populations, which could allow for a more stable resource of *E. connexa* for parasitoid populations to build on over time. The major known parasitoids present in North America have a broad host range, and the parasitism levels in North America will depend on the abundance of other hosts, which will likely differ from that in Europe.

An unexpected outcome of the parasitoid survey was the frequent occurrence of *Contarinia asclepiadis*. I found *C. asclepiadis* in all localities in Switzerland, and in two sites for which I dissected seedpods about 9% of the seedpods contained *C. asclepiadis*. In Solbreck (2000), Widenfalk and Solbreck (2002), and Weed et al. (2011b) *C. asclepiadis* was reported to be rare. It is not clear if the difference between Weed et al. (2011) and my results are due to the time of sampling. It is certain that the species was

rare in Sweden between 2000-2002 (Widenfalk and Solbreck 2002), but it is not known if the difference in the results between my study and theirs is because of geographic or temporal variation or both. I also detected three parasitoid morphospecies that appear to attack *C. asclepiadis* and at least two of them would be new host records for this species as only one parasitoid species has been previously reported. *Contarinia asclepiadis* may not be an ideal candidate for biological control as it is only found in seedpods associated with *E. connexa*. If further research were to show that the presence of *C. asclepiadis* in the seedpods infested with *E. connexa* enhanced the negative effects on *Vincetoxicum* spp., it could be considered as a biological control agent to be introduced with or following *E. connexa*.

There are several directions that future research on *E. connexa* can take. One of which is to investigate further the natural host range of *E. connexa* in Europe. The natural host of *E. connexa* is *V. hirundinaria* and *E. connexa* has never been reported on *V. rossicum* or *V. nigrum* in Europe (Weed et al. 2011b) . Populations of *V. hirundinaria* are in close proximity to *V. nigrum* (René Sforza, personal communication) and *V. rossicum* (Weed, unpublished data). If the areas where *V. hirundinaria* is in close proximity are surveyed, a population of *E. connexa* may be found on one of the target weeds. For biological control, this population could be more suited to release as a biological control agent in North America because it could be more adapted to the target weeds.

Future work is needed on the parasitoids of *E. connexa* and *C. asclepiadis* and their interactions. Although not directly related to the potential control by *E. connexa,* gathering biological information on *C. asclepiadis* is of considerable scientific interest. The current study focused on larval and larval-pupal parasitoids, and the egg and pupal parasitoid assemblages should be investigated. For the assessment of egg parasitoids it is

suggested that seedpods of *V. hirundinaria* be dissected to collect the eggs of *E. connexa* and of *C. asclepiadis*. In this thesis, only *E. connexa* pupa where overwintered to detect larval-pupal parasitoids. The same technique of overwintering could be applied to *C. asclepiadis*, and could yield new larval-pupal parasitoid host associations. Pupal parasitoids were not investigated in this thesis. Because both *E. connexa* and *C. asclepiadis* leave the seedpod to pupate in the soil, pupal parasitoids can be assessed through field surveys. This can be done by the removal of pupae from soil underneath highly infested plant patches: 1) starting immediately after pupal drop, 2) in late September, 3) in late October, 4) following snow melt, and 5) prior to adult emergence. The pupae collected at these five times can be reared through until the emergence of either a host or parasitoid.

A next step in the progression of the *E. connexa* biological control program is to assess the impact of *E. connexa* on the target weeds. *Euphranta connexa* has not been reported on *V. rossicum* or *V. nigrum* in the field, but under experimental conditions *E. connexa* can oviposit and complete development on both of these species. Further testing is needed to determine the impact of *E. connexa* on the three species of *Vinceotxicum*, and so provide evidence that may support the case for testing and release of *E. connexa* to control the invasive species in North America.

The most important next step of the assessment of *E. connexa* for the biological control of *Vincetoxicum* spp. is the assessment of risk to non-target species. The biological information on *E. connexa* gained from this thesis will enable the synchronization of oviposition with seedpod availability on non-target plant species, and the construction of biologically appropriate oviposition and larval host range testing. Infortmation on fundamental and ecological host range will enable decisions about

whether *E. connexa* should be released into North America to slow the spread of *Vincetoxicum* spp.

CITED LITERATURE

Adler, P.B., D'Antonio, C.M. and Tunison, J.T. 1998. Understory succession following a dieback of *Myrica faya* in Hawai'i Volcanoes National Park. Pacific Science **52**: 69–78.

Ågren, J., Ehrlén, J. and Solbreck, C. 2008. Spatio-temporal variation in fruit production and seed predation in a perennial herb influenced by habitat quality and population size. Journal of Ecology **96**: 334–345.

Allen, T.C. and Fluke, C.L. 1933. Notes on the life history of the apple maggot in Wisconsin. Journal of Economic Entomology **26**: 1108–1112.

Alley, H., Humburg, N., Fornstrom, J. and Ferell, M. 1984. Leafy spurge repetitive herbicide treatments. University of Wyoming Agricultural Experiment Station Research Journal **192**: 90–93.

Alonso-Pimentel, H., Korer, J.B., Nufio, C. and Papaj, D.R. 1998. Role of colour and shape stimuli in host-enhanced oogenesis in the walnut fly, *Rhagoletis juglandis*. Physiological Entomology **23**: 97–104.

Askew, R.R., Blasco-Zumeta, J. and Pujade-Villar, J. 2001. Chalcidoidea and Mymarommatoidea (Hymenoptera) of a *Juniperus thurifera* L. forest of Los Monegros region, Zaragoza. Monografias SEA **4**: 1–76.

Askew, R.R., Plantard, O., Gomez, J.F., Hernandez Nieves, M. and Nieves-Aldrey, J.L. 2006. Catalogue of parasitoids and inquilines in galls of Aylacini, Diplolepidini and Pediaspidini (Hym., Cynipidae) in the West Palaearctic. Zootaxa **1301**: 1–60.

Averill, K.M., DiTommaso, A., Mohler, C.L. and Milbrath, L.R. 2011. Survival, growth, and fecundity of the invasive swallowworts (*Vincetoxicum rossicum* and *V. nigrum*) in New York State. Invasive Plant Science and Management **4**: 198–206.

Averill, K.M., DiTommaso, A. and Morris, S.H. 2008. Response of pale swallow-wort (*Vincetoxicum rossicum*) to triclopyr application and clipping. Invasive Plant Science and Management **1**: 196–206.

Baliraine, F.N., Bonizzoni, M., Guglielmino, C.R., Osir, E.O., Lux, S.A., Mulaa, F.J., Gomulski, L.M., Zheng, L., Quilici, S., Gasperi, G. and others. 2004. Population genetics of the potentially invasive African fruit fly species, *Ceratitis rosa* and *Ceratitis fasciventris* (Diptera: Tephritidae). Molecular Ecology **13**: 683–695.

Bangsund, D.A., Leistritz, F.L. and Leitch, J.A. 1999. Assessing economic impacts of biological control of weeds: The case of leafy spurge in the northern Great Plains of the United States. Journal of environmental management **56**: 35–43.

Barnes, H. 1933. A cambium miner, of basket willows (Agromyzidae) and its inquiline gall midge (Cecidomyiidae). Annals of Applied Biology **20**: 498–519.

Barratt, B.I. 2012. Assessing safety of biological control introductions. *In* Plant Sciences Reviews 2011. *Edited by* D. Hemming. CABI, Wallingford, UK. pp. 167–178.

Belcher, J.W. and Wilson, S.D. 1989. Leafy spurge and the species composition of a mixed-grass prairie. Journal of Range Management **42**: 172–175.

Benelli, G., Gennari, G. and Canale, A. 2013. Host discrimination ability in the tephritid parasitoid *Psyttalia concolor* (Hymenoptera: Braconidae). Journal of Pest Science **86**: 245–251.

Bjureke, K. 2007. *Vincetoxicum rossicum* on the islands of the Inner Oslo fjord: transferred from a red-listed species to a pest species. Blyttia **65**: 76–85.

Blay, S. and Yuval, B. 1999. Oviposition and fertility in the Mediterranean fruit fly (Diptera: Tephritidae): Effects of male and female body size and the availability of sperm. Annals of the Entomological Society of America **92**: 278–284.

Blossey, B. 1995. Host specificity screening of insect biological weed control agents as part of an environmental risk assessment. *In* Biological Control: Benefits and Risks. *Edited by* H. M. T. Hokkanen, J. M. Lynch. Cambridge University Press, Cambridge,UK. pp. 84–89.

Blossey, B., Schroeder, D., Hight, S.D. and Malecki, R.A. 1994. Host-specificity and environmental-impact of the weevil *Hylobius transversovittatus*, a biological-control agent of purple-loosestrife (*Lythrum salicaria).* Weed Science **42**: 128–133.

Boucek, Z. 1977. A faunistic review of the Yugoslavian Chalcidoidea parasitic Hymenoptera. Acta Entomologica Yugoslavica **13**: 5–26.

Boucek, Z. and Graham, M.W.R.D. 1978. British checklist of Chalcidoidea Hymenoptera taxonomic notes and additions. Entomologist's Gazette **29**: 225–235.

Bourchier, R.S. and Van Hezewijk, B.H. 2013. *Euphorbia esula* L., Leafy spurge (Euphorbiaceae). *In* Biological Control Programs in Canada 2001–2012. *Edited by* P. G. Mason, D. R. Gillespie. CABI, Wallingford, UK. pp. 315–320.

Bourchier, R.S., Weed, A., Casagrande, R., Gassmann, A., Smith, S.M. and Cappuccino, N. 2013. *Vincetoxicum nigrum* (l.) Moench, *V. rossicum* (Kleopow) Barbar., swallow-worts, dog strangling vine (Apocynaceae). *In* Biological Control Programs in Canada 2001–2012. *Edited by* P. G. Mason, D. R. Gillespie. CABI, Wallingford, UK. pp. 402–407.

Briese, D.T. 2005. Translating host-specificity test results into the real world: The need to harmonize the yin and yang of current testing procedures. Biological Control **35**: 208–214.

Broad, G. 2009. Checklist of British and Irish Ichneumonidae (Hymenoptera). [online] Available from http://www.nhm.ac.uk/resources-rx/files/ichneumonidae-checklist-54448.pdf [accessed July 9, 2013].

Bruun, H.H., Jorgensen, J. and Skuhrava, M. 2012. Nineteen species of gall midges (Diptera: Cecidomyiidae) new to Denmark. Entomologiske Meddelelser **80**: 87–98.

Buffington, M.L. and Polaszek, A. 2009. Recent occurrence of *Aphanogmus dictynna* (Waterston) (Hymenoptera: Ceraphronidae) in Kenya — an important hyperparasitoid of the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae). Zootaxa **2214**: 62–68.

Capo, M. and Saa, J.M. 1989. (-)-Antofine a phenanthroindolizidine from *Vincetoxicum nigrum*. Journal of Natural Products **52**: 389–390.

Cappuccino, N. 2004. Allee effect in an invasive alien plant, pale swallow-wort *Vincetoxicum rossicum* (Asclepiadaceae). Oikos **106**: 3–8.

Carroll, L.E., White, I.M., Freidberg, A., Norrbom, A.L., Dallwitz, M.J. and Thompson, F.C. 2002. Pest fruit flies of the world. [online] Available from http://delta-intkey.com/ffa/www/eup_conn.htm [accessed April 22, 2013].

Casagrande, R.A. and Dacey, J.E. 2007. Monarch butterfly oviposition on swallow-worts (*Vincetoxicum* spp.). Environmental Entomology **36**: 631–636.

Casagrande, R.A., Weed, A.S., Hazlehurst, A., Tewksbury, L., Gassmann, A. and Bourchier, R. 2011. A petition for experimental open-field release of *Hypena*

opulenta and *Abrostola asclepiadis* two potential biological control agents of swallow-worts (*Vincetoxicum nigrum* and *V. rossicum*) in North America DRAFT VERSION. pp. 35.
Chapman, T., Miyatake, T., Smith, H.K. and Partridge, L. 1998. Interactions of mating, egg production and death rates in females of the Mediterranean fruit fly, *Ceratitis capitata*. Proceedings of the Royal Society B. **265**: 1879–1894.
Christensen, T. 1998. Swallow-worts, the ecology and control of *Vincetoxicum* spp. Wildflower **14**: 21–25.
Christenson, L.D. and Foote, R.H. 1960. Biology of fruit flies. Annual Review of Entomology **5**: 171–192.
Clausen, C.P. 1978. Introduced parasites and predators of arthropod pests and weeds: A world review. U.S.D.A. Agricultural Research Service, Agricultural Handbook No. 480.
Colautti, R.I. and MacIsaac, H.J. 2004. A neutral terminology to define 'invasive' species. Diversity and Distributions **10**: 135–141.
Colautti, R.I., Ricciardi, A., Grigorovich, I.A. and MacIsaac, H.J. 2004. Is invasion success explained by the enemy release hypothesis? Ecology Letters **7**: 721–733.
Copeland, R.S., White, I., Okumu, M., Machera, P. and Wharton, R.A. 2004. Insects associated with fruits of the *Oleaceae* (Asteridae, Lamiales) in Kenya, with special reference to the Tephritidae (Diptera). Bishop Museum Bulletin of Entomology **12**: 135–164.
Cronk, Q.C.B. and Fuller, J.L. 1995. Plant Invaders. Chapman and Hall, Cambridge, UK.
Daane, K.M. and Johnson, M.W. 2010. Olive fruit fly: managing an ancient pest in modern times. Annual Review of Entomology **55**: 151–169.
De Bach, P. 1964. Biological Control of Insect Pests and Weeds. Chapman & Hall Ltd., London, UK.
De Clerck-Floate, R., Mason, P., Parker, D., Gillespie, D., Broadbent, A. and Boivin, G. 2006. Guide for the Importation and Release of Arthropod Biological Control Agents in Canada. Agriculture and Agri-Food Canada, Ottawa, ON.
De Groote, H., Ajuonu, O., Attignon, S., Djessou, R. and Neuenschwander, P. 2003. Economic impact of biological control of water hyacinth in Southern Benin. Ecological Economics **45**: 105–117.
De Meyer, M., Robertson, M.P., Peterson, A.T. and Mansell, M.W. 2008. Ecological niches and potential geographical distributions of Mediterranean fruit fly (*Ceratitis capitata*) and Natal fruit fly (*Ceratitis rosa*). Journal of Biogeography **35**: 270–281.
Denoth, M., Frid, L. and Myers, J.H. 2002. Multiple agents in biological control: improving the odds? Biological Control **24**: 20–30.
Denslow, J.S. and D'Antonio, C.M. 2005. After biocontrol: assessing indirect effects of insect releases. Biological Control **35**: 307–318.
Ding, J., Mack, R.N., Lu, P., Ren, M. and Huang, H. 2008. China's booming economy is sparking and accelerating biological invasions. BioScience **58**: 317–324.
DiTomaso, J.M. 2000. Invasive weeds in rangelands: species, impacts, and management. Weed Science **48**: 255–265.
DiTommaso, A., Brainard, D.C. and Webster, B.R. 2005a. Seed characteristics of the invasive alien vine *Vincetoxicum rossicum* are affected by site, harvest date, and storage duration. Canadian Journal of Botany **83**: 102–110.

DiTommaso, A., Lawlor, F.M. and Darbyshire, S.J. 2005b. The biology of invasive alien plants in Canada. 2. *Cynanchum rossicum* (Kleopow) Borhidi [= *Vincetoxicum rossicum* (Kleopow) Barbar.] and *Cynanchum louiseae* (L.) Kartesz & Gandhi [= *Vincetoxicum nigrum* (L.) Moench]. Canadian Journal of Plant Science **85**: 243–263.

DiTommaso, A. and Losey, J.E. 2003. Oviposition preference and larval performance of monarch butterflies (*Danaus plexippus*) on two invasive swallow-wort species. Entomologia Experimentalis et Applicata **108**: 205–209.

Doorduin, L.J. and Vrieling, K. 2011. A review of the phytochemical support for the shifting defence hypothesis. Phytochemistry Reviews **10**: 99–106.

Douglass, C.H., Weston, L.A. and DiTommaso, A. 2009. Black and pale swallow-wort (*Vincetoxicum nigrum* and *V. rossicum*): The biology and ecology of two perennial, exotic and invasive vines. *In* Management of Invasive Weeds. *Edited by* X. Inderjit. Springer Science + Business Media B.V., Dordrecht, Netherlands. pp. 261–277.

Douglass, C.H., Weston, L.A. and Wolfe, D. 2011. Phytotoxicity and potential allelopathy in pale (*Cynanchum rossicum*) and black swallowwort (*C. nigrum*). Invasive Plant Science and Management **4**: 133–141.

Dubbert, M., Tscharntke, T. and Vidal, S. 1998. Stem-boring insects of fragmented *Calamagrostis* habitats: herbivore-parasitoid community structure and the unpredictability of grass shoot abundance. Ecological Entomology **23**: 271–280.

Duyck, P.F. and Quilici, S. 2002. Survival and development of different life stages of three *Ceratitis* spp. (Diptera: Tephritidae) reared at five constant temperatures. Bulletin of Entomological Research **92**: 461–469.

Duyck, P.F., Sterlin, J.F. and Quilici, S. 2004. Survival and development of different life stages of *Bactrocera zonata* (Diptera: Tephritidae) reared at five constant temperatures compared to other fruit fly species. Bulletin of Entomological Research **94**: 89–93.

Dzhanokmen, K.A. 1978. Identification of the insects of the European part of the USSR. Volume 3. Hymenoptera. Second part. Pteromalidae. Opredeliteli po Faune SSSR: 57–228.

Edwards, P.B., Adair, R.J. and Holtkamp, R.H. 1996. Seedhead tephritids and their parasitoids - can predictions be made about likely parasitism of tephritids introduced into Australia for biological control of Asteraceae? *In* Proceedings of the 9th International Symposium on Biological Control of Weeds 19-26 January 1996, Stellenbosch, South Africa. *Edited by* V. C. Moran, J. H. Hoffmann. University of Cape Town, Cape Town, South Africa. pp. 153–164.

Ehler, L.E. and Hall, R.W. 1982. Evidence for competitive-exclusion of introduced natural enemies in biological control. Environmental Entomology **11**: 1–4.

Eibler, E., Tanner, U., Mayer, K.K., Wiegrebe, W. and Reger, H. 1995. HPLC-analysis of alkaloids from *Cynanchum vincetoxicum*. Acta Pharmaceutica **45**: 487–493.

El Fels, M.A.E.A., Roques, A. and Boumezzough, A. 1999. The arthropods related to cones and seeds of incense-juniper, *Juniperus thurifera* L., in Atlas mountains of Morocco. Ecologia Mediterranea **25**: 95–110.

Elzinga, J.A., Zwakhals, K., Harvey, J.A. and Biere, A. 2007. The parasitoid complex associated with the herbivore *Hadena bicruvis* (Lepidoptera : Noctuidae) on

Silene latifolia (Caryophyllaceae) in the Netherlands. Journal of Natural History **41**: 101–123.
Endress, M. and Bruyns, P. 2000. A revised classification of the Apocynaceae s.l. The Botanical Review **66**: 1–56.
Ernst, C.M. and Cappuccino, N. 2005. The effect of an invasive alien vine, *Vincetoxicum rossicum* (Asclepiadaceae), on arthropod populations in Ontario old fields. Biological Invasions **7**: 417–425.
Fischer, M. 1982. Description and redescription of Opiinae Hymenoptera Braconidae from the Palearctic region. Annalen des Naturhistorischen Museums in Wien Serie B Botanik und Zoologie **86**: 85–132.
Fischer, M. and Koponen, M. 1999. A survey of *Opiinae* (Hymenoptera: Braconidae) of Finland, part 2. Entomologica Fennica **10**: 129–160.
Fischer, M., Tormos, J., Pardo, X. and Asis, J.D. 2008. New citations of Alysiini from Spain, with a description of *Dinotrema mediocornis hispanicum* nov.ssp and of the females of *Aspilota inflatinervis* and *Synaldis azorica* (Hymenoptera, Braconidae, Alysiinae). Linzer Biologische Beitraege **40**: 1449–1466.
Fisher, J.P. 1965. A contribution to the biology of *Eurytoma curculionum* Mayr (Hym., Eurytomidae). Entomophaga Paris **10**: 317–318.
Fitt, G.P. 1986. The influence of a shortage of hosts on the specificity of oviposition behavior in species of *Dacus* (Diptera: Tephritidae). Physiological Entomology **11**: 133–143.
Fitton, M.G., Graham, M.W.R.d.V., Boucek, Z.R.J., Fergusson, N.D.M., Huddleston, T., Quinlan, J. and Richards, O.W. 1978. A check list of British insects. Second edition (completely revised). Part 4: Hymenoptera. Handbooks for the Identification of British Insects **11**: i–ix, 1–159.
Flanders, S.E. 1942. Oosorption and ovulation in relation to oviposition in the parasitic Hymenoptera. Annals of the Entomological Society of America **35**: 251–266.
Flanders, S.E. 1950. Regulation of ovulation and egg disposal in the parasitic Hymenoptera. Canadian Entomologist **82**: 134–139.
Fletcher, B.S. and Prokopy, R.J. 1991. Host location and oviposition in tephritid fruit flies. *In* Reproductive Behaviour of Insects. *Edited by* W. J. Bailey, J. Ridsdill-Smith. Chapman and Hall, London, UK. pp. 139–171.
Forare, J. 1995. The biology of the noctuid moth *Abrostola asclepiadis* Schiff. (Lepidoptera, Noctuidae) in Sweden. Entomologisk Tidskrift **116**: 179–186.
Fournier, P. 1977. Les quatre flores de la France, corse comprise (Générale, Alpine, Méditerranéene, Littorale).2. Editions Lechevalier S.A.R.L., Paris, France.
Fusu, L. 2009. Romanian Eupelmidae (Hymenoptera: Chalcidoidea): new cytogenetic, faunistic and host records. North-Western Journal of Zoology **5**: 307–320.
Garrido Torres, A.M. and Nieves-Aldrey, J.L. 1999. Pteromalids from the autonomous community of Madrid (CAM) (Spain): faunistics and catalogue (Hymenoptera: Chalcidoidea, Pteromalidae). Graellsia **55**: 9–147.
Gaskin, J.F., Bon, M.-C., Cock, M.J., Cristofaro, M., Biase, A.D., De Clerck-Floate, R., Ellison, C.A., Hinz, H.L., Hufbauer, R.A. and Julien, M.H. 2011. Applying molecular-based approaches to classical biological control of weeds. Biological Control **58**: 1–21.

Gassmann, A., Bennett, M., Penic, M. and Leroux, A. 2012. Biological control of swallow-worts, *Vincetoxicum rossicum* and *V. nigrum*. *In* CABI Switzerland Annual Report 2011. CABI Delémont, Switzerland. pp. 25.

Gassmann, A., Leroux, A.M., Mosyakin, A., Edelmann, L. and Weed, A.S. 2008. Evaluating the potential for biological control of swallow-worts, *Vincetoxicum rossicum* and *V. nigrum*. *In* Annual Report 2007. CABI Europe – Switzerland, Delémont, Switzerland. pp. 19.

Gassmann, A., Leroux, A.M. and Weed, A.S. 2009. Evaluating the potential for biological control of swallow-worts, *Vincetoxicum nigrum* and *V. rossicum*. *In* Annual Report 2008. CABI Europe - Switzerland, Delémont, Switzerland. pp. 25.

Gassmann, A., Schwan, A., Mosyakin, A., Häflinger, N., Dejong, R. and Tosevski, I. 2011. Biological control of swallow-worts, *Vincetoxicum rossicum* and *V. nigrum*. *In* Annual Report 2010. CABI, Delémont, Switzerland. pp. 21.

Gheorghiu, V. 1984. La famille des Tephritidae (Diptera) dans les collections du Muséum d'Histoire Naturelle "Grigore Antipa".Travaux du Muséum d'Histoire Naturelle "Grigore Antipa". **26**: 209–217.

Gibson, D.M., Krasnoff, S.B., Biazzo, J. and Milbrath, L. 2011. Phytotoxicity of antofine from invasive swallow-worts. Journal of Chemical Ecology **37**: 871–879.

Gibson, G.A., Gates, M.W. and Buntin, G.D. 2006a. Parasitoids (Hymenoptera: Chalcidoidea) of the cabbage seedpod weevil (Coleoptera: Curculionidae) in Georgia, USA. Journal of Hymenoptera Research **15**: 187–207.

Gibson, G.A.P. 1995. Parasitic wasps of the subfamily Eupelminae: classification and revision of world genera (Hymenoptera: Chalcidoidea: Eupelmidae). Memoirs on Entomology, International **5**: 1–421.

Gibson, G.A.P., Gillespie, D.R. and Dosdall, L. 2006b. The species of Chalcidoidea (Hymenoptera) introduced to North America for biological control of the cabbage seedpod weevil, and the first recovery of *Stenomalina gracilis* (Chalcidoidea: Pteromalidae). Canadian Entomologist **138**: 285–291.

Gilbert, M.G., Stevens, W. and Ping-tao, L. 1995. Notes on the Asclepiadaceae of China. Novon **5**: 1–16.

Glass, E.H. 1960. Apple maggot fly emergence in western New York. Bulletin of the New York State Agricultural Experiment Station **789**: 1–29.

Glavendekic, M. and Kolarov, J. 1994. Fauna of Yugoslavian Ichneumonidae Pimplinae, Xoridinae, Acaenitinae (Insecta, Hymenoptera, Ichneumonidae). Entomofauna **15**: 1–12.

Goeden, R.D. and Louda, S.M. 1976. Biotic interference with insects imported for weed control. Annual Review of Entomology **21**: 325–342.

Goulet, H. and Huber, J.T. 1993. Hymenoptera of the World: an Identification Guide to Families. Agriculture Canada Publication **1894E**, Ottawa, ON. pp. i–vii, 1–668.

Graham, M.W.R.d.V. 1969. The Pteromalidae of north-western Europe (Hymenoptera: Chalcidoidea). Bulletin of the British Museum Natural History Entomology **16**: 1–908.

Groenteman, R., Fowler, S.V. and Sullivan, J.J. 2011. St. John's wort beetles would not have been introduced to New Zealand now: a retrospective host range test of New Zealand's most successful weed biocontrol agents. Biological Control **57**: 50–58.

Gullan, P.G. and Cranston, P.S. 2010. The insects: an outline of entomology.4th edition. Wiley-Blackwell. Oxford, UK. 560 p.

Gultekin, L. 2005. New ecological niche for weevils of the genus *Lixus* Fabricius and biology of *Lixus obesus* Petri (Coleoptera: Curculionidae, Lixinae). Weevil News **24**: 1–3.

Harker, K.N. and O'Donovan, J.T. 2013. Recent weed control, weed management, and integrated weed management. Weed Technology **27**: 1–11.

Harris, K.M. 1970. Coffee berry blister midge, *Contarinia coffeae* sp. n., a new pest from Uganda. Bulletin of Entomological Research **59 pt. 4**: 671–674.

Harris, P. 1971. Current approaches to biological control of weeds. *In* Biological Control Programs Against Insects and Weeds in Canada 1969–1980. *Edited by* J. S. Kelleher, M. A. Hulme. Commonwealth Agricultural Bureaux, Farnham Royal, UK. pp. 95–103.

Harris, P. 1979. Cost of biological control of weeds by insects in Canada. Weed Science **27**: 242–250.

Harris, P. and Zwolfer, H. 1968. Screening of phytophagous insects for biological control of weeds. Canadian Entomologist **100**: 295–303.

Hazlehurst, A.F., Weed, A.S., Tewksbury, L. and Casagrande, R.A. 2012. Host specificity of *Hypena opulenta*: a potential biological control agent of *Vincetoxicum* in North America. Environmental Entomology **41**: 841–848.

Henderson, C.E. and Rutz, D.A. 1991. Species composition of parasitoids attacking house flies, *Musca domestica* Linnaeus, in high-rise poultry farms in New York State, USA. Journal of Agricultural Entomology **8**:51–58.

Herr, J.C. 2000. Evaluating non-target effects: the thistle story. Center for Biological Control, College of Natural Resources, University of California, Berkeley, CA. pp. 12–17.

Herting, B. 1973. A Catalogue of Parasites and Predators of Terrestrial Arthropods.Section A. Host or prey/enemy. Volume 3 Coleoptera - Strepsiptera. Commonwealth Agricultural Bureaux., Farnham Royal, UK.

Herting, B. 1975. Lepidoptera, Part 1 (Microlepidoptera). A Catalogue of Parasites and Predators of Terrestrial Arthropods. Section A. Host or Prey/Enemy.6. Commonwealth Agricultural Bureaux., Farnham Royal, UK.

Herting, B. 1977. Hymenoptera. A Catalogue of Parasites and Predators of Terrestrial Arthropods. Section A. Host or Prey/Enemy. Commonwealth Agricultural Bureaux., Farnham Royal, UK.

Herting, B. 1978. Neuroptera, Diptera, Siphonaptera. A Catalogue of Parasites and Predators of Terrestrial Arthropods. Section A. Host or Prey/Enemy. Commonwealth Agricultural Bureaux., Farnham Royal, UK.

Hill, R. 1999. Minimising uncertainty in support of no choice tests. *In* Host specificity testing in Australasia: towards improved assays for biological control. *Edited by* T. M. Withers, L. Browne, J. Stanley. CRC for Tropical Pest Managment, Brisbane, Australia. pp. 1–10.

Hill, R.W., Wyse, G.A. and Anderson, M. 2004. Animal Physiology.2nd edition. Sinauer Associates Inc., Sunderland, MA. 770 p.

Hoffmann, J.H. and Moran, V.C. 1998. The population dynamics of an introduced tree, *Sesbania punicea*, in South Africa, in response to long-term damage caused by different combinations of three species of biological control agents. Oecologia **114**: 343–348.

Hokkanen, H. and Pimentel, D. 1984. New approaches for selecting biological control agents. Canadian Entomologist **116**: 1109–1121.
Hokkanen, H.M.T. and Pimentel, D. 1989. New associations in biological control theory and practice. Canadian Entomologist **121**: 829–840.
Hooker, J.D. 1885. Asclepiadeae. *In* The Flora of British India Volume 4. *Edited by* J. D. Hooker. L. Reeve, London, UK. pp. 1–78.
Hopkins, M.J.G. 1984. The parasite complex associated with stem boring *Apion* (Col., Curculionidae) feeding on *Rumex* species (Polygonaceae). Entomologist's Monthly Magazine **120**: 187–192.
Hopper, K.R. 1999. Risk spreading and bet hedging in insect populations biology. Annual Review of Entomology **44**: 535–560.
Horstmann, K. 2010. Revision of ichneumon fly species XIV (Hymenoptera: Ichneumonidae). Mitteilungen Muenchener Entomologischen Gesellschaft **100**: 119–130.
Howarth, F.G. 1983. Classical biocontrol: panacea or pandora's box? Proceedings of the Hawaiian Entomological Society **24**: 239–244.
Huber, J. 1998. The importance of voucher specimens, with practical guidelines for preserving specimens of the major invertebrate phyla for identification. Journal of Natural History **32**: 367–385.
Inderjit, S. 2005. Plant invasions: habitat invasibility and dominance of invasive plant species. Plant Soil **277**: 1–5.
Janzon, L.A. 1982. Description of the egg and larva of *Euphranta connexa* (Fabricius) (Diptera: Tephritidae) and of the egg of its parasitoid *Scambus brevicornis* (Gravenhorst) (Hymenoptera: Ichneumonoidea). Entomologica Scandinavica **13**: 313–316.
Judd, G.J.R., Whitfield, G.H. and Maw, H.E.L. 1991. Temperature dependent development and phenology of pepper maggots (Diptera: Tephritidae) associated with pepper and horsenettle. Environmental Entomology **20**: 22–29.
Kandybina, M.N. 1977. Larvae of fruit flies. Opredeliteli Po Faune SSSR, Leningrad: Nauka, Leningradskoe otd-nie. **114**: 212.
Katsoyannos, B.I., Boller, E.F. and Benz, G. 1987. Reproductive biology of *Rhagoletis cerasi* L. preoviposition period, daily rhythm of oviposition, and influence of mating, on fecundity and fertility of single or grouped females (Diptera: Tephritidae). Mitteilungen der Schweizerischen Entomologischen Gesellschaft **60**: 3–13.
Keane, R.M. and Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology and Evolution **17**: 164–170.
Kirby, D.R., Lym, R.G., Sterling, J.J. and Sieg, C.H. 2003. Observation: leafy spurge control in western prairie fringed orchid habitat. Journal of Range Management **56**: 466–473.
Kolar, C.S. and Lodge, D.M. 2001. Progress in invasion biology: predicting invaders. Trends in Ecology and Evolution **16**: 199–204.
Korner, C. 2007. The use of 'altitude' in ecological research. Trends in Ecology and Evolution **22**: 569–574.
Kronberg, S.L., Muntifering, R.B., Ayers, E.L. and Marlow, C.B. 1993. Cattle avoidance of leafy spurge: a case of conditioned aversion. Journal of Range Management: 364–366.

Lamb, R.J. 1992. Developmental rate of *Acyrthosiphon pisum* (Homoptera: Aphididae) at low temperatures implications for estimating rate parameters for insects. Environmental Entomology **21**: 10–19.
Landgraf, B.K., Fay, P.K. and Havstad, K.M. 1984. Utilization of leafy spurge (*Euphorbia esula*) by sheep. Weed Science **32**: 348–352.
Lavault, M., Richomme, P. and Bruneton, J. 1999. Acetophenones and new pregnane glycosides from the roots of *Vincetoxicum hirundinaria*. Fitoterapia **70**: 216–220.
Lawlor, F.M. 2000. Herbicidal Treatment of the Invasive Plant *Cynanchum rossicum* and Experimental Post Control Restoration of Infested Sites. MS Thesis. State University of New York, Syracuse, NY.
Lawlor, F.M. and Raynal, D.J. 2002. Response of swallow-wort to herbicides. Weed Science **50**: 179–185.
Leimu, R. 2004. Variation in the mating system of *Vincetoxicum hirundinaria* (Asclepiadaceae) in peripheral island populations. Annals of Botany **93**: 107–113.
Leimu, R. and Lehtilä, K. 2006. Effects of two types of herbivores on the population dynamics of a perennial herb. Basic and Applied Ecology **7**: 224–235.
Leimu, R. and Mutikainen, P. 2005. Population history, mating system, and fitness variation in a perennial herb with a fragmented distribution. Conservation Biology **19**: 349–356.
Leimu, R. and Syrjänen, K. 2002. Effects of population size, seed predation and plant size on male and female reproductive success in *Vincetoxicum hirundinaria* (Asclepiadaceae). Oikos **98**: 229–238.
Liede-Schumann, S., Kong, H., Meve, U. and Thiv, M. 2012. *Vincetoxicum* and *Tylophora* (Apocynaceae: Asclepiadoideae: Asclepiadeae) two sides of the same medal: independent shifts from tropical to temperate habitats. Taxon **61**: 803–825.
Liede, S. 1996. *Cynanchum*, *Rhodostegiella*, *Vincetoxicum, Tylophora* (Asclepiadaceae): new considerations on an old problem. Taxon **45**: 193–211.
Liu, X. and Ye, H. 2009. Effect of temperature on development and survival of *Bactrocera correcta* (Diptera: Tephritidae). Scientific Research and Essays **4**: 467–472.
Louda, S.M. 2000. Negative ecological effects of the musk thistle biological control agent, *Rhinocyllus conicus*. *In* Nontarget Effects of Biological Control. *Edited by* P. A. Follett, J. J. Duan. Kluwer Academic Publishers, Boston, MA. pp. 215–243.
Lumer, C. and Yost, S.E. 1995. The reproductive biology of *Vincetoxicum nigrum* (L.) Moench (Asclepiadaceae), a Mediterranean weed in New York State. Bulletin of the Torrey Botanical Club **122**: 15–23.
Lyons, L.A. 1970. Some population features of reproductive capacity in *Neodiprion swainei* (Hymenoptera: Diprionidae). Canadian Entomologist **102**: 68–84.
Mack, R. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications **10**: 689–710.
Magidow, L.C., DiTommaso, A., Ketterings, Q.M., Mohler, C.L. and Milbrath, L.R. 2013. Emergence and performance of two invasive swallowworts (*Vincetoxicum* spp.) in contrasting soil types and soil pH. Invasive Plant Science and Management **6**: 281–291.
Maguire, D., Sforza, R. and Smith, S.M. 2011. Impact of herbivory on performance of *Vincetoxicum* spp., invasive weeds in North America. Biological Invasions **13**: 1229–1240.

Markgraf, F. 1972. Asclepiadaceae. *In* Flora Europaea. *Edited by* T. G. Tutin, V. H. Heywood, N. A. Burges, D. H. Valentine, S. M. Walters, D. A. Webb. Cambridge University Press, Cambridge, UK. pp. 70–73.

Masner, L. 1993. Superfamily Platygasteroidea. *In* Hymenoptera of the World: An Identification Guide to Families. *Edited by* H. Goulet, J. Huber. Research Branch Agriculture Canada, Ottawa, ON. pp. 548–569.

McClay, A.S. 1996. Biological control in a cold climate: temperature responses and climatic adaptation of weed biocontrol agents. *In* Proceedings of the 9th International Symposium on Biological Control of Weeds, Stellenbosch, South Africa. *Edited by* V. C. Moran, J. H. Hoffmann. pp. 377–383.

McConnachie, A.J., de Wit, M.P., Hill, M.P. and Byrne, M.J. 2003. Economic evaluation of the successful biological control of *Azolla filiculoides* in South Africa. Biological Control **28**: 25–32.

McFadyen, R.E.C. 1998. Biological control of weeds. Annual Review of Entomology **43**: 369–393.

McFadyen, R.E.C. and Spafford-Jacob, H. 2003. Insects for the biocontrol of weeds: Predicting parasitism levels in the new country. *In* Proceedings of the 11th International Symposium on Biological Control of Weeds, Canberra, Australia. *Edited by* J. M. Cullen, D. T. Briese, D. J. Kriticos, W. M. Lonsdale, L. Morin, J. K. Scottpp. 135–140.

McFayden, R. 2008. Return on investment: determining the economic impact of biological control programmes. *In* Proceedings of the 12th International Symposium on the Biological Control of Weeds, Wallingford, United Kingdom: CAB International. *Edited by* M. Julien, R. Sforza, M. Bon, H. Evans, P. Hatcher, H. Hinz, B. Rector. pp. 67–74.

McKague, C.I. and Cappuccino, N. 2005. Response of pale swallow-wort, *Vincetoxicum rossicum*, following aboveground tissue loss: implications for the timing of mechanical control. Canadian Field-Naturalist **119**: 525–531.

Medvedev, G.S., Skarlato, O.A., Likharev, I.M. and Kryzhanovskii, O.L. 1995. Keys to the Insects of the European Part of the USSR. Volume 3. Hymenoptera. Part Four. Braconidae. [OpredeliteV Nasekomykh Evropeiskoi Chasti SSSR, Tom III, Pereponchatokrylye, Chetvertaia Chasf]. Baba Barkha Nath Printers, New Delhi, India.

Messenger, P.S. and van den Bosch, R. 1971. The adaptability of introduced biological control agents. *In* Theory and Practice of Biological Control. *Edited by* C. B. Huffaker, P. S. Messenger. Academic Press, New York, NY. pp. 68–92.

Messersmith, C.G. and Lym, R.G. 1983. Distribution and economic impacts of leafy spurge in North Dakota. North Dakota Farm Research **40**: 8–13.

MeteoSwiss. 2012a. Normes 1981–2010: Temperature de l`air a 2m. [online] Available from http://www.meteosuisse.admin.ch/files/kd/normwerte/norm8110/nvrep_tre200m0_fr.pdf.

MeteoSwiss. 2012b. Normes 1981–2010: Temperature moyenne maximale de l`air a 2m. [online] Available from http://www.meteosuisse.admin.ch/files/kd/normwerte/norm8110/nvrep_tre2dymx_fr.pdf

MeteoSwiss. 2012c. Normes 1981–2010: Temperature moyenne minimale de l`air a 2m. [online] Available from http://www.meteosuisse.admin.ch/files/kd/normwerte/norm8110/nvrep_tre2dymn_fr.pdf.

Milbrath, L.R. 2010. Phytophagous arthropods of invasive swallow-wort vines (*Vincetoxicum* spp.) in New York. Environmental Entomology **39**: 68–78.

Milbrath, L.R. and Biazzo, B.J. 2007. Proposed host specificity test plant list for testing potential biological control agents of swallow-worts. Unpublished report submitted to USDA-APHIS-PPQ. pp. 41.

Mitroiu, M. 2001. [Revision of the collections of Chalcidoidea Pteromalidae (Hymenoptera) of the Institut royal des Sciences naturelles de Belgique (Royal Institute of Natural Sciences of Belgium) and the discovery of 31 species that are new for Belgium.]. Bulletin de la Societe Royale Belge d'Entomologie **137**: 91–97.

Mlonyeni, X.O., Wingfield, B.D., Wingfield, M.J., Ahumada, R., Klasmer, P., Leal, I., de Groot, P. and Slippers, B. 2011. Extreme homozygosity in Southern Hemisphere populations of *Deladenus siricidicola*, a biological control agent of *Sirex noctilio*. Biological Control **59**: 348–353.

Mooney, H.A. and Cleland, E.E. 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences **98**: 5446–5451.

Moore, R.J. 1959. The dog-strangling vine *Cynanchum medium*, its chromosome number and its occurrence in Canada. Canadian Field-Naturalist **73**: 144–147.

Moraiti, C.A., Nakas, C.T. and Papadopoulos, N.T. 2012. Prolonged pupal dormancy is associated with significant fitness cost for adults of *Rhagoletis cerasi* (Diptera: Tephritidae). Journal of Insect Physiology **58**: 1128–1135.

Muola, A., Mutikainen, P., Laukkanen, L., Lilley, M. and Leimu, R. 2010a. Genetic variation in herbivore resistance and tolerance: the role of plant life-history stage and type of damage. Journal of Evolutionary Biology **23**: 2185–2196.

Muola, A., Mutikainen, P., Laukkanen, L., Lilley, M. and Leimu, R. 2011. The role of inbreeding and outbreeding in herbivore resistance and tolerance in *Vincetoxicum hirundinaria*. Annals of Botany **108**: 547–555.

Muola, A., Mutikainen, P., Lilley, M., Laukkanen, L., Salminen, J.P. and Leimu, R. 2010b. Associations of plant fitness, leaf chemistry, and damage suggest selection mosaic in plant-herbivore interactions. Ecology **91**: 2650–2659.

Myers, J.H., Jackson, C., Quinn, H., White, S.R. and Cory, J.S. 2009. Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada. Biological Control **50**: 66–72.

Narendran, T., David, B. and Selvaraj, P. 2005. A new species of *Aprostocetus* Westwood (Hymenoptera: Eulophidae) parasitic on *Melanagromyza obtusa* (Malloch)(Diptera: Agromyzidae) from India. Entomon **30**: 221–225.

Nazar, N., Goyder, D.J., Clarkson, J.J., Mahmood, T. and Chase, M.W. 2013. The taxonomy and systematics of Apocynaceae: where we stand in 2012. Botanical Journal of the Linnean Society **171**: 482–490.

Neilson, W.T.A. 1975. Fecundity of virgin and mated apple maggot (Diptera: Tephritidae) females confined with apple and black ceresin wax domes. Canadian Entomologist **107**: 909–911.

Norrbom, A.L. 1993a. New synonymy of *Epochra* Loew with *Euphranta* (*Racochlaena* Loew) (Diptera:Tephritidae) and description of a new species from Mexico. Proceedings of the Entomological Society of Washington **95**: 189–194.
Norrbom, A.L. 1993b. New synonymy of *Epochra* with *Euphranta* (Rhacochlaena Loew) (Diptera: Tephritidae) and description of a new species from Mexico. Proceedings of the Entomological Society of Washington **95**: 189–194.
Noyes, J.S. 1994. The reliability of published host-parasitoid records: a taxonomist's view. Norwegian Journal of Agricultural Sciences **Supplement No. 16**: 59–69.
Noyes, J.S. 2013. Universal Chalcidoidea Database. [online] Available from http://www.nhm.ac.uk/chalcidoids.
Ortega, Y.K., Pearson, D.E. and McKelvey, K.S. 2004. Effects of biological control agents and exotic plant invasion on deer mouse populations. Ecological Applications **14**: 241–253.
Ovruski, S., Aluja, M., Sivinski, J. and Wharton, R. 2000. Hymenopteran parasitoids on fruit-infesting Tephritidae (Diptera) in Latin America and the southern United States: diversity, distribution, taxonomic status and their use in fruit fly biological control. Integrated Pest Management Reviews **5**: 81–107.
Pagola-Carte, S. and Peydro, R.J. 2012. *Euphranta* (*Euphranta*) *connexa* (Fabricius, 1794) (Diptera: Tephritidae): First record for the Iberian Peninsula with data on its biology. Heteropterus Revista de Entomologia **12**: 129–134.
Pailer, M. and Streiche.W. 1965. Alkaloide aus *Vincetoxicum officinale* Moench. (Gemeine Schwalbenwurz). Monatshefte Fur Chemie Und Verwandte Teile Anderer Wissenschaften **96**: 1094–1102.
Papp, J. 2012. A revision of the *Bracon* Fabricius species in Wesmael's collection deposited in Brussels (Hymenoptera: Braconidae: Braconinae). European Journal of Taxonomy **21**: 1–154.
Paynter, Q., Fowler, S.V., Hugh Gourlay, A., Groenteman, R., Peterson, P.G., Smith, L. and Winks, C.J. 2010. Predicting parasitoid accumulation on biological control agents of weeds. Journal of Applied Ecology **47**: 575–582.
Pearson, D.E. and Callaway, R.M. 2006. Biological control agents elevate hantavirus by subsidizing deer mouse populations. Ecology Letters **9**: 443–450.
Pemberton, R.W. 1995. *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in the United States: an immigrant biological control agent or an introduction of the nursery industry? American Entomologist **41**: 230–232.
Perkins, J.F. 1943. Preliminary notes on the synonymy of the European species of the *Ephialtes*-complex (Hym.), Ichneumonidae. Annals and Magazine of Natural History **10**: 249–273.
Petrova, A.S. 2010. First records of *Vincetoxicum nigrum* and *Avena byzantina* in Bulgaria. Phytologia Balcanica **16**: 75–78.
Pimentel, D. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. Agriculture, Ecosystems and Environment **84**: 1–20.
Pimentel, D., Zuniga, R. and Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics **52**: 273–288.
Pringle, J.S. 1973. The spread of *Vincetoxicum* species (Asclepiadaceae) in Ontario. Canadian Field-Naturalist **87**: 27–33.

Pyšek, P. and Richardson, D.M. 2010. Invasive species, environmental change and managment and health. Annual Review of Environment and Resources **35**: 25–55.
Raspi, A. and Canale, A. 2000. Effect of superparasitism on *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae) second instar larvae by *Psyttalia concolo*r (Szepligeti) (Hymenoptera Braconidae). Redia **83**: 123–131.
Rejmánek, M. 2000. Invasive plants: approaches and predictions. Austral Ecology **25**: 497–506.
Ricalde, M.P., Nava, D.E., Loeck, A.E. and Donatti, M.G. 2012. Temperature-dependent development and survival of Brazilian populations of the Mediterranean fruit fly, *Ceratitis capitata*, from tropical, subtropical and temperate regions. Journal of Insect Science. **12**: 1–11.
Richardson, D.M., Pyšek, P., Rejmánek, M., Barbour, M.G., Panetta, F.D. and West, C.J. 2000. Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions **6**: 93–107.
Robinson, B.L. and Fernald, M.L. 1908. Gray's New Manual of Botany. American Book Company, New York, NY.
Room, P.M., Harley, K.L.S., Forno, I.W. and Sands, D.P.A. 1981. Successful biological-control of the floating weed *Salvinia*. Nature **294**: 78–80.
Rose, R., Weeks, R. and Usnick, S. 2011. Cactus moth, *Cactoblastis cactorum* 2011 survey plan for PPQ and State Cooperators. . USDA-APHIS Plant Health, Plant Protection and Quarantine, Washington, DC. pp. 1–18.
Schaffner, U. 2001. Host range testing of insects for biological weed control: how can it be better interpreted? BioScience **51**: 951–959.
Schooler, S., Baron, Z. and Julien, M. 2006. Effect of simulated and actual herbivory on alligator weed, *Alternanthera philoxeroides*, growth and reproduction. Biological Control **36**: 74–79.
Seguy, E. 1934. Faune de France. 28. Dipteres (Brachyceres) (Muscidae Acalypterae et Scatophagidae). Paris Lechevalier **1934**: 27.
Selleck, G.W., Frankton, C. and Coupland, R.T. 1962. Leafy spurge in Saskatchewan. Ecological Monographs **32**: 1–45.
Senger, S.E., Roitberg, B.D. and Thistlewood, H.M.A. 2007. Relative flight responses of *Rhagoletis indifferens* as influenced by crowding, sex, and resources. Entomologia Experimentalis et Applicata **123**: 91–100.
Senger, S.E., Roitberg, B.D. and Thistlewood, H.M.A. 2008. Ovarian response to resource availability in female *Rhagoletis indifferens*. Entomologia Experimentalis et Applicata **129**: 26–31.
Serbesoff-King, K. 2003. *Melaleuca* in Florida: a literature review on the taxonomy, distribution, biology, ecology, economic importance and control measures. Journal of Aquatic Plant Management **41**: 98–112.
Severin, H.P. 1917. Life history, habits, natural enemies and methods of control of the currant fruit–fly (*Epochra canadenbis* Loew). Bulletin Maine Experimental Statation Technical Bulletin **264**: (177–247).
Sexton, J.P., Sala, A. and Murray, K. 2006. Occurrence, persistence, and expansion of saltcedar (*Tamarix* spp.) populations in the great plains of Montana. Western North American Naturalist **66**: 1–11.

Sheeley, S.E. 1992. Life History and Distribution of *Vincetoxicum rossicum* (Asclepiadaceae): An Exotic Plant in North America. MS Thesis. Syracuse University of New York, NY.

Sheeley, S.E. and Raynal, D.J. 1996. The distribution and status of species of *Vincetoxicum* in Eastern North America. Bulletin of the Torrey Botanical Club **123**: 148–156.

Sheppard, A.W. 2003. Prioritising agents based on predicted efficacy: beyond the lottery approach. *In* Improving the Selection, Testing and Evaluation of Weed Biological Control Agents. *Edited by* H. Spafford Jacob, D. T. Briese. CRC for Australian Weed Management, Glen Osmond, Australia. pp. 11–21.

Sheppard, A.W., van Klinken, R.D. and Heard, T.A. 2005. Scientific advances in the analysis of direct risks of weed biological control agents to nontarget plants. Biological Control **35**: 215–226.

Silva, C.C.A., Laumann, R.A., Barbosa Cavalcante Ferreira, J., Blassioli Moraes, M.C., Borges, M. and Cokl, A. 2012. Reproductive biology, mating behavior, and vibratory communication of the brown-winged stink bug, *Edessa meditabunda* (Fabr.) (Heteroptera: Pentatomidae). Psyche **2012**: 1–9.

Simberloff, D. 1996. How risky is biological control? Ecology **77**: 1965–1974.

Simberloff, D. 2005. Non-native species do threaten the natural environment! Journal of Agricultural and Environmental Ethics **18**: 595–607.

Sime, K.R., Daane, K.M., Messing, R.H. and Johnson, M.W. 2006. Comparison of two laboratory cultures of *Psyttalia concolor* (Hymenoptera: Braconidae), as a parasitoid of the olive fruit fly. Biological Control **39**: 248–255.

Simova-Toi, D. and Dobrivojevi, K. 1966. The raspberry stem gall midge (*Lasioptera rubi*) a little known raspberry pest. Zast Bilja **17**: 323–330.

Sivinski, J., Aluja, M., Dodson, G., Freidberg, A., Headrick, D.H., Kaneshiro, D.K. and Landolt, P. 2000. Topics in evolution of sexual behaviour in the Tephritidae. *In* Fruit Flies (Tephritidae): Phylogeny and the Evolution of Behavior. *Edited by* M. Aluja, A. L. Norrbom. CRC Press, Florida. pp. 751–792.

Sivinski, J.M. and Calkins, C.O. 1990. Sexually dimorphic developmental rates in the Caribbean fruit-fly (Diptera: Tephritidae). Environmental Entomology **19**: 1491–1495.

Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods 6th edition.6. Iowa State, Ames, IA.

Solbreck, C. 2000. Ecology and biology of *Euphranta connexa* (Fabr.) (Diptera: Tephritidae) a seed predator on *Vincetoxicum hirundinaria* Med. (Asclepiadaceae). Entomologisk Tidskrift **121**: 23–30.

Solbreck, C. and Ives, A.R. 2007. Density dependence vs. independence, and irregular population dynamics of a swallow-wort fruit fly. Ecology **88**: 1466–1475.

Solbreck, C. and Kugelberg, O. 1972. Field observations on the seasonal occurrence of *Lygaeus equestris* (L.) (Het., Lygaeidae) with special reference to food plant phenology. Insect Systematics and Evolution **3**: 189–210.

Solbreck, C. and Sillén-Tullberg, B. 1986a. The role of variable weather for the dynamics of a seed-seed predator system. Oecologia **71**: 59–62.

Solbreck, C. and Sillén-Tullberg, B. 1986b. Seed production and seed predation in a patchy and time-varying environment. Dynamics of a milkweed — tephritid fly system. Oecologia **71**: 51–58.

Solbreck, C. and Sillén-Tullberg, B. 1990. Population dynamics of a seed feeding bug, *Lygaeus equestris*. 1. Habitat patch structure and spatial dynamics. Oikos **58**: 199–209.

Spencer, L. and Whitfield, J.B. 1999. Revision of the nearctic species of *Rhysipolis* Forster (Hymenoptera : Braconidae). Transactions of the American Entomological Society **125**: 295–324.

St Denis, M. and Cappuccino, N. 2004. Reproductive biology of *Vincetoxicum rossicum* (Kleo.) Barb. (Asclepiadaceae), an invasive alien in Ontario. Journal of the Torrey Botanical Society **131**: 8–15.

Stiling, P. 1993. Why do natural enemies fail in classical biological control programs? American Entomologist **39**: 31–37.

Stinson, K., Kaufman, S., Durbin, L. and Lowenstein, F. 2007. Impacts of garlic mustard invasion on a forest understory community. Northeastern Naturalist **14**: 73–88.

Stinson, K.A., Campbell, S.A., Powell, J.R., Wolfe, B.E., Callaway, R.M., Thelen, G.C., Hallett, S.G., Prati, D. and Klironomos, J.N. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biology **4**: 140.

Streito, J.C. and Nibouche, S. 1997. First observations on the parasitoids associated with lepidopterous pests of cotton in Burkina Faso. Entomophaga **42**: 543–557.

Sullivan, C.R. and Wallace, D.R. 1967. Interaction of temperature and photoperiod in induction of prolonged diapause in *Neodiprion sertifer*. Canadian Entomologist **99**: 834–850.

Systat. 2009. Systat 13 Statistics. Systat Software Inc., Chicago, IL.

Taylor, F. 1981. Ecology and evolution of physiological time in insects. American Naturalist **117**: 1–23.

Tewksbury, L., Casagrande, R.A. and Gassmann, A. 2002. Swallow-worts. *In* Biological Control of Invasive Plants in the Eastern United States *Edited by* R. van Driesche, B. Blossey, M. Hoddle, S. Lyon, R. Reardon. U.S. Forest Service-FHTET-2002-04, Morgantown, WV. pp. 209–216.

Thomas, M.B. and Reid, A.M. 2007. Are exotic natural enemies an effective way of controlling invasive plants? Trends in Ecology and Evolution **22**: 447–453.

Thompson, J.D. 1991. The biology of an invasive plant. BioScience **41**: 393–401.

Timonin, A.K. and Savitskii, M.Y. 1997. Pollination efficiency of *Vincetoxicum hirundinaria* (Asclepiadaceae s. str.). Botanicheskii Zhurnal **82**: 45–52.

Tóth, P. and Lukas, J. 2005. Parasitic Ichneumonoidea on the horse chestnut leaf miner, *Cameraria ohridella* (Lepidoptera : Gracillariidae) in Slovakia. Journal of Pest Science **78**: 151–154.

USDA-APHIS. 1998. Reviewer`s Manual for the Technical Advisory Group for Biological Control Agents of Weeds: Guidelines for Evaluating the Safety of Candidate Biological Control Agents. *Edited by* USDA-APHIS-PPQ. Marketing and Regulatory Programs.

USDA and NRCS. 2011. The PLANTS Database, Version 3.5 [online] Available from http://plants.usda.gov [accessed 20 August 2013].

Valéry, L., Fritz, H., Lefeuvre, J.-C. and Simberloff, D. 2008. In search of a real definition of the biological invasion phenomenon itself. Biological Invasions **10**: 1345–1351.

van Achterberg, C. 1988. The genera of the *Aspilota*-group and some descriptions of fungicolous *Alysiini* from the Netherlands. (Hymenoptera: Braconidae: Alysiinae). Zoologische Verhandelingen (Leiden): 3–88.
van Driesche, R., Hoddle, M. and Center, T. 2008. Control of Pests and Weeds by Natural Enemies: An Introduction to Biological Control. Blackwell Publishing, Malden, MA. pp. 473.
van Driesche, R.G. 1983. Meaning of "percent parasitism" in studies of insect parasitoids. Environmental Entomology **12**: 1611–1622.
van Klinken, R.D. 1999. Host specificity testing: why do we do it and how we can do it better. *In* Proceedings of Session: Host Specificity Testing of Exotic Arthropod Biological Control Agents: The Biological Basis for Improvement in Safety 10th International Symposium on Biological Control of Weeds, Bozeman, Montana. *Edited by* R. Van Driesche, T. A. Heard, A. S. McClay, R. Reardon. pp. 54–68.
van Klinken, R.D. and Raghu, S. 2006. A scientific approach to agent selection. Australian Journal of Entomology **45**: 253–258.
van Wilgen, B., Moran, V. and Hoffmann, J. 2013. Some perspectives on the risks and benefits of biological control of invasive alien plants in the management of natural ecosystems. Environmental Management **52**: 531–540.
Vidal, S. 1997. Determination list of entomophagous insects nr. 13. IOBC-WPRS Bulletin **20**: 1–53.
Vlug, H.J. 1995. Catalogue of the Platygastridae (Platygastroidea) of the World (Insecta: Hymenoptera). SPB Academy, Amsterdam, The Netherlands.
von Hausner, G. 1976. Embryogenese und nucellar-polyembryonie bie *Cynanchum - Vincetoxicum*. Arten Beitrage Biologie Pflanzen **52**: 101–126.
Wagner, D.L. and van Driesche, R.G. 2010. Threats posed to rare or endangered insects by invasions of nonnative species. Annual Review of Entomology **55**: 547–568.
Walker, J.W., Kronberg, S.L., Alrowaily, S.L. and West, N.E. 1994. Comparison of sheep and goat preferences for leafy spurge. Journal of Range Management **47**: 429–434.
Wang, S. and Yue, S. 1995. New parasitical ichneumonids on *Pissodes validrostris* Sahlberg in China (Hymenoptera: Ichneumonidae, Pimplinae). Acta Entomologica Sinica **38**: 228–233.
Wang, X.-G., Johnson, M.W., Yokoyama, V.Y., Pickett, C.H. and Daane, K.M. 2011. Comparative evaluation of two olive fruit fly parasitoids under varying abiotic conditions. Biocontrol **56**: 283–293.
Wapshere, A.J. 1974. A comparison of strategies for screening biological control organisms for weeds. Commonwealth Institute of Biological Control Miscellaneous Publication **6**: 151–160.
Wapshere, A.J. 1985. Effectiveness of biological control agents for weeds: present quandaries. Agriculture, Ecosystems and Environment **13**: 261–280.
Wapshere, A.J. 1989. A testing sequence for reducing rejection of potential biological-control agents for weeds. Annals of Applied Biology **114**: 515–526.
Waterhouse, D.F. 1998. Foreword. *In* Biological Control of Weeds: World Catalogue of Agents and Their Target Weeds. *Edited by* M. H. Julien, M. W. Griffiths. CAB International, Wallingford, UK.

Weed, A.S. 2010. Biology and Ecology of European Natural Enemies of Swallow-worts (*Vincetoxicum*) and the Potential for Biological Control. Ph.D. Dissertation, University of Rhode Island, Kingston, RI.

Weed, A.S. and Casagrande, R. 2010. Biology and larval feeding impact of *Hypena opulenta* (Christoph)(Lepidoptera: Noctuidae): a potential biological control agent for *Vincetoxicum nigrum* and *V. rossicum*. Biological Control **53**: 214–222.

Weed, A.S. and Casagrande, R.A. 2011. Evaluation of host range and larval feeding impact of *Chrysolina aurichalcea asclepiadis* (Villa): considerations for biological control of *Vincetoxicum* in North America. Environmental Entomology **40**: 1427–1436.

Weed, A.S. and Gassmann, A. 2007. Evaluating the potential for biological control of swallow-worts, *Vincetoxicum rossicum* and *V. nigrum*. *In* Annual Report 2006 CABI Europe - Switzerland, Delemont, Switzerland. pp. 18.

Weed, A.S., Gassmann, A. and Casagrande, R.A. 2011a. Effects of leaf and root herbivory by potential insect biological control agents on the performance of invasive *Vincetoxicum* spp. Biological Control **56**: 50–58.

Weed, A.S., Gassmann, A., Leroux, A.M. and Casagrande, R.A. 2011b. Performance of potential European biological control agents of *Vincetoxicum* spp. with notes on their distribution. Journal of Applied Entomology **135**: 700–713.

Weston, L.A., Barney, J.N. and DiTommaso, A. 2005. A review of the biology and ecology of three invasive perennials in New York State: Japanese knotweed (*Polygonum cuspidatum*), mugwort (*Artemisia vulgaris*) and pale swallow-wort (*Vincetoxicum rossicum*). Plant and Soil **277**: 53–69.

Wharton, R.A. and Yoder, M.J. 2013. Parasitoids of Fruit-Infesting Tephritidae. [online] Available from http://paroffit.org/public/site/paroffit/home [accessed 1 December 2013, 2013].

White, I.M. and Elson-Harris, M.M. 1992. Fruit Flies of Economic Significance: Their Identification and Bionomics. CAB International, Wallingford, UK.

Widenfalk, O., Gyllenstrand, N., Sylven, E. and Solbreck, C. 2002. Identity and phylogenetic status of two sibling gall midge species (Diptera: Cecidomyiidae: *Contarinia*) on the perennial herb *Vincetoxicum hirundinaria*. Systematic Entomology **27**: 519–528.

Wingsanoi, A. and Siri, N. 2012. The oviposition of the chili fruit fly (*Bactrocera latifrons* Hendel) (Diptera: Tephritidae) with reference to reproductive capacity. Songklanakarin Journal of Science and Tehcnology **34**: 475–478.

Xiao, H. and Huang, D.W. 1999. A taxonomic study of *Stenomalina* (Hym.: Pteromalidae) from China. Acta Zootaxonomica Sinica **24**: 336.

Yokoyama, V.Y., Renden, P.A. and Sivinski, J. 2008. *Psyttalia cf. concolor* (Hymenoptera: Braconidae) for biological control of olive fruit fly (Diptera: Tephritidae) in California. Environmental Entomology **37**: 764–773.

Young, J.A. and Longland, W.S. 1996. Impact of alien plants on Grant [Sic] Basin rangelands. Weed Technology **10**: 384–391.

Zamora D.L. and Thill, D.C. 1999. Early detection and eradication of new weed infestations. *In* Biology and Management of Noxious Rangeland Weeds. *Edited by* R.L. Sheley and J.K. Petroff.Oregon State University Press, Corvallis, OR. pp. 73–84.

Zavaleta, E.S. 2000. Valuing ecosystem services lost to *Tamarix* invasion in the United

States. *In* Invasive Species in a Changing World. *Edited by* H.A.,Mooney and R.J. Hobbs. Island Press. Washington, DC. pp 261–300.
Zhao, L.-Q. and Zhu, D.-H. 2011. Effect of mating status on the fecundity of a cricket, *Teleogryllus emma*. Insect Science **18**: 320–324.