HOST RANGE AND MULTITROPHIC INTERACTIONS BETWEEN THE PARASITOID *COTESIA VANESSAE* (HYMENOPTERA: BRACONIDAE) AND NOCTUIDAE (LEPIDOPTERA) HOSTS IN NORTH AMERICA

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To my parents and my wife,

for having always supported my passion for entomology

ABSTRACT

Caterpillars in the family Noctuidae are important crop pests. After discovering the parasitoid *Cotesia vanessae* in the Nearctic region, which was previously only known from the Palearctic and Afrotropic regions, I investigated its fundamental host range on North American Lepidoptera and found that it could parasitize a large number of species of Noctuidae. Its fitness varied among host species, and Plusiinae species appeared to be the best hosts. Nearly all hosts in this experiment were reared on McMorran diet, which appeared to have varying suitability among Lepidoptera species. Through a second experiment, I showed that the fitness of the parasitoid and that of its host varied according to host food quality. Thus, parasitoid fitness on different hosts is partly conditioned by diet quality. This study shows that *C. vanessae* can parasitize a large number of Noctuidae and can be easily mass-reared, which could make it an ideal biological control agent.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAFC: Agriculture and Agri-Food Canada
AB: Alberta
AK: Alaska
AL: Alabama
ANCOVA: Analysis of Covariance
ANOVA: Analysis of variance
AR: Arkansas
AZ: Arizona
BC: British Columbia
BOLD: Barcode of Life Data Systems
CA: California
CAD: Canadian Dollar
CO: Colorado
CO1: Cytochrome c oxidase subunit 1
CT: Connecticut
DC: District of Columbia
DNA: Desoxyribonucleic Acid
FL: Florida
GA: Georgia
HI: Hawaii
HSD: Honest Significant Difference
IA: Iowa
ID: Idaho
IL: Illinois
IN: Indiana
IPS: Insect Production Services
IQR: Interquartile Range
K2P: Kimura 2-Parameter distance
KOH: Potassium hydroxide
KS: Kansas
KY: Kentucky
L:D: Light:Dark cycle
LA: Louisiana
MA: Massachusetts
MB: Manitoba
MD: Maryland
ME: Maine
MI: Michigan
MN: Minnesota
MO: Missouri
MS: Mississippi
MT: Montana
NB: New Brunswick
NC: North Carolina
ND: North Dakota
NE: Nebraska
NH: New Hampshire
NJ: New Jersey
NL: Newfoundland and Labrador
NM: New Mexico
NS: Nova Scotia
NT: Northwest Territories
NU: Nunavut
NY: New York
OH: Ohio
OK: Oklahoma
ON: Ontario
OR: Oregon
PA: Pennsylvania
PE: Prince Edward Island
Q1: 1st quartile
Q3: 3rd quartile
QC: Quebec
RI: Rhode Island
SC: South Carolina
SD: South Dakota
SE: Standard error of the mean
SK: Saskatchewan
Sp.: species (singular)
Spp.: species (plural)
TN: Tennessee
TX: Texas
US: United States
USA: United States of America
USD: American Dollar
USDA: United States Department of Agriculture
UT: Utah
UV: Ultraviolet
VA: Virginia
VT: Vermont
WCCP: Western Committee on Crop Pests
WA: Washington
WI: Wisconsin
WV: West Virginia
YT: Yukon

CHAPTER 1: GENERAL INTRODUCTION

1.1. Context

Larvae of many species of moths in the family Noctuidae are important pests of cultivated plants in North America and elsewhere in the world. For example, the pale western cutworm, *Agrotis orthogonia* Morrison, caused an average annual loss of over 2-million USD in 1920 and 1921 in Montana (Mabee, 1929), $4.7-million (likely CAD) annually between 1929 and 1932 in the prairies of Canada (McMillan, 1935), and 17-million USD annually between 1955 to 1975 in eight US states combined (Idaho, Montana, South Dakota, Wyoming, Nebraska, Colorado, Kansas, and Oklahoma). From about 1955 to 1975, the army cutworm, *Euxoa auxiliaris* (Grote), caused an annual loss of nearly 8-million USD in four US states combined (Montana, South Dakota, Colorado, and Oklahoma) (USDA, 1977); and in 1990 in southern Alberta, over 10,000 ha had to be either sprayed with insecticide or reseeded due to damage from this pest (Jones et al., 1990). In recent years, the cotton bollworm, *Helicoverpa armigera* (Hübner), caused an estimated annual worldwide loss of 3-billion USD (Sharma et al., 2014). Cutworms have been an enduring problem on the Canadian prairies for over a century (Jacobson, 1969, 1971; Jacobson et al., 1961; Strickland, 1916, 1923). In recent years, particularly damaging outbreaks have renewed interest in these pests (WCCP, 2012, 2013), which was the stimulus for initiating this thesis research.

Larvae of these pests often go by common names such as cutworms, armyworms and loopers, according to the life history of the larva, and dart moth as adults. While armyworms and loopers are usually a problem on mature plants, as they feed on developing fruiting bodies, cutworms are mainly a problem in the spring when crops start to grow. Cutworms can be classified as subterranean, above-ground, or climbing species (Walkden, 1950). Subterranean species feed exclusively on the small part of the stem below the ground; thus, a single cutworm can destroy numerous individual plants. Above-ground species are mainly foliage feeders, feeding primarily at dawn and dusk, and hide in the soil and under debris during the day, and late instars can cut the stems of seedlings. Climbing cutworms, armyworms, and loopers usually remain on the crown of plants where they feed primarily on the foliage and fruiting bodies. Armyworms have a tendency to congregate and move together "as an army" from one area to a next as plants become consumed (Capinera, 2001; Walkden, 1950). Loopers move forward by drawing their posterior end toward their anterior end, thus forming a partial loop. Loopers are also called "semi-loopers" to avoid confusion with caterpillars of the family Geometridae that make a full loop with their body. In this thesis, Geometridae are not dealt with and for practicality the term "looper" is used when referring to noctuid species in the subfamily Plusiinae.

The abundance of cutworms and their natural enemies, both in time and in space, is largely conditioned by biotic and abiotic conditions. Cutworms can generally be found at low densities throughout their range, but during outbreaks they can completely destroy a crop (personal observation). Specific conditions during oviposition time, mainly of soil texture, structure, humidity, and cover, drive cutworm moths to oviposit in certain fields rather than others; e.g., *Euxoa* spp., *Agrotis orthogonia*, and *Agrotis segetum* (Denis & Schiffermüller) prefer to lay eggs in dry loose sandy or dusty soil; thus fields tilled in late summer to early fall are of higher risk of cutworm outbreaks in the spring, and outbreaks are often composed of multiple species (Esbjerg et al., 2009; Jacobson, 1971; Jacobson et al., 1961; Wagner et al., 2012). In some species, spring rains contribute to the mortality of cutworm larvae; e.g., wet conditions increase mortality rates of *A. segetum* and *A. orthogonia* (Cook, 1926; Esbjerg et al., 2009; Seamans, 1935). Cutworm outbreaks will in turn induce a build-up of the populations of their natural enemies that will then slowly reduce pest populations (Jacobson, 1971). Moist spring conditions have been reported to be detrimental to cutworms by increasing incidence of parasitism and disease (Jacobson, 1971; Seamans, 1935). Prolonged severe winds also may be detrimental to parasitoids (Strickland, 1923). These conditions generally drive important outbreaks to occur episodically. Cutworm outbreaks can be prevented, reduced, or stopped by agronomic practices, chemical control, and naturally occurring biological control agents (Jacobson, 1971; Strickland, 1916). The latter, also referred to as “natural enemies” include pathogens, predators, and parasitoids (Capinera, 2001; Jacobson, 1971). Many species of natural enemies have been reported to kill cutworm pest species in North America, including pests of particular economic importance such as the redbacked cutworm, *Euxoa ochrogaster* (Guenée), darksided cutworm, *Euxoa messoria* (Harris), and army cutworm, *Euxoa auxiliaris* (Grote) (Lepidoptera: Noctuidae) (Tables 1.1, 1.2, 1.3) (Gavloski et al., 2013). Parasitoids have long been identified as important naturally occurring biological control agents of cutworms in the prairies of Canada (Jacobson, 1971; Strickland, 1923).

The term “parasitoid” was coined in 1913 in German by O. M. Reuter and in 1914 in English by W. M. Wheeler (Wheeler, 1914) to define parasitic insects that, unlike parasites, only use a single host throughout their larval stage, that always end up killing the host, and whose adults are free living. The parasitoid lifestyle has evolved independently multiple times in the insect orders Hymenoptera, Diptera, Coleoptera, Neuroptera, Lepidoptera, and Trichoptera (Godfray, 1994). Most hymenopteran parasitoids use their ovipositor to insert eggs within the body of their host. During oviposition, parasitoids may inject other substances into the host, such as venom, calyx fluid, and viruses, which can interfere with the development of the host and combat its immune system to aid the development of their offspring (Lanzrein et al., 2012). Knowledge of host-parasitoid interactions can guide use of parasitoids in different types of biocontrol programs targeting pest species. Common biological control strategies include innundative (flooding the environment with biological control agents; a strategy mainly used in greenhouses), classical (introducing an exotic biological control agent to control an exotic invasive pest), neoclassical (introducing a foreign biological control agent to control a native pest), and conservation (preservation or modification of the environment to enhance populations of biological control agents) programs (Bellows et al., 1999; van den Bosch et al., 1982).

Multitrophic interactions (in this case between plant-host and host-parasitoids) drive host suitability and fitness of parasitoids. To locate suitable hosts, parasitoids rely on cues produced by the environment that their hosts live in, and many studies have found that parasitoids of herbivorous insects are attracted to herbivore-induced plant volatiles. For example, *Cotesia rubecula* (Marshall) and *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) are attracted to volatiles produced by damaged leaves of cabbage, *Brassica oleracea* L. (Brassicaceae) (Agelopoulos et al., 1994; Mattiacci et al., 1994, 1995; Steinberg et al., 1993), and *Cotesia marginiventris* (Cresson) is attracted to corn, *Zea mays* L. (Poaceae), damaged by *Spodoptera exigua* (Hübner) larvae (Turlings et al., 1990). In addition, parasitoid fitness can be affected when hosts feed on poor quality diet, either because of the presence of plant defensive secondary plant compounds such as tannins, terpenes, flavones, phenols, and glucosinolates (Agrawal et al., 2015; Barbosa, et al. 1986; Gols et al., 2008a, 2008b, 2008c; Harvey 2005; Harvey et al., 2007, 2011; Ode 2006; Rhoades et al., 1976; Soler et al., 2005), or because the diet is nutritionally unbalanced (Bloem et al., 1990; Pimentel, 1966).

The fitness of parasitoids can be assessed by measuring different parameters. These can include development time, survival rate, adult longevity, fertility, fecundity, body size, body mass, efficiency in locating hosts, host patch-exploitation strategy, offspring sex ratio, and offspring performance (Boivin et al., 2004; Harvey, 2005; Roitberg et al., 2001; Vos et al., 2003). Because of limited time and resources, normally only a subset of these parameters can be estimated experimentally. Body size and mass are the most commonly used proxies of fitness because their increase is often positively correlated with longevity and fecundity (Harvey, 2005; Roitberg et al., 2001). However, a too large increase in one parameter can result in a decrease of other parameters of fitness; e.g., too large body size can result in increased predation (West et al., 1996). Hence, multiple parameters should be measured to increase the assessment accuracy of the fitness of individuals (Roitberg et al., 2001).

1.2. Goals of the study

The current research was initiated following the discovery in the Nearctic region of the parasitoid *Cotesia vanessae* (Reinhard) (Braconidae) (Chapter 2). This discovery arose from recovery of *C. vanessae* from the pest species tomato looper, *Chrysodeixis chalcites* (Esper), and cabbage looper, *Trichoplusia ni* (Hübner), collected in greenhouses and fields in southern Ontario, Canada (Hervet et al., 2014). This parasitoid species was previously only known from the Palearctic and parts of the Afrotropic regions where it has been reared from a number of Noctuidae species. In fact, it has been reported from 33 species in seven families (Yu et al., 2016), but some of these reports are considered doubtful (Hervet et al., 2014). Preliminary laboratory observations showed that this species was able to parasitize North American noctuid pests, which provided rationale for the study of *C. vanessae* as a potential control agent for cutworms (Chapter 3), and its mass-rearing in the lab for potential future redistribution programs.

For this study, nearly all Lepidoptera species were reared on an artificial diet known as McMorran diet (McMorran, 1965), using the recipe modified by Grisdale (1973). This artificial diet has been used by previous authors to rear a number of Lepidoptera species but it has not previously been used for the rearing of most of the species reared in the Chapter 3 study. Finding a suitable diet for the rearing of insects in the laboratory can greatly ease and enhance investigations of a species. For this reason, I report the species that I was able to rear on McMorran diet and species that are mentioned to have been reared on this diet in the literature (Chapter 4).

The McMorran diet may not be equally suitable for all species, even if they complete development; e.g., some individuals had deformed wings, which can be indicative of a nutritionally unbalanced diet (Morris, 1967). Unbalanced diet for a caterpillar can affect the fitness of its parasitoid (Bloem et al., 1990; Pimentel, 1966). Thus, the diet used may confound parasitoid performance on different host species. One of the most important and often limiting elements in the diet of phytophagous insects is nitrogen (Harrison et al., 2012). To investigate how an unbalanced diet affects the fitness of a caterpillar and that of its parasitoid, in Chapter 5 I investigated the fitness of a caterpillar, *Trichoplusia ni*, and that of its parasitoid, *C. vanessae*, when the host was reared on artificial diets containing different concentrations of protein.

Except for the General Introduction and General Discussion (Chapters 1 & 6), each chapter is written as a stand-alone manuscript intended to be published, or already published, as individual articles. The tables in Chapter 1 are published in Gavloski et al. (2013), Chapter 2 is published as Hervet et al. (2014), and Chapter 4 is published as Hervet et al. (2016b). An appendix (Appendix 1) summarizes some observations of the life history of *C. vanessae* that have not previously been reported.

Table 1.1. Parasitoids of army cutworm, *Euxoa auxiliaris*, darksided cutworm, *Euxoa messoria*, and redbacked cutworm*, Euxoa ochrogaster* (published in Gavloski et al., 2013).

Parasitoid species	**Known distribution in North America**	***Euxoa auxiliaris***	***Euxoa messoria***	***Euxoa ochrogaster***
INSECTA				
Diptera				
Bombyliidae				
Poecilanthrax halcyon (Say)	Canada: SK			(King et al., 1928)
Poecilanthrax willistoni (Coquillet)	Canada: SK			(King et al., 1928)
Villa alternata (Say)	Canada: AB, ON, SK			(Brooks, 1952)
Villa fulviana (Say)	Canada: SK			(King et al., 1928)
Villa lateralis (Say)	Canada: SK			(King et al., 1928)
Muscidae				
Muscina stabulans (Fallén)	Worldwide			(Cheng, 1977)
Sarcophagidae				
Sarcophaga cimbicis Townsend	Canada: AB, MB, NS, ON, QC, SK; USA: Throughout		(Crumb, 1929; Dahlem et al., 1996)	
Tachinidae				
Aphria ocypterata Townsend	Canada: AB, BC, MB, NB, ON, QC, SK, YT; USA: WA to ME, South to CA, NM and VA		(Arnaud, 1978; Cheng, 1977, 1981; O'Hara et al., 2004)	
Bonnetia comta (Fallén)	Canada: AB, BC, MB, NB, NS, NT, ON, PE, QC, SK, YT; Mexico: Throughout; USA: AK, throughout continental USA	(Arnaud, 1978; Capinera, 2001; O'Hara et al., 2004)	(Arnaud, 1978; Cheng, 1977, 1981)	(Arnaud, 1978; King et al., 1928; O'Hara et al., 2004)

Chetogena claripennis (Macquart)	Canada: AK to ON; but probably a mixture with *edwardsii*, and QC; Throughout USA and Mexico	(Arnaud, 1978; Capinera, 2001; O'Hara et al., 2004)		
Chetogena edwardsii (Williston) (probably)	Distribution confused with that of *C. claripennis*		(Arnaud, 1978; O'Hara et al., 2004)	(Arnaud, 1978; O'Hara et al., 2004)
Gonia aldrichi Tothill	Canada: BC to NB; USA: South to CA and VA		(O'Hara et al., 2004)	(Arnaud, 1978; King et al., 1928)
Gonia breviforceps Tothill	Canada: BC to QC; USA: MT, South to CA and AZ, also MI and KS			(Arnaud, 1978; O'Hara et al., 2004)
Gonia fuscicollis Tothill	Canada: MB, SK; USA: South to NE and TN, also MD and IN			(Arnaud, 1978; King et al., 1928; O'Hara et al., 2004)
Gonia spp.				(Arnaud, 1978)
Panzeria spp.		(Arnaud, 1978; Capinera, 2001)		
Peleteria rubescens Robineau-Desvoidy		(Arnaud, 1978)		
Peleteria texensis Curran	USA only	(Arnaud, 1978; O'Hara et al., 2004)		
Periscepsia cinerosa (Coquillett)	Canada: BC to MT, also AB and YT; USA: South to CA, AZ, CO	(Arnaud, 1978; Capinera, 2001)		
Periscepsia helymus (Walker)	Canada: BC to NS, also NU and YT; USA: South to CA, NM, KS, CT and AK	(Arnaud, 1978; Capinera, 2001; O'Hara et al., 2004)		(Arnaud, 1978; O'Hara et al., 2004)
Periscepsia laevigata (Wulp)	Canada: AK to NU; also BC, NB, NL, NS, and QC; Mexico ; USA: South to CA, TX and VA;	(Arnaud, 1978; O'Hara et al., 2004)		

Tachina algens Wiedemann	Canada: BC; NT to NL, NS and QC; USA: South to CA, AZ, NM, MA and AK		(Arnaud, 1978; O'Hara et al., 2004)	
Winthemia deilephilae (Osten Sacken)	Canada: ON		(Cheng, 1977)	
Winthemia rufopicta (Bigot)	Canada: ON		(Cheng, 1977)	
Winthemia quadripustulata Fabricius	Canada: NT, YT; BC to NS; USA: South to CA, AZ, KS and NJ, also WV and VA			(Arnaud, 1978; O'Hara et al., 2004)
Hymenoptera				
Braconidae				
Bracon erucarum (Cushman)	USA: AZ, CA, CO, ID, MT, OK, OR, UT, WY	(Yu et al., 2016)		
Chelonus insularis Cresson	Mexico; USA: AR, AZ, CA, CO, FL, GA, HI, IL, KS, LA, MO, MS, NM, OK, SC, TX, UT	(Capinera, 2001; Krombein et al., 1979)		
Cotesia acronyctae (Riley)	Canada: AB, ON, SK; USA: CA, CO, CT, IA, IL, IN, MA, MD, ME, MO, NH, NJ, OH			(Yu et al., 2016)
Cotesia griffini (Viereck)	Canada: AB, NB, QC; USA: AR, FL, KS, MA, NY, OK, SC, SD, TX, WA	(Krombein et al., 1979; Soteres et al., 1984; Yu et al., 2016)		(Schaaf, 1972; Yu et al., 2016)
Cotesia laeviceps (Ashmead)	Canada: AB, BC, MB, NB, ON, QC, SK; USA: CA, CO, CT, GA, IA, IL, MO, NM, NY, UT	(Krombein et al., 1979; Strickland, 1923; Yu et al., 2016)	(Cheng, 1977, 1981; Yu et al., 2016)	(Krombein et al., 1979; Strickland, 1923; Yu et al., 2016)
Cotesia vanessae (Reinhard)	Canada: AB, ON	This study (see Chapter 3)	This study (see Chapter 3)	This study (see Chapter 3)
Glyptapanteles alticola (Ashmead)	Canada: BC, NB; USA: AK, CA, CO, ID, ME, NH, OR, UT			

Apatia truncator (Say)	Canada: AB, BC, MB, NB, NS, ON, QC, SK; Mexico; USA: Throughout	(Capinera, 2001; Krombein et al., 1979)		
Macrocentrus incompletus Muesebeck	Canada: AB, SK; North of Mexico; USA: AZ, CA, CO, KS, MT, NE, NM, OK, OR, SD, UT, WY	(Capinera, 2001; Krombein et al., 1979; Yu et al., 2016)		
Meteorus dimidiatus (Cresson)	Canada: AB, BC, MB, NS, ON; USA: AK, AZ, CA, CO, DC, IL, KS, MA, ME, MI, MN, MO, NH, NJ, NY, OH, OR, PA, UT, VT, VA, WA	(Strickland, 1923; Yu et al., 2016)		(Strickland, 1923)
Meteorus pendulus (Müller)	Canada: BC, NB, NS, ON, QC; USA: AK, CA, CO, CT, DC, IL, KS, MA, ME, MI, MO, NC, NH, NJ, NY, OR, PA, RI, TX, VA, VT, WV		(Cheng, 1977, 1981; Krombein et al., 1979; Yu et al., 2016)	
Meteorus rubens (Nees)	Canada: AB, BC, MB, NB, NT, NS, ON, QC, SK; Mexico; USA: Throughout	(Cheng, 1977; Krombein et al., 1979; Soteres et al., 1984; Yu et al., 2016)	(Cheng, 1977; Krombein et al., 1979; Yu et al., 2016)	(Schaaf, 1972; Yu et al., 2016)
Microplitis feltiae Muesebeck	USA: AL, AZ, CA, CO, ID, IL, IN, KS, LA, MO, ND, OK, SC, TN, TX, WA	(Capinera, 2001; Krombein et al., 1979); (Yu et al., 2012)[1]		
Microplitis kewleyi Muesebeck	Canada: AB, MB, NB, NL, NS, ON, PE, QC; USA: CA, DC, IA, MD, ME, MI, NJ, NY, WI			(Schaaf, 1972; Yu et al., 2016)
Microplitis melianae Viereck	Canada: AB, ON; USA: IA, IL, KS, MI, MN, NY, OH, OK, TN, TX	(Capinera, 2001; Krombein et al., 1979)		

Protapanteles militaris (Walsh)	Canada: MB, NB, ON, QC; Mexico; USA: AR, AZ, CA, CT, DC, FL, HI, IA, IL, IN, KS, LA, MA, MD, MI, MN, MO, NJ, NM, NY, OK, TN, TX, VA	(Krombein et al., 1979; Yu et al., 2016)	(Cheng, 1977; Yu et al., 2016)	
Rogas sp.		(Capinera, 2001)		
Encyrtidae				
Copidosoma bakeri (Howard)	Canada: AB, NB, ON, SK; USA: AZ, CO, KS, MA, MT, NM, UT	(Byers et al., 1993; Noyes, 2011; Yu et al., 2016)	(Cheng, 1977; Noyes, 2011; Peck, 1963; Yu et al., 2016)	(Cheng, 1977; Noyes, 2011; Peck, 1963; Schaaf, 1972; Yu et al., 2016)
Ichneumonidae				
Arenetra canadensis Cresson	Canada: AB, NB, ON, SK; USA: CA, CO, IA, ID, IL, KS, MI, MT, NM, OR, SD, UT, WA, WI, WY	(Yu et al., 2016)		
Arenetra fumipennis Townes	Canada: AB; USA: CO, MT, ND			(Yu et al., 2016)
Arenetra rufipes Cresson	Canada: AB, BC, MB, NL, NS, ON, QC, SK; USA: AK, AZ, CA, CO, ID, KS, MA, ME, MI, MN, MT, ND, NH, NM, NY, OR, PA, SD, WA, WY		(Cheng, 1977; Yu et al., 2016)	(Yu et al., 2016)
Campoletis atkinsoni (Viereck)	Canada: AB, MB, SK; USA: ID, OR, WA			(Schaaf, 1972; Yu et al., 2016)
Campoletis australis (Viereck)	Canada: AB; USA: NM			(Schaaf, 1972; Yu et al., 2016)
Campoletis flavicincta (Ashmead)	Canada: AB, BC, NB, ON; Mexico; USA: AR, CA, CO, DE, FL, GA, IL, IN, KS, NC, OH, OK, SC, TN, VA	(Capinera, 2001)	(Cheng, 1977; Yu et al., 2016)	

Campoletis sonorensis (Cameron)	Canada: AB, BC, ON; Mexico; USA: AL, AZ, CA, FL, GA, KY, MS, NC, OK, SC, TN, TX, WA	(Capinera, 2001; Murillo, 2008); (Yu et al., 2012)[1]	This study[2]	This study[2]
Diphyus apiculatus (Walkley)	Canada: AB; USA: CO, ID, MT, OR, WY			(Schaaf, 1972; Yu et al., 2016)
Diphyus euxoae Heinrich	Canada: AB, BC; USA: CA, CO, ME, NJ, NM	(Yu et al., 2016)	(Cheng, 1977, 1981; Yu et al., 2016)	(Schaaf, 1972; Yu et al., 2016)
Diphyus nuncius (Cresson)	Canada: AB, BC; USA: CA, NJ, NY, UT	(Capinera, 2001; Yu et al., 2016)		
Diphyus subfuscus (Cresson)	Canada: AB, BC; USA: CA, CO, NV, NJ, NY	(Strickland, 1923; Yu et al., 2016)		(Strickland, 1923; Yu et al., 2016)
Enicospilus sp.	Canada: ON		(Cheng, 1977)	
Erigorgus ambiguus (Norton)	Canada: AB, MB, NB, ON, QC, SK; USA: AR, AZ, CA, CO, KS, KY, MA, ME, MI, MN, MO, MS, MT, NC, ND, NH, NM, NY, OH, PA, SD, TX			(Schaaf, 1972; Yu et al., 2016)
Eutanyacra suturalis (Say)	Canada: AB, BC, MN, NB, NL, NU, ON, QC, SK; Mexico; USA: CO, DE, GA, IL, KS, MA, NH, NJ, NM, NY, PA, UT, VA		(Cheng, 1977; Yu et al., 2016)	(Schaaf, 1972; Yu et al., 2016)
Exetastes lasius Cushman	USA: CA, KS, NE, WY	(Capinera, 2001; Yu et al., 2016)		
Exetastes obscurus Cresson	Canada: AB, MB; Mexico; USA: AL, CO, CT, DC, GA, ID, IL, KS, MD, MA, MI, MO, NC, NH, NJ, NY, OH, OR, PA, SC, TX, VA, WV			(Schaaf, 1972; Yu et al., 2016)
Ichneumon longulus Cresson	Canada: AB, BC; USA: AZ, CA, CO, GA, IL, KS, NJ, NM, UT	(Yu et al., 2016)		

Netelia sp.	Canada: AB			(Schaaf, 1972)
Spilichneumon inconstans (Cresson)	Canada: AB, BC, NL, NT, QC, YT; USA: CA, CO, CT, IL, MA, ME, MI, NJ, NY, VA	(Yu et al., 2016)		
Spilichneumon superbus (Provancher)	Canada: AB, BC, MB, NL, NS, NT, ON, QC, SK; USA: CO, HI, TX	(Capinera, 2001; Yu et al., 2016)	(Cheng, 1977, 1981; Krombein et al., 1979; Yu et al., 2016)	(Krombein et al., 1979; Schaaf, 1972; Yu et al., 2016)
ADENOPHOREA				
Mermithida				
Mermithidae				
Agamermis sp. (probably)	Canada: AB			(Schaaf, 1972)

[1] Information previously appeared in Yu et al. (2012), but not anymore in Yu et al. (2016).

[2] *Campoletis sonorensis* (GenBank accession number: KF640230) originally reared from *Chrysodeixis includens* and *Trichoplusia ni* collected in southwestern Ontario and maintained at the University of Windsor before being transferred to us. The three hosts reported here were exposed to *C. sonorensis* using a protocol identical to that described in Chapter 3.

Table 1.2. Predators of army cutworm, *Euxoa auxiliaris*, darksided cutworm, *Euxoa messoria*, and redbacked cutworm*, Euxoa* ochrogaster (published in Gavloski et al., 2013).

Predator species	**Known distribution in North America**	***Euxoa auxiliaris***	***Euxoa messoria***	***Euxoa ochrogaster***
ARACHNIDA				
Opiliones				
Phalangiidae (kill moths)				
Leiobunum vittatum (Say)	Canada		(Cheng, 1984)	
Phalangium opilio (Linnaeus)	Canada		(Cheng, 1984)	
Odiellus pictus (Wood)	Canada		(Cheng, 1984)	
Sclerosomatidae				
Hadrobunus maculosus (Wood)	Canada		(Cheng, 1984)	
AVES (kill karvae)				
Charadriiformes				
Laridae				
Leucophaeus pipixcan (Wagler)	Throughout North America			(King et al., 1928)
Passeriformes				
Icteridae				
Quiscalus quiscalus (L.)	Canada: ON		(Cheng, 1984)	
Sturnidae				
Sturnis vulgaris L.	Canada: ON		(Cheng, 1984)	
INSECTA				
Coleoptera				
Carabidae (kill larvae and eggs)				
Agonum cupreum Dejean	Canada, USA			(Frank, 1971; Larochelle, 1990)
Agonum placidum (Say)	Canada, USA			(Frank, 1971; Larochelle, 1990)
Amara apricaria (Paykull)	Canada, USA			(Frank, 1971; Larochelle, 1990)
Amara avida (Say)	Canada			(Frank, 1971; Larochelle, 1990)
Amara ellipsis (Casey)	Canada			(Frank, 1971; Larochelle, 1990)

Amara latior (Kirby)	Canada, USA		(Frank, 1971; Larochelle, 1990)
Amara patruelis Dejean	Canada		(Frank, 1971; Larochelle, 1990)
Amara quenseli (Schönherr)	Canada		(Frank, 1971; Larochelle, 1990)
Amara torrida (Panzer)	Canada		(Frank, 1971; Larochelle, 1990)
Bembidion bimaculatum (Kirby)	Canada		(Frank, 1971; Larochelle, 1990)
Bembidion canadianum Casey	Canada		(Frank, 1971; Larochelle, 1990)
Bembidion mutatum Gemminger & Harold	Canada		(Frank, 1971; Larochelle, 1990)
Bembidion nitidum (Kirby)	Canada		(Frank, 1971; Larochelle, 1990)
Bembidion obscurellum (Motschulsky)	Canada, USA		(Frank, 1971; Larochelle, 1990)
Bembidion quadrimaculatum oppositum Say	Canada, USA		(Frank, 1971; Larochelle, 1990)
Bembidion rupicola (Kirby)	Canada		(Frank, 1971; Larochelle, 1990)
Bembidion versicolor (LeConte)	Canada		(Frank, 1971; Larochelle, 1990)
Calosoma calidum (Fabricius)	Canada, USA	(King et al., 1928; Larochelle, 1990)	(King et al., 1928; Larochelle, 1990)
Carabus serratus Say	Canada, USA		(Frank, 1971; Larochelle, 1990)
Carabus taedatus Fabricius	Canada, USA		(Frank, 1971; Larochelle, 1990)
Harpalus amputatus Say	Canada, USA		(Frank, 1971; Larochelle, 1990)
Harpalus caliginosus (Fabricius)	Canada, USA	(Cheng, 1984; Larochelle, 1990)	
Harpalus funerarius Csiki	Canada		(Frank, 1971; Larochelle, 1990)
Poecilus lucublandus (Say)	Canada, USA		(Frank, 1971; Larochelle, 1990)
Pterostichus adstrictus Eschscholtz	Canada		(Frank, 1971; Larochelle, 1990)
Trichocellus cognatus (Gyllenhal)	Canada		(Frank, 1971; Larochelle, 1990)
Staphylinidae (kill eggs)			
Leptacinus batychrus (Gyllenhal)	Canada		(Frank, 1971; Larochelle, 1990)
Philonthus occidentalis Horn	Canada		(Frank, 1971; Larochelle, 1990)
Tachyporus sp.	Canada		(Frank, 1971; Larochelle, 1990)
Hymenoptera			
Formicidae (kill eggs)			
Lasius niger neoniger Emery	Temperate region of North Americ a		(King et al., 1928; Wang et al., 1995)

MAMMALIA		
Carnivora		
Ursidae (kill moths)		
Ursus arctos horribilis Ord	USA: WY, ID, MT (Yellowstone National Park area)	(French et al., 1994)

Table 1.3. Pathogens of army cutworm, *Euxoa auxiliaris*, darksided cutworm, *Euxoa messoria*, and redbacked cutworm, *Euxoa ochrogaster* (published in Gavloski et al., 2013).

Pathogen species	***Euxoa auxiliaris***	***Euxoa messoria***	***Euxoa ochrogaster***
(BACTERIA)			
BETAPROTEOBACTERIA			
Burkholderiales			
Alcaligenaceae			
Achromobacter spp.[2]		(Cheng, 1984)	
BACILLI			
Bacillales			
Bacillaceae			
Bacillus cereus Frankland & Frankland		(Cheng, 1984)	
Bacillus sphaericus Neide[2,3]		(Cheng, 1984)[3]	
Streptococcus faecalis (Andrewes & Horder)[2]		(Cheng, 1984)	
GAMMAPROTEOBACTERIA			
Enterobacteriales			
Enterobacteriaceae			
Enterobacter cloacae (Jordan)[2]		(Cheng, 1984)	
Enterobacter aerogenes (Kruse)[1]		(Cheng, 1984)	
Klebsiella pneumonia (Schroeter)[2]		(Cheng, 1984)	
Pseudomonadales			
Pseudomonadaceae			
Pseudomonas fluorescens (Migula)[1]		(Cheng, 1984)	
(FUNGI)			
DIHAPLOPHASEA			
Dissociodihaplophasida			
Nosematidae			
Nosema sp.		(Cheng, 1984)	
SORDARIOMYCETES			
Hypocreales			
Sorosporella uvella (Krass.)		(Cheng, 1984)	
(VIRUSES)			
Baculoviridae			
Granulosis virus	(Jackson et al., 1985)	(Cheng, 1984)	(Cheng, 1984)[3]
Nuclear polyhedrosis virus	(Jackson et al., 1985; McCarthy et al., 1975)	(Cheng, 1984)	(Cheng, 1984)[3]
Nonoccluded nuclear polyhedrosis virus	(Sutter, 1973)		
Poxviridae			
Entomopoxvirus	(Sutter, 1973)		

[1] Detected in haemocoel, potential pathogens

[2] Detected in haemocoel, unlikely pathogens

[3] Information that does not appear in Gavloski et al. (2013)

CHAPTER 2: IDENTIFICATION AND BIOLOGY OF *COTESIA VANESSAE*. FIRST REPORT OF THIS SPECIES IN NORTH AMERICA

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2.1. Abstract

This chapter reports for the first time the occurrence of the well-known Eurasian and north African parasitoid *Cotesia vanessae* (Reinhard) (Hymenoptera: Braconidae) in North America. Specimens were reared from *Chrysodeixis chalcites* (Esper) and *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) recovered from several locations in southwestern Ontario, and detected by DNA sequencing from one *Autographa californica* (Speyer) (Lepidoptera: Noctuidae) in southern Alberta, Canada.

2.2. Introduction

Cotesia is a genus of braconid wasps that are parasitoids of Lepidoptera larvae. Several species are of interest as biological control agents of pest caterpillars. For example, *Cotesia urabae* Austin & Allen was released into New Zealand in 2011 for the biological control of the eucalyptus pest *Uraba lugens* Walker (Lepidoptera: Nolidae) (Avila et al., 2011). *Cotesia flavipes* Cameron has been studied for many years and has been introduced in many countries as a biological control agent for the sugarcane borer *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) on cereal crops (Poaceae) (Jiang et al., 2004; Overholt et al., 1997). *Cotesia rubecula* (Marshall) has been released in the United States as a natural enemy of *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae) on cabbage, *Brassica* spp. (Brassicaceae) (van Driesche et al., 2002).

In the most recent checklist of *Cotesia* spp. in Canada and Alaska, Fernández-Triana (2010) reported 55 species (including four undescribed species) with the expectation that many more species in the genus remained unreported. The current work adds to this list the first report of *Cotesia vanessae* (Reinhard) in Canada and, more broadly, North America (Whitfield, 1995).

Cotesia vanessae is a gregarious endoparasitoid that primarily attacks species of Vanessini (Nymphalidae) and certain Noctuidae. It has also been reported from Notodontidae, Lasiocampidae, Crambidae, and Pterophoridae (Yu et al., 2012), but these reports are considered doubtful. Host records from the Old World here consider reliable include the vanessines *Vanessa cardui* (Linnaeus), *Vanessa atalanta* (Linnaeus) and *Aglais urticae* (Linnaeus), and the noctuids *Actebia praecox* (Linnaeus) and *Mythimna litoralis* (Curtis) (specimens in the British Museum of Natural History and the National Museum of Scotland). Nixon (1974) recorded *Spodoptera exigua* (Hübner) as a host, but in the present study *C. vanessae* couldn't be reared on this species in laboratory experiments (see Chapter 3). In Britain, *C. vanessae* overwinters within overwintering noctuid host larvae because its vanessine hosts are available as larvae only in the summer and the parasitoid adults always emerge from the cocoons in the year of their formation (Nixon, 1974).

Genetic analyses, as well as morphological and biological observations, show the existence of populations of *C. vanessae* in Ontario and Alberta, Canada. This parasitoid species has previously been reported throughout Europe and scattered locations in Asia and North Africa (Stefanescu et al., 2012; Yu et al., 2012). European populations of *C. vanessae* may be bisexual or, in the Mediterranean region, comprise only thelytokous females (Stefanescu et al., 2012).

2.3. Collections

2.3.1. From Ontario

Cotesia vanessae was first reared from the tomato looper, *Chrysodeixis chalcites* and cabbage looper, *Trichoplusia ni,* collected in 2009 in southwestern Ontario. *C. chalcites* has already been reported as a host of *C. vanessae* (Messelink, 2002), but *T. ni* is a new host record for *C. vanessae*.

For *Chrysodeixis chalcites*, parasitized loopers were collected from tomato plants, *Solanum lycopersicum* Linnaeus (Solanaceae), in fields around the cities of Harrow and Leamington in the summers of 2009 and 2010 (42°05'07.25"N, 82°36'08.65"W; 42°02'11.54"N, 82°57'03.78"W; 42°02'36.45"N, 82°38'05.60"W; 42°05'00.78"N, 82°37'07.26"W; 42°02'00.82"N, 82°53'58.49"W). Parasitized *C. chalcites* were also collected from tomato, pepper, *Capsicum annuum* Linnaeus (Solanaceae), and cucumbers, *Cucumis sativus* Linnaeus (Cucurbitaceae), in greenhouses around Leamington and Chatham-Kent in the fall of 2009, and in the late summer and fall of 2010. For *T. ni*, a parasitized larva was collected from a broccoli plant in a field west of Leamington in August 2009 (42°02'36.45"N, 82°38'05.60"W) and from tomato plants in fields around Harrow and Chatham-Kent in the summer of 2010 (42°02'11.54"N, 82°57'03.78"W; 42°23'26.93"N, 82°09'27.13"W) (Fig. 2.1).

Parasitoids from these original field collections initially were reared at the Sunrite Greenhouses Biocontrol laboratory, Ontario (by Henry Murillo, University of Windsor), and subsequently at the Lethbridge Research Centre, Alberta (by VH). Colonies were maintained on *T. ni* purchased from the Insect Production Services of the Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, which were reared on McMorran diet (McMorran, 1965) with Grisdale’s modification (Grisdale, 1973). Only females have been observed in our laboratory colony, reproducing by thelytokous parthenogenesis over multiple generations. From a subsample of 26 *T. ni* (these included the ten *T. ni* previously mentioned here in Hervet et al., 2014), each exposed to a single *C. vanessae* for 48 hours, an average of 95 adult parasitoids emerged per host (range: 5-176) (see Fig. 3.5 in Chapter 3). From another subsample of 31 *T. ni*, each oviposited into only once by a *C. vanessae*, an average of 92 adult parasitoids emerged per host (range: 16-199) (see Fig. 5.9 in Chapter 5). These hosts were parasitized at their early 4th instar and reared on the diet previously mentioned.

To identify the parasitoid species, CO1 sequences were obtained from laboratory colony specimens using primers and general methods as described in Hebert et al. (2003). All the sequences were identical, one of which (KF640231) was deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/). This sequence was compared with existing sequences from GenBank, using phylogenetic analyses that identified these specimens as *Cotesia vanessae* (Fig. 2.2).

To characterize the morphology of this species, photographs of adult and immature *C. vanessae* are provided in Figures 2.3 and 2.4. There are many similar *Cotesia* species, thus these pictures are not meant to be used to identify an unknown specimen. Reinhard's descriptions of *C. vanessae* (Reinhard, 1880, 1881) and Nixon's (1974) diagnosis correspond with our observations. However, the Ontario specimens tend to have lighter metafemur, metatibia, and metasoma (especially the laterotergites) compared to the specimens that were examined (by Mark R. Shaw) from northwestern Europe. Specimens from Libya and Ethiopia (perhaps really Eritrea) also have been reported to be lighter in color than European specimens (Nixon, 1974; Papp, 1987). Nixon (1974) also drew attention to minor differences, that he considered to be of little significance, between European series reared from vanessines and noctuids – presumably merely season- and/or host-related.

2.3.2. From Alberta

A DNA sequence for *C. vanessae*, but not specimens, was recovered from a final instar larva of alfalfa looper, *Autographa californica* (Speyer) (Lepidoptera: Noctuidae), that had also produced a brood of a different species of *Cotesia*. The larva was one of about thirty

A. californica collected for an unrelated study on 9 and 10 July 2012 from three canola fields; i.e., 12 km NE of Claresholm (50°04’45”N, 113°26’21”W), 17 km NE of Claresholm (50°07’23”N, 113°26’21”W), 30 km E of Calgary (50°57’27”N, 113°31’42”W). Larvae were held indoors in a Plexiglas cage (30 x 30 x 30 cm) at 22 °C and fed canola leaves, *Brassica napus* Linnaeus (Brassicaceae) for three days after which an unidentified gregarious species of *Cotesia* emerged from most individuals. Based on morphology and CO1 sequences (KF640233), the parasitoid was not *C. vanessae*. However, in sequencing parasitized caterpillars to verify their identity, a CO1 sequence (KF640229) of one of these hosts showed to be identical to that for *C. vanessae* from Ontario (Fig. 2.2).

It is speculated that this *A. californica* was parasitized by both the unknown *Cotesia* sp. and by *C. vanessae*, but only the former emerged from the host. Cases of caterpillars being parasitized by two *Cotesia* species and progeny of both species successfully developing within the host have previously been documented (Ngi-Song et al., 2001).

2.4. Current known range

Cotesia vanessae is widely distributed throughout the Palearctic (Fig. 2.5). Nixon (1974) recorded it from Eritrea (Asmara), Libya (Benghazi), Turkey, Italy, and “northwest Europe”. Stefanescu et al. (2012) reported it from locations in central-western Morocco and north-east Spain. Specimens in the Natural Museum of Scotland also include records from: Israel (Negev Mountains), Bulgaria (East Rhodope Mountains), Greece (N. Peloponnese), Crete, Canary Islands (Lanzarote, Fuerteventura), Spain (Girona, Lleida, Barcelona, Córdoba, Aragon), France (Charente), Finland (Åland), and most of the counties in the southern half of England. Additional records (Fig. 2.5) are from Yu et al. (2012).

2.5. Conclusion

The first record of *Cotesia vanessae* in North America is here documented by morphological, biological and genetic evidence. Where, when and how this parasitoid may have been introduced to North America is unknown. It may have been introduced into North America while developing inside a host in a shipment of imported plants.

Recovery of this parasitoid from three pest species thus far (*Chrysodeixis chalcites, Trichoplusia ni* and *Autographa californica*) warrants further study of *C. vanessae* as a potential biological control agent against noctuid crop pest caterpillars. However, this parasitoid is also reported to parasitize nymphalid butterflies such that non-pest species are likely to be at risk. Benzon et al. (Benson et al., 2003) showed that the deliberate introduction of *Cotesia rubecula* (Marshall) for the biological control of pestiferous *Pieris* butterflies had a negative impact on a native non-pest congener. Hence, monitoring the establishment and spread of *C. vanessae* in North America and its possible colonization of native vanessines and noctuids is advised, before any deliberate manipulations in the interests of crop protection are attempted.

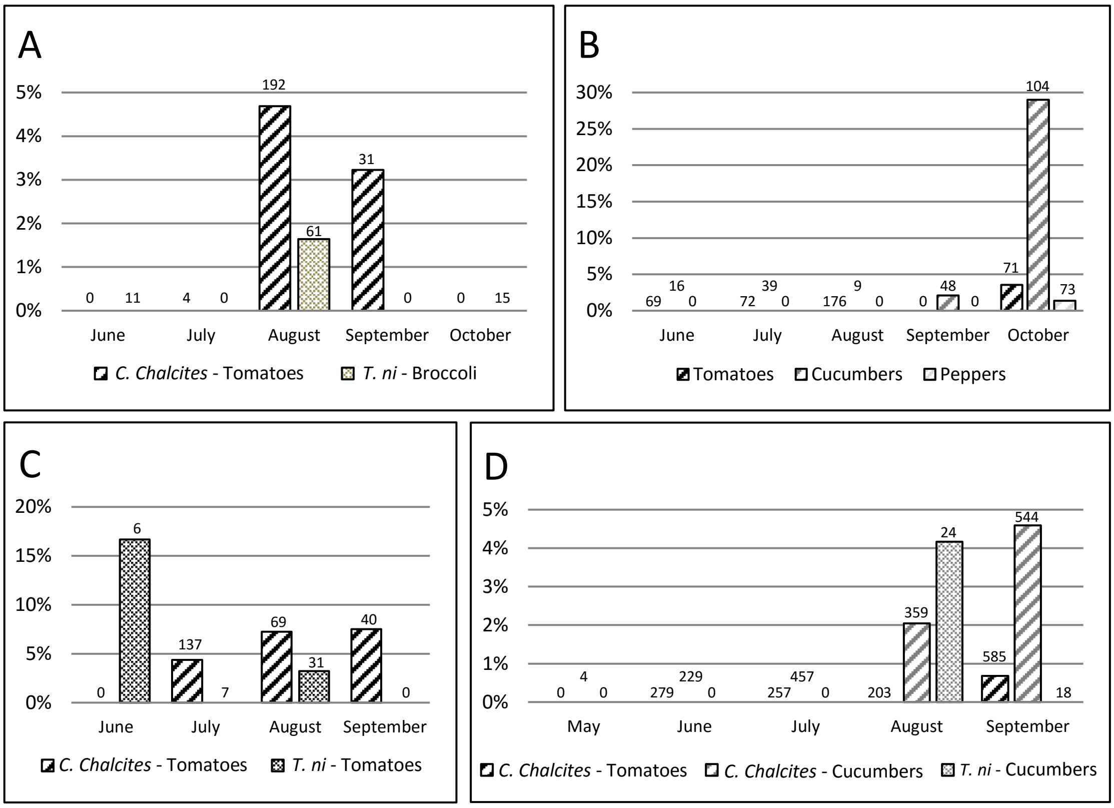
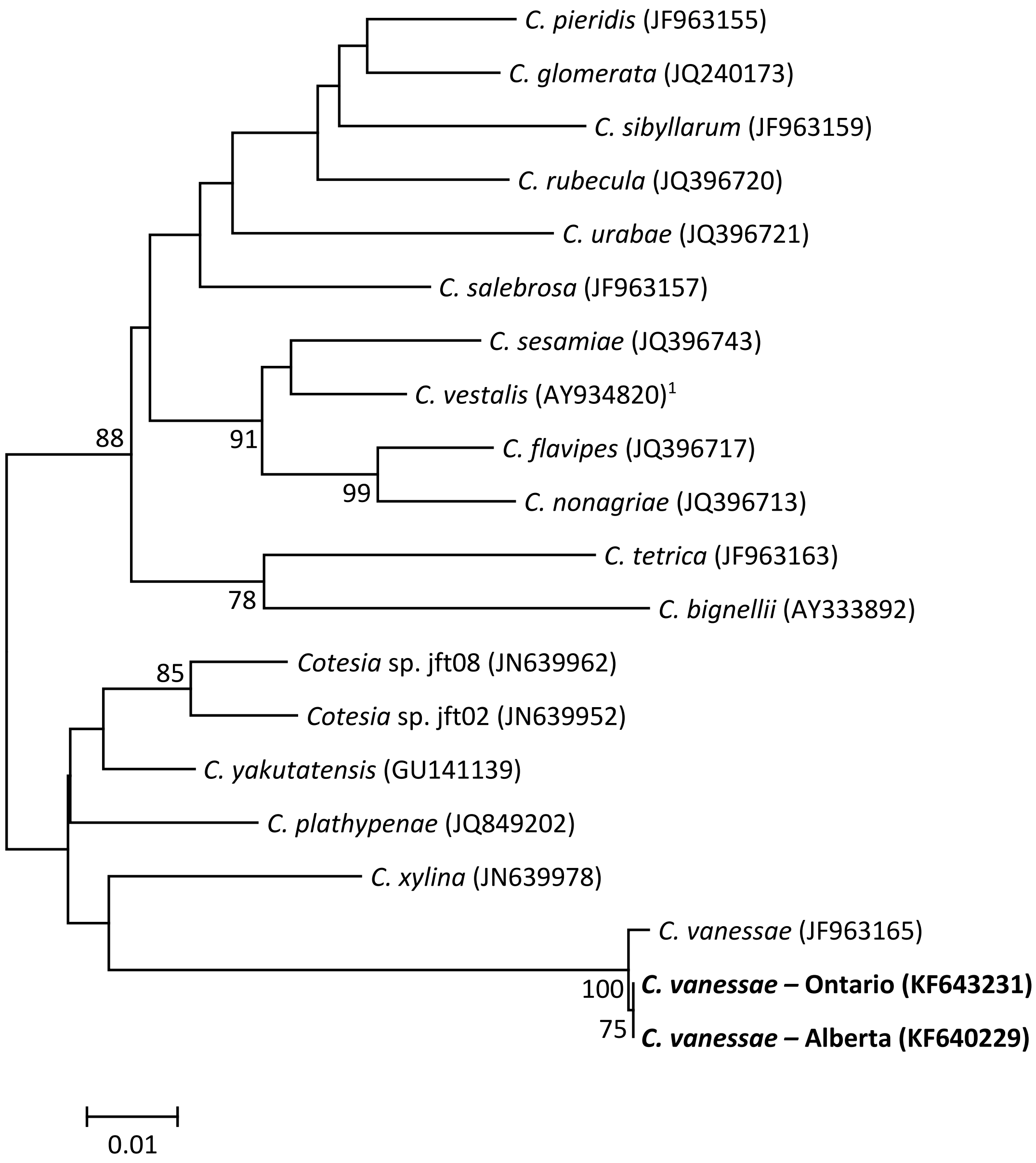


Figure 2.1. Parasitism (%) of *Chrysodeixis chalcites* and *Trichoplusia ni* by *Cotesia vanessae* collected from fields and commercial greenhouses in southwestern Ontario, Canada in 2009 and 2010. The numbers represent the total number of caterpillars collected each month on the indicated plants: **A.** 2009 in fields; **B.** 2009 in greenhouses; **C.** 2010 in fields; **D.** 2010 in greenhouses.



[1]Although the name *Cotesia plutellae* (Kurdjumov) is used for this sequence in GenBank, the valid name for this species is *Cotesia vestalis* (Haliday) (see Shaw (2003)).

Figure 2.2. Neighbour-joining tree, K2P distance model for CO1 sequences for *Cotesia* species generated using Mega 5.2. Sequences obtained from GenBank. Bootstrap values < 70 are not reported.

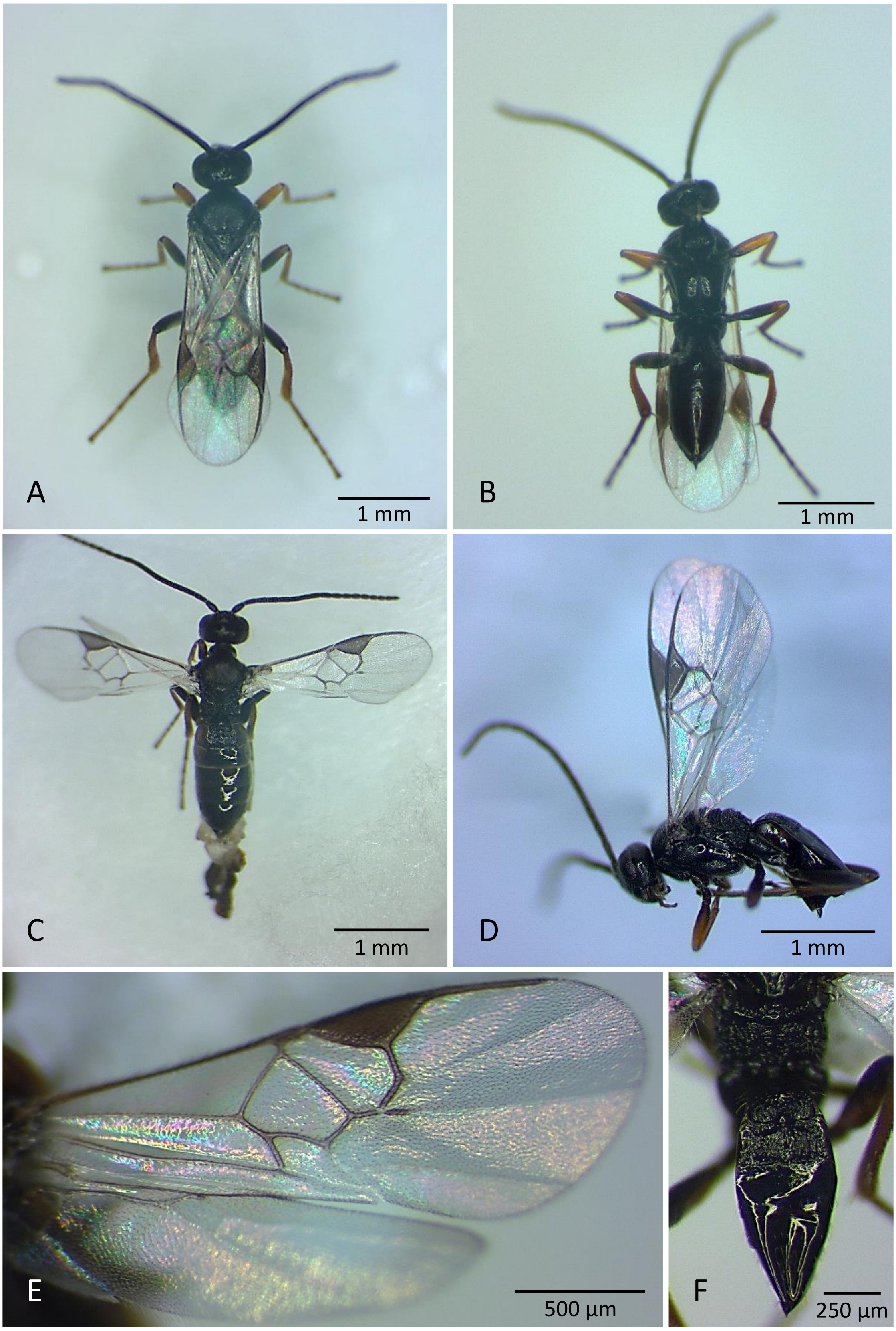


Figure 2.3. *Cotesia vanessae* adults (females): **A.** live, dorsal side; **B.** live, ventral side; **C.** live, emerging from pupal exuvia, dorsal side; **D.** dead, pointed, left lateral side; **E.** dead, upper surface of right wings; **F.** dead, dorsal side of abdomen.

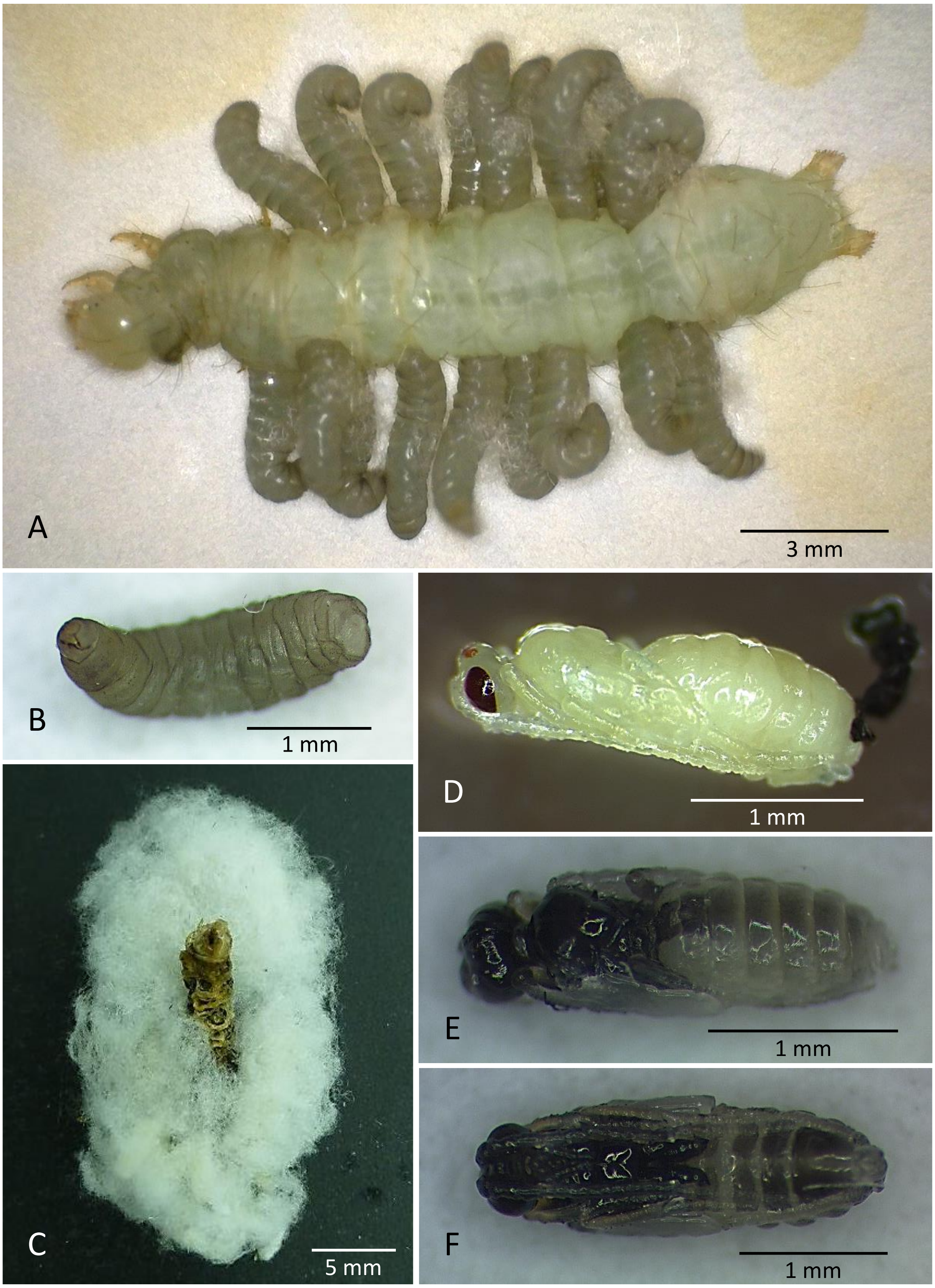


Figure 2.4. *Cotesia vanessae* immature stages: **A.** larvae (prepupae) erupting from a *T. ni* larva and starting to spin cocoons; **B.** prepupa just emerged from a Noctuidae host; **C.** cocoon mass of about 80 cocoons around dead host; **D.** one-day-old pupa, left lateral side, with cast larval exuvia and meconium (black mass at posterior end); **E.** three-day-old pupa, dorsolateral side; **F.** five-day-old pupa, ventral side.

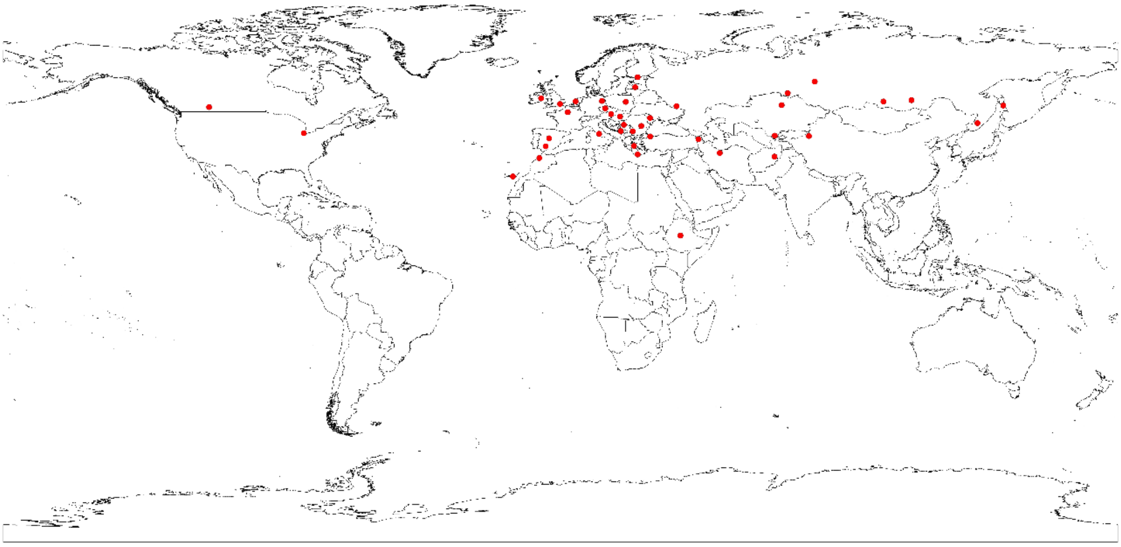


Figure 2.5. Reported distribution of *Cotesia vanessae*. The North American reports are from this study. The Old World distribution includes unpublished data (specimens in the National Museums of Scotland). Figure generated using SimpleMappr.

CHAPTER 3: EVALUATION OF THE HOST RANGE AND FITNESS OF THE PARASITOID *COTESIA VANESSAE* ON NORTH AMERICAN LEPIDOPTERA, PARTICULARLY PESTIFEROUS NOCTUIDAE

> *"I have a friend who's an artist and has sometimes taken a view which I don't agree with very well. He'll hold up a flower and say "Look how beautiful it is," and I'll agree, I think. And he says– "you see, I as an artist can see how beautiful this is, but you as a scientist, oh, take this all apart and it becomes a dull thing." And I think that he's kind of nutty. First of all, the beauty that he sees is available to other people and to me, too, I believe, although I may not be quite as refined aesthetically as he is; but I can appreciate the beauty of a flower. At the same time I see much more about the flower than he sees. I can imagine the cells in there, the complicated actions inside which also have a beauty. I mean it's not just beauty at this dimension of one centimeter, there is also beauty at smaller dimensions, the inner structure. Also the processes, the fact that the colors in the flower evolved in order to attract insects to pollinate it is interesting–it means that insects can see the color. It adds a question: Does this aesthetic sense also exist in the lower forms? Why is it aesthetic? All kinds of interesting questions which a science knowledge only adds to the excitement and mystery and the awe of a flower."*
> Richard P. Feynman (1981). The pleasure of finding things out.

3.1. Abstract

Noctuidae are major pests of cultivated plants worldwide, including in North America. Natural enemies can help reduce damage, both in greenhouses and in fields. The occurrence of a parthenogenetic population of the gregarious braconid parasitoid *Cotesia vanessae* in Canada is evidenced by Chapter 2. This species was already known from the Palearctic region where it is reported to use Noctuidae and Nymphalini as hosts. The potential North American host range of this species was assessed in a laboratory experiment by testing its ability to complete its development using Noctuidae, Nymphalidae, and Lepidoptera from other families. Of the 47 species tested, I found 30 Noctuidae and one Nymphalidae species not previously known as hosts, and confirmed three Noctuidae that were already known hosts of *C. vanessae*. For most of these host species, parasitoid fitness was investigated by measuring rate of successful parasitism, development time (in host, in cocoon, and total), brood size, individual parasitoid mass, and estimated brood mass. Noctuidae in the subfamily Plusiinae were found to generally be the best hosts. Species of similar host quality were usually, but

not always, closely related. Thus, we can likely predict non-tested species from certain genera to be good hosts (e.g., *Euxoa, Apamea*) or non-hosts (e.g., *Lacinipolia, Spodoptera*). Evidence of scramble and contest competition among parasitoid larvae was also observed, manifested by a trade-off between parasitoid brood size and individual mass, and siblicidal behaviour, respectively. Finally, *C. vanessae* was also observed to cause indirect death of some parasitized Lepidoptera larvae, of up to 100% for some species, by suppressing their ability to pupate and to excrete food, even though the immune system of these individuals killed all parasitoid larvae at an early stage. The ability of *C. vanessae* to develop in different species of pestiferous Noctuidae suggests a potential role for this species as a biocontrol agent in pest control programs.

3.2. Introduction

With about 25,000 described species, including 3,250 in America north of Mexico, the family Noctuidae is the most species-rich family of Lepidoptera, and it contains the greatest number of horticultural and agricultural pest species (Zahiri, 2012; Zhang, 1994). Many noctuid species – known by common names such as cutworms, armyworms, and semi-loopers – cause significant economic losses in North America and elsewhere. For example, between 1955 to 1975 in Idaho, Montana, South Dakota, Wyoming, Nebraska, Colorado, Kansas, and Oklahoma, the pale western cutworm, *Agrotis orthogonia* Morrison, caused an average annual loss of 17-million USD (the equivalent of ~90-million USD in 2016). During the same time period in Montana, South Dakota, Colorado, and Oklahoma, the army cutworm, *Euxoa auxiliaris* (Grote), caused a total annual average loss of nearly 8-million USD (~60-million USD in 2016) (USDA, 1977). Global losses due to cotton bollworm, *Helicoverpa armigera* (Hübner), are estimated to be 2-billion USD annually, in addition to over 1-billion USD spent on insecticide applications (Sharma et al., 2014). Over the last decade a noticeable increase in cutworm outbreaks in the agricultural region of the Canadian prairies has renewed interest in these pests, and biological control techniques are being investigated (WCCP, 2012, 2013).

Cotesia (Hymenoptera: Braconidae) is a speciose genus of both gregarious and solitary parasitoids within the subfamily Microgastrinae. All microgastrines are koinobiont endoparasitoids of Lepidoptera larvae. Adult females lay small, hydropic eggs (= ostensibly yolk-free eggs that develop and expand within the host, enabled by an extraembryonic membrane that permits absorption of nutrients from the host's haemolymph (Grbić et al. 1998; Harvey et al. 2013; King et al., 1969)). Larvae of many genera (e.g., *Cotesia*, *Microplitis,* and *Protomicroplitis*) feed essentially on the host's haemolymph and fat tissues, and egress from the host's sides prior to pupating. These characteristics, which are putatively apomorphic compared to solitary tissue feeding, allow these species to parasitize a wide range of host sizes, develop rapidly, and, for some species, utilize the moribund host as a body guard for their cocoons (Flanders, 1942; Harvey et al. 2008; Michel-Salzart et al., 2004; Shaw et al. 1991).

Species in the genus *Cotesia* have been used in previous biological control programs; e.g., *C. rubecula* (Marshall) against *Pieris rapae* (Linnaeus) (Lepidoptera: Pieridae) (van Driesche et al., 2002), *C. urabae* Austin & Allen against *Uraba lugens* Walker (Lepidoptera: Nolidae) (Avila et al., 2011; Avila et al., 2013), *C. flavipes* (Cameron) against *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and its relatives (Cugala et al., 2001), and *Cotesia marginiventris* (Cresson) against *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) and other Noctuidae (Gillespie et al., 1999; Messelink, 2002). Only recently has the Palearctic species *Cotesia vanessae* (Reinhard) (Hymenoptera: Braconidae) been recognized as occurring in North America (Hervet et al., 2014). Because this species is known to parasitize species of Noctuidae and Nymphalidae in the western Palearctic region (Hervet et al., 2014; Nixon, 1974; Papp, 2011-2012; Stefanescu et al., 2012), it may help suppress pestiferous species in North America.

Cotesia vanessae has many attributes that enhance its potential as a biocontrol agent. It is easy to mass rear because it usually parasitizes hosts as soon it is exposed to them, it can parasitize multiple hosts consecutively (e.g., 50 *Euxoa ochrogaster* (Guenée) (Lepidoptera: Noctuidae) larvae were parasitized by a single parasitoid in 20 minutes), it successfully parasitizes any host instar except the first, it is gregarious, it can be kept alive as adult usually up to a month, sometimes up to a month and half (using rearing conditions described below) and it has a facultative diapause that allows for continuous rearing in the laboratory. Further, maintaining thelytokous *C. vanessae* eliminates the problem of male-biased laboratory colonies and every individual can parasitize hosts.

In the current study, *C. vanessae* host range and its fitness (parasitism rate, development time, brood size, brood mass, and individual parasitoid mass) was tested on possible host species to assess its potential use as a biological control agent against noctuid pests. Non-pest species and species in other families were also included to investigate the limit of its host range and potential non-target effects. Closely related species were tested to assess the robustness of a phylogenetic approach to help predict whether species closelyrelated to known hosts would also be hosts. Most species tested were pestiferous Noctuidae, partly because these were target species, but also because they represented the highest number of catches using UV light traps, and also because some of these species were commercially available.

I also investigated the occurrence of scramble competition (i.e., “the finite resource is shared equally amongst the competitors so that the quantity of food per individual declines with increasing population density” (Den Berg et al., 2006)) and contest competition (i.e., “each successful competitor obtains all resources it requires for survival or reproduction; the remaining competitor being deprived of its resources, will not be able to function anymore” (Den Berg et al., 2006)) among parasitoid larvae within hosts.

3.3. Materials and Methods

3.3.1. Insect collection

The research was conducted at the Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada (AAFC), Lethbridge, AB, Canada. The *C. vanessae* laboratory colony was initiated at the University of Windsor (Windsor, ON) and transferred to AAFC in 2011. Parasitoids originated from southern Ontario, where they were reared from larvae of tomato looper, *Chrysodeixis chalcites* (Esper), and cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae: Plusiinae), collected on vegetables in commercial greenhouses and fields in 2009 and 2010 (Hervet et al., 2014). In the laboratory, parasitoids were maintained on *T. ni* purchased from Insect Production Service (IPS), Natural Resources Canada, Sault Ste Marie, ON, Canada. *Trichoplusia ni* larvae were reared on an artificial diet, the McMorran diet with Grisdale's modification (Grisdale, 1973; McMorran, 1965), also purchased from IPS.

Species of Lepidoptera tested as potential hosts for *C. vanessae* were obtained using different methods. Adults were collected with two UV light traps in summer and fall of 2012 to 2014 in and near Lethbridge, Alberta (traps were usually at 49.6924 °N -112.8152 °E and 49.6953 °N -112.7674 °E) (Table 3.1). Eggs were obtained from gravid females, males were released. Other species were purchased as eggs from Benzon Research Inc., Carlisle, PA, USA (Table 3.1). To import species from the United States, Canadian Food Inspection Agency Import Permits (No. P-2011-04397, P-2013-02134, and P-2014-02394) were obtained. An additional ~50 species were collected by hand and sweep net as eggs or larvae, in and near Lethbridge, and near Magrath (Cardston County, AB) (49.4327 °N -112.8626 °E) (i.e., Noctuidae, Erebidae, Lasiocampidae, Plutellidae, Hesperidae, Nymphalidae, Pieridae, Papilionidae, and Tortricidae). Although these were tested as potential hosts of *C. vanessae*, many of them were either too diseased or too close to pupation to remain as larva long enough to support parasitoid development and were thus not included in the analyses.

3.3.2. Insect identification

The identity of the Lepidoptera species was determined based on the morphology of the adult and larval forms and confirmed using molecular identification. For morphological identification, I used taxonomic keys (Eichlin, 1975; Eichlin et al., 1978; Schmidt, 2015) and comparison with preserved adults and larvae in collections held at the Lethbridge Research and Development Centre. For molecular identification, mitochondrial cytochrome oxidase 1 (CO1) sequences were obtained from specimens using primers and general methods as described in Hebert et al. (2003). DNA extraction and amplification was done at AAFC Lethbridge; sequencing was done at the University of Calgary. Sequencher software was used to trim and align the sequences. The CO1 sequences thus obtained were compared with sequences deposited in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). Field-collected larvae may have been parasitized prior to collection. Therefore, parasitoids emerging in the laboratory were also identified with this molecular method if necessary to confirm the identity of *C. vanessae*.

3.3.3. Colony rearing

Field-collected lepidopteran larvae were handled in one of two ways depending on their number. Species collected in low numbers were usually reared to the next generation to increase the number of larvae available, to produce parasitoid-free individuals, and reduce disease pressure. Species that were collected in large numbers (~50 individuals per species) were used directly in the experiment; i.e.*,* *Apamea sordens* (Hufnagel), *Dargida diffusa* (Walker) (Lepidoptera: Noctuidae), *Aglais milberti* (Godart) (Lepidoptera: Nymphalidae), *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), and *Pieris rapae*. Between experiments, Lepidoptera of all stages were reared in Plexiglas cages (40 x 34 x 30 cm [length, width, height]). The cages had a 10 cm diameter screen on opposing sides for ventilation and on the front a 15 cm diameter hole to which a screen sleeve was attached for accessing the cage. Adult Lepidoptera were provided with water, crystal sucrose, and oviposition substrates: fine sand, cheese cloth, paraffin film, brown paper, and paper towel. When eggs were laid, the oviposition substrate was cut into small squares and placed in clean cages on top of blocks (22 ml) of McMorran diet. Sand was sieved to recover the eggs, and the eggs were placed in a shallow 20 cm diameter plastic lid in the middle of the cage. Blocks of McMorran diet were mainly placed along the sides and corners of the cages to ease caterpillar access and were replaced with fresh blocks every 5 to 7 days as needed. To slow evaporation, pieces of paraffin film (Parafilm®) (3 x 3 cm) were placed on the bottom and top of each diet block. Species that couldn't be reared on McMorran diet were reared on their natural host plants (details in Chapter 4). Crumpled pieces of brown paper bag were placed in cages as shelter. Larvae were either used in experiments or used to generate a subsequent generation. In the latter case, pupae were placed in clean cages with paper towel for emerging moths to climb onto and provided with water, sucrose, and oviposition substrates. Both sexes were kept together to ensure that females were fertilized. Their eggs were handled as described above. Lepidoptera were reared in a growth cabinet at 12:12 h light:dark cycle, at constant 20 °C, and at 70 to 80% humidity to reduce evaporation of artificial diet and prevent desiccation of insect pupae. These conditions were monitored using a data logger (HOBO Pendant® UA-002-08) placed into an empty experimental cup and a thermo- and hygrometer (TempMinder® Remote Thermo-Hygro EMGR819LR(MRC800)) placed into an empty cage.

The parasitoid colony of *C. vanessae* was reared in the same type of cages that was used for Lepidoptera. To increase their life span, adult parasitoids were maintained at 10 °C, 12:12 h light:dark cycle under low light intensity, with access to honey, water, and a piece of crumpled brown paper for shelter. The cage was moved to 20 °C for two hours to allow parasitoids to feed and drink each week. At the beginning of that time fresh drops of honey were placed on the upper corner of the cage where parasitoids spent the majority of their time and water was sprayed on the walls and ceiling of the cage. For offspring production, adult parasitoids (1 to 3 weeks post-emergence) were exposed to 4th instar *Trichoplusia ni*. To ensure that all larvae were parasitized, they were exposed individually to parasitoids inside of the parasitoid cage until one or two parasitism events were observed. Parasitized larvae were then transferred to clean cages (~20 larvae per cage) containing blocks of McMorran diet and reared in the same conditions as non-parasitized individuals.

When individuals of a given Lepidoptera species were no longer required, they were typically exposed to several dozen parasitoids in cages (for 20 to 100 caterpillars per cage) to help regenerate the parasitoid colony. When placed into cages containing hosts, parasitoids didn’t seem to be able to locate them and the percent of parasitism was always very low (< 10%). However, when placed in direct contact with a Lepidoptera larva, and especially when dropped onto it, parasitism generally occurred instantly. Hence, to produce high in-cage percent parasitism, hosts were usually placed into a pile. Parasitoids were gathered in a vial with a mouth aspirator, then dropped onto the pile of caterpillars. To reduce incidence of diseases, all material (e.g., cages, experimental cups, and forceps) was thoroughly cleaned and disinfected with 70% bleach for five minutes between uses.

3.3.4. Host-range and host-fitness testing

Cotesia vanessae host-range and fitness in different host species was tested using no-choice tests. Based on preliminary observations, 4th instars were selected to be parasitized as these appeared to yield the best parasitism results; i.e., compared to earlier instars, parasitoids egressed (= emerged as larvae from a host; term here used to prevent confusion with the “emergence” of adults from cocoons) from fully grown host larvae so they produced larger broods, parasitized hosts had higher survival rates, and they were more attractive to parasitoids – and compared to later instars, 4th instars displayed lower physical resistance and a higher percentage of successful parasitism. When too few 4th instars were available, other instars (usually 5th instar) of similar size were used. Because some species were larger than others, larvae parasitized were of different sizes between species.

For Lepidoptera reared to the next generation in the laboratory, the goal was to use 30 to 35 larvae per species in the experiment. For some species, fewer individuals were used due to lack of availability. Field-collected larvae had a high mortality rate (from 51% with *Aglais milberti*, n = 49, to 100% with *Malacosoma disstria*, n = 60), due to diseases and field parasitism. Hence, for these species, 50 individuals were used whenever possible. The highest number of replicates used was 75 for *Helicoverpa zea* (Boddie). Initially 25 *H. zea* were exposed to *C. vanessae*, none of which produced parasitoids. However, exposing caged individuals to parasitoids revealed that a low percentage of *H. zea* were suitable hosts for *C. vanessae*. To capture the parasitism rate for this species, the experiment was repeated using another 50 *H. zea*.

To obtain a parasitized Lepidoptera larva, an individual was picked up from a colony cage using thin soft metal forceps, then, still held with forceps, placed into the colony cage of parasitoids and put in contact with a parasitoid's antennae. This usually resulted in immediate parasitism (i.e., insertion of the ovipositor for at least one second, and position of its wings and middle and hind legs upward (Fig. 3.1), a display that I previously observed with other braconid species and was assumed to be indicative of oviposition). When a parasitoid didn't respond, the larva was presented to another parasitoid, until one eventually responded. Sometimes when few parasitoids were available, none of them were willing to parasitize hosts. In this case, a parasitoid was collected using a mouth aspirator, the host was placed in an experimental cup, and the parasitoid was rapidly thrown onto it. This usually triggered immediate parasitism.

Parasitoids were quick to parasitize some species of lepidopterans, but required repeated exposure over extended periods before they parasitized other species. Eventually, however, they parasitized all exposed lepidopteran species with the exception of *Spilosoma virginica* Fabricius (Lepidoptera: Erebidae: Arctiinae) (~10 individuals exposed in 2013 and 30 in 2014). The heavy pilosity of these larvae probably prevented parasitism (although six individuals of another hairy Arctiinae, *Estigmene acrea* (Drury), were parasitized on their hairless ventral side). Larvae were slowly removed from the cage while being parasitized. When the parasitoid detached, both insects were quickly placed into an experimental container to allow for further parasitism. This was rarely observed. Parasitoids subsequently were removed after 48 h and returned to the colony, or discarded when dead. The experimental container was a translucent plastic cup (240 ml capacity, Polar Plastic Ltd.) containing a disc of filter paper (55 mm diameter) at the bottom, on top of which a block of diet was placed (7.3 ml). Each diet block had a square of paraffin film (3 x 3 cm) placed on top and below to reduce evaporation. A small drop of honey was placed on top of the upper paraffin film and some water was sprayed into the cup to sustain the parasitoid. Beforehand, numerous small holes (~0.5 mm diameter) were pierced in the bottom of the cups and on the lids to allow airflow and prevent the accumulation of moisture and the development of mold within the cups. Diet blocks were replaced approximately every 5 days as they were consumed or became too dry. At the same time, frass was discarded. For species that did not develop on McMorran diet; i.e., *Aglais milberti*, *Pieris rapae*, *Habrosyne scripta* (Gosse) (Lepidoptera: Drepanidae), and *Hyles euphorbiae* (Linnaeus) (Lepidoptera: Sphingidae), pieces of plant stems with a few leaves attached were used instead. Excised plant parts were placed onto a piece of paraffin film to avoid contact with the filter paper and sprayed with water daily to reduce wilting. Plant parts were replaced every 2 to 4 days as they were consumed or wilted. Tests were done in a growth cabinet (a different one than that used for colonies) at 12:12 h light:dark cycle, at constant 20 °C, and at 70 to 80% humidity. These conditions were monitored using a digital device measuring temperature and humidity and a data logger measuring temperature, humidity, and light intensity, which were each placed into two empty experimental containers (240 ml) to ensure that these conditions corresponded to those to which insects were exposed.

Parasitized caterpillars remained in these conditions until they either died or completed development to adults. This last outcome took several months for diapausing species (up to 8 months for *Mamestra configurata* Walker (Lepidoptera: Noctuidae)). Of all the Lepidoptera species tested, only *Hyles euphorbiae* and *Habrosyne scripta* did not emerge from their pupae, which suggests that these two species require a cold treatment to break their winter diapause. All other species contained specimens that successfully matured to adults in the conditions described above, given sufficient time, although maturity was not synchronized among all individuals.

The fitness of parasitoids on different host species was measured using a suite of parameters, including:

- The percent parasitism (number of caterpillars from which parasitoids egressed / [total number of parasitized larvae - larvae that died prior to pupating without producing parasitoids]).
- The development time of parasitoid broods within the host (from ovipotition to egression of larvae from the host).
- The development time of parasitoid broods within their cocoon masses (from egression of larvae from the host to first adult emergence from the cocoon mass).
- The number of adult parasitoids that emerged from each host, here referred to as “brood size” (parasitoids that didn’t fully emerge from cocoon masses were not counted in order to only consider individuals that could contribute to the next generation).
- The dry mass of individual adult parasitoids (parasitoids were dried in their experimental container in a rearing room at 20 °C, 20% relative humidity).

To measure parasitoid dry mass, the individual mass of 10 haphazardly selected parasitoids in pristine conditions from each brood was measured. However, to ensure completion of the data collection on time, for about half of the host species it was necessary to instead measure the mass of 30 parasitoids simultaneously from each brood; i.e., *Abagrotis reedi* Buckett, *Actebia balanitis* (Grote), *Agrotis ipsilon* (Hufnagel), *Anagrapha falcifera* (Kirby), *Anaplectoides prasina* (Denis & Schiffermüller), *Apamea devastator* (Brace), *Apamea lignicolora* (Guenée), *Caradrina morpheus* (Hufnagel), *Chrysodeixis includens* (Walker), *Dargida diffusa*, *Feltia herilis* (Grote), *Helicoverpa zea*, *Heliothis virescens* (Fabricius), *Lacanobia grandis* (Guenée), *Mythimna unipuncta* (Haworth), and *Spaelotis clandestina* (Harris) (Lepidoptera: Noctuidae).

3.3.5. Phylogeny of lepidopteran species

Genetic relatedness of the Noctuoidea used in this experiment was compared using CO1 barcodes generated from specimens used in this experiment and others from GenBank (Benson et al., 2005) and Barcode of Life Data Systems (BOLD) (Ratnasingham et al., 2007) for species that were not tested in this experiment, but which are considered reliable hosts for *C. vanessae* (Hervet et al., 2014). A neighbour-joining tree (Kimura 2-parameter distance) was generated to assess the host quality of different clades of Noctuidae according to the maximum likelihood of evolutionary distance among species based on their CO1 sequences (Kimura et al., 1980), and to predict the suitability of non-tested species as hosts for *C. vanessae*.

3.3.6. Investigating parasitized caterpillars that did not produce parasitoids

Many parasitized caterpillars survived and metamorphosed to adults. To verify whether parasitoid eggs were actually laid in these larvae, *H. zea* (a host with a low rate of successful parasitism; *n* = 20) and *Trichoplusia ni* (a host with a high rate of successful parasitism; *n* = 20) larvae were exposed to *C. vanessae* until the parasitoid displayed the putative oviposition posture on each caterpillar for more than one second. Five larvae from each of these two species were dissected immediately following parasitism to allow for observation of parasitoid eggs. The remaining caterpillars were maintained in individual cups in conditions previously described, and were dissected throughout the following 11 days to observe different stages of the parasitoid and of the hosts' immune reaction. To prevent gut contents from clouding the haemolymph, the head and the last rear abdominal segments of each caterpillar were excised and the digestive system removed. Haemolymph was then squeezed from the hemocoel onto a microscope slide, diluted with one drop of saline solution, and topped with a coverslip. The haemolymph was checked for eggs using a compound light microscope (400 x). Two *H. zea* dissected had too much fat, which clouded the haemolymph and rendered potential parasitoid eggs undetectable. To overcome this issue, remaining larvae were kept 2 days in a growth chamber without food to reduce their amount of fat tissues.

Parasitized caterpillars occasionally died without producing parasitoids. These caterpillars are referred to as having died of "premature death". Potential causes include: diseases, unsuitable rearing conditions, an indirect effect of parasitism by *C. vanessae* (discussed later), and death by other parasitoid species (for field-collected larvae only). Because it was unknown if these dead larvae would have produced *C. vanessae* under different conditions (some dissected dead caterpillars contained dead *C. vanessae* larvae), they were excluded from analyses. Premature death was observed in nearly all species tested, but was particularly evident in a few species; e.g., *Spodoptera frugiperda* (J. E. Smith) and *Anarta trifolii* (Hufnagel) (Lepidoptera: Noctuidae), all of which died following parasitism by *C. vanessae*, but without producing parasitoids, while conspecific non-parasitized individuals in the colony cages they came from were healthy. Some of these caterpillars were dissected upon death and their haemolymph observed with a dissecting microscope (15 x), but no parasitoid larvae were detected. New specimens of *A. trifolii* were obtained the following year using UV light traps to test experimentally whether *C. vanessae* induced premature death of these caterpillars. Larvae (*n* = 70) were reared individually in experimental containers (240 ml) using the setup previously described. Half of these larvae (*n* = 35) were parasitized by *C. vanessae* while the other half were not parasitized (control group). However, this time nearly all *A. trifolii* that were exposed to *C. vanessae* produced parasitoids (results of this test were included in the host-range and host-fitness experiment previously described). These different outcomes raised concerns regarding the conspecificity of individuals used in both tests. To verify the identity of specimens, CO1 sequences of specimens from both tests were compared (GenBank accession numbers: KX281193 and KX281194). They showed to be 100% identical and to match sequences of *A. trifolii* voucher specimens in GenBank. Since *C. vanessae* didn't induce premature death of *A. trifolii* in the second test, the test was then repeated using the same protocol but this time with *S. frugiperda*. Time to death of parasitized *S. frugiperda* larvae and time to pupation and adult emergence of non-parasitized individuals were recorded. Haemolymph of dead *S. frugiperda* larvae was observed with a compound light microscope (400 x) immediately following death of individuals.

3.3.7. Data analyses

A lepidopteran species was defined as a host if it produced at least one adult parasitoid. A χ^2 test was used to compare percentages of parasitism among host species, followed by post hoc comparisons between pairs of species using Fisher's exact tests with Bonferroni correction to detect which species differed. A χ^2 test was also used to compare the percentage of premature death between parasitized and non-parasitized *S. frugiperda* to test whether *C. vanessae* induced premature death of this species. A Welsh's approximate two-sample test (alternative to *t*-test since standard deviations of samples were unequal) was used to compare time to death of parasitized *S. frugiperda* and time to metamorphosis to adults of non-parasitized *S. frugiperda*.

One-way analysis of variance (ANOVA) tests were performed to examine the effect of host species on parasitoid brood size and on parasitoid development time within cocoons. When results detected a significant effect of host species, Tukey's range tests were performed to identify which host species had significant differences. Assumptions of normal distribution and homoscedasticity of variables were verified using residual-by-predicted plots. Kruskal-Wallis tests were performed to analyze *C. vanessae* development time in the host, total development time, and estimated total brood mass because ANOVA assumptions of homoscedasticity between host species and normality in a few species were violated. When Kruskal-Wallis tests detected a significant difference, post hoc tests were performed using Dunn tests (pairwise multiple comparisons using rank sums) to detect differences between groups.

It was hypothesized that differences in parasitoid fitness parameters between host species might be confounded by other factors. For example, if there is scramble competition within hosts, larger parasitoid broods may result in smaller individuals. In addition, considering that smaller individuals have less body mass to produce, smaller parasitoids may develop faster than larger parasitoids, thus brood size may directly influence parasitoid development time. Thirdly, if smaller parasitoids develop faster within the host, they may also develop faster within their cocoons for the same reason. Conversely, the opposite may also occur: due to the fact that parasitoids from the same brood egress simultaneously from their host, broods that develop rapidly in the host may comprise less-developed individuals. Thus, parasitoids that have a short development time within their host may compensate by having a longer development time within their cocoon. For these reasons, three analyses of covariance (ANCOVA) tests were performed to test for an effect of: host species and parasitoid brood size on average individual parasitoid mass, host species and brood size on *C.*

vanessae total development time, and host species and *C. vanessae* development time in the host on the development time of *C. vanessae* in cocoons. When a significant interaction between the main effects was detected, scatterplots and post hoc Pearson product-moment correlations with Bonferroni correction were used to identify the nature of the effects and which treatments differed.

All analyses were conducted in R version 3.3.0 (Dinno, 2016; Fox et al., 2011; R Core Team, 2016), which was also used to generate the figures. For figures displaying results of statistical analyses, species were ordered along the x axis starting with the species providing best fitness for *C. vanessae* for the variable analyzed, and in such way that the lines showing lack of significant difference would be the least interrupted. Unless otherwise stated, all means are reported ± SE; in some instances, ranges are provided.

3.4. Results

3.4.1. Fitness of *C. vanessae* in relation to host species

The experimental testing identified 31 previously unknown hosts of *C. vanessae* (see Table 3.1). The morphology of the species "*Abagrotis* sp." closely matches that of *Abagrotis orbis* (Grote), but CO1 sequences from four specimens put it closer to *Abagrotis baueri* McDunnough (Lepidoptera: Noctuidae) than to *A. orbis*. It may be either one of those two species, or is an undescribed species. Larvae of this species feed on buds of grapevines, *Vitis* spp. (Vitaceae), and it appears to be an important pest of vineyards in British Columbia, Canada. Lepidoptera species that were already known hosts of *C. vanessae* confirmed to be hosts in this experiment were *Anarta trifolii*, *Apamea sordens*, and *Trichoplusia ni* (Hervet et al., 2014; Tobias, 1971, 1976, 1986). Species that did not produce *C. vanessae* in this study (n = 13) are listed in Tables 3.1 and 3.2.

Parasitism rate was significantly different among host species ($\chi^2_{32} = 451.98$, $P < 0.001$) (Fig. 3.2). Result from *Amphipyra tragopoginis* (Clerck) (Lepidoptera: Noctuidae) is not displayed in Fig. 3.2 because only a single individual was exposed to *C. vanessae*, which produced parasitoids. Results indicate that suitable hosts can be found in the families Noctuidae and Nymphalidae, and that hosts of similar parasitism rate are often closely-related; e.g., Plusiinae, Apameini, *Euxoa* spp., *Abagrotis* spp., *Agrotis* spp., *Lacinipolia* spp., and *Spodoptera* spp. However, there were exceptions; e.g., Noctuini, Hadenini and *Feltia* spp. (Fig. 3.3). The wide range of parasitism success detected among Noctuini species is likely an effect of the high number of species tested in this group. Non-Noctuoidea species for which CO1 sequences were obtained – thus are not displayed in Fig. 3.3, are *Aglais milberti* (GenBank accession number: KX281189) and *Habrosyne scripta* (GenBank accession number: KX281205).

Development time of *C. vanessae* varied among host species in the host (Kruskal-Wallis, $\chi^2_{24} = 415.94$, $P < 0.001$), in the cocoon (ANOVA, $F_{24, 474} = 7.08$, $P < 0.001$), and total development time (Kruskal-Wallis, $\chi^2_{24} = 383.41$, $P < 0.001$) (Fig. 3.4). Total parasitoid development time for species from which fewer than five individuals produced parasitoids are not displayed in Fig. 3.4 because, due to a lack of power stemming from too few replicates, significant differences could not be detected; i.e., *Agrotis ipsilon* (26.8 ± 0.19 days, *n* = 4), *Apamea sordens* (30.7 ± 0.75 days, *n* = 2), *Dargida diffusa* (31.3 ± 1.32 days, *n* = 3), *Euxoa messoria* (Harris) (29 ± 2.52 days, *n* = 3), *Helicoverpa zea* (27.6 ± 0.61 days, *n* = 3), *Lacanobia grandis* (28.2 ± 24.5 days, *n* = 2), *Mamestra configurata* (27.82 ± 0.02 days, *n* = 2), and *Spaelotis clandestina* (28.5 ± 0.18 days, *n* = 2).

Regarding the potential effect of brood size on parasitoid development time, there was a significant interaction between host species and *C. vanessae* brood size on *C. vanessae* total development time (ANCOVA, $F_{24, 445} = 1.67$, $P = 0.03$). There was a significant effect of species (ANCOVA, $F_{24, 445} = 19.5$, $P < 0.001$) (Fig. 3.5), but not of total development time (ANCOVA, $F_{1, 445} = 0.31$, $P = 0.58$) on brood size. Scatterplots and Bonferroni-corrected Pearson product-moment correlation test showed no effect of parasitoid brood size on total development time (all $P \geq 0.006$). Parasitoid brood size for species from which fewer than five individuals produced parasitoids were not displayed in Fig. 3.5 because of low power; i.e., *A. ipsilon* (71.5 ± 17.97, $n = 4$), *D. diffusa* (28 ± 23.07, $n = 3$), *H. zea* (24 ± 16.33, $n = 3$), *L. grandis* (130 ± 24.50, $n = 2$), *M. configurata* (119 ± 16, $n = 2$), *S. clandestina* (120 ± 20.50, $n = 2$), *A. tragopoginis* (42, $n = 1$), and *A. sordens* (1, $n = 1$).

Host species associated with longer parasitoid development time in the host were usually also associated with longer parasitoid cocoon development time; e.g., parasitoids from *Feltia herilis* showed both the longest development time in the host (32.7 ± 1.4 days) and in the cocoon (11.1 ± 0.1 days). There was a significant interaction between *C. vanessae* development time in the host and host species on the development time of *C. vanessae* in-cocoons (ANCOVA, $F_{24, 449} = 4.09$, $P < 0.001$). There was also a significant effect of host species (ANCOVA, $F_{24, 449} = 8.20$, $P < 0.001$), but not of development time in the host (ANCOVA, $F_{1, 449} = 1.61$, $P = 0.21$) on *C. vanessae* in-cocoon development time. Post hoc Pearson product-moment correlation tests and scatterplots detected a significant negative correlation between *C. vanessae* development time in the host versus development time in the cocoon only for a few host species; e.g., *Agrotis vancouverensis*, *Anaplectoides prasina*, and *Noctua pronuba* (Linnaeus) (all $P < 0.001$). There was no interaction between *C. vanessae* development time in the host and in the cocoon for the other host species.

Average individual parasitoid mass per brood was different among host species (one-way ANOVA, $F_{24, 484} = 13.13$, $P < 0.001$) (Fig. 3.6). There was a significant interaction between host species and parasitoid brood size on individual parasitoid mass (ANCOVA, $F_{24, 464} = 4.47$, $P < 0.001$). There were also significant effects of host species (ANCOVA, $F_{24, 464} = 18.17$, $P < 0.001$) and brood size (ANCOVA, $F_{1, 464} = 105.38$, $P < 0.001$) on the mass of individual parasitoids. Scatterplots showed negative correlations between brood sizes and parasitoid individual mass for six species, as did Bonferroni-corrected Pearson product-moment correlation tests; i.e., *Trichoplusia ni, Autographa californica* (Speyer), *Chrysodeixis includens,* *Actebia balanitis, Abagrotis reedi, and Euxoa satis* (Harvey) (all r ≤ -0.70, *df* = 15 to 32, all $P < 0.001$). These species were amongst those that produced both the heaviest and the largest broods (Figs. 3.5 & 3.7). These six hosts also produced among the highest estimated brood masses, surpassed only by *Anarta trifolii* (22.94 ± 1.21 mg), *Noctua pronuba* (22.07 ± 1.50 mg), and *Anaplectoides prasina* (19.91 ± 1.25 mg), which were the three host species that also produced that largest parasitoid broods. Average individual parasitoid mass per brood for species from which fewer than five individuals produced parasitoids is not displayed in Fig. 3.6 due to low power; i.e., *A. ipsilon* (0.15 ± 0.003 mg, *n* = 4), *D. diffusa* (0.14 ± 0.023 mg, *n* = 3), *H. zea* (0.17 ± 0.023 mg, *n* = 3), *L. grandis* (0.13 ± 0.004, *n* = 2), *M. configurata* (0.15 ± 0.010 mg, *n* = 2), and *S. clandestina* (0.16 ± 0.024 mg, *n* = 2), and *A. sordens* (0.24 mg, *n* = 1). For similar reasons, estimated parasitoid brood mass for these species is not displayed in Fig. 3.7: *A. ipsilon* (10.60 ± 2.44 mg, *n* = 4), *D. diffusa* (3.90 ± 3.09 mg, *n* = 3), *H. zea* (4.35 ± 3.11 mg, *n* = 3), *L. grandis* (17.15 ± 3.74 mg, *n* = 2), *M. configurata* (18.42 ± 3.59 mg, *n* = 2), and *S. clandestina* (18.66 ± 0.48 mg, *n* = 2), and *A. sordens* (0.24 mg, *n* = 1).

Even if host size was not measured, it was evident that the largest host species didn’t always produce the largest broods or the heaviest parasitoids; e.g., *Eurois occulta* (Linnaeus) larvae were noticeably larger than *Anarta trifolii* larvae, although *A. trifolii* produced significantly larger broods than *E. occulta*. In addition, *E. occulta* and *N. pronuba* were the largest species used in this experiment, but many other species of noticeably smaller size produced parasitoids of comparable mass (Fig. 3.6). In addition, brood size varied considerably both within and among host species (Fig. 3.5). For example, *A. trifolii* larvae produced parasitoid broods ranging from one to 250 individuals (250 being the largest brood observed in this experiment). Other hosts that produced broods of one were: *A. sordens* (one parasitoid larva egressed and formed a cocoon, which produced an adult), *Feltia jaculifera* (Guenée), and *H. virescens* (in both cases a few parasitoid larvae egressed and formed a cocoon mass from which only one adult emerged). Three *H. virescens* individuals produced one, one, and five parasitoid larvae, which failed to produce cocoons and eventually dried out and died. These *H. virescens* were considered to have died producing parasitoids, although they didn't have an associated brood size.

There was little variation in the mass of individual parasitoids from the same brood. For example, average individual parasitoid mass from *T. ni* hosts was 0.18 ± 0.0025 mg (*n* = 251 individuals from 26 broods). Their dry mass varied between 0.08 mg to 0.33 mg (individuals from two different broods). However, out of 26 broods, the average difference between the heaviest and the lightest of 10 individuals chosen randomly from the same brood was 0.06 ± 0.0048 mg. The smallest difference of dry mass between two individuals emerged from a brood was 0.02 mg (with 0.11 mg and 0.13 mg, *n* = 10), and the greatest difference was 0.12 mg (with 0.14 mg and 0.26 mg, *n* = 10).

3.4.2. Oviposition testing

All larvae into which *C. vanessae* inserted its ovipositor (20 *T. ni* and 17 *H. zea*) contained either parasitoid eggs (observed up to 4 days following parasitism, at which time embryos were visible within) or larvae (observed starting 5 days after parasitism). Parasitoid eggs and larvae were observed within the haemolymph of each dissected *T. ni* (Fig. 3.8 A & B). None of them were encapsulated by the host's hemocytes, which would have been a normal immune response for lepidopteran larvae to parasitism (Lavine et al., 2002). *Cotesia vanessae* larvae were observed to produce teratocytes (Fig. 3.8 B), which are macrocells known to have trophic, immunosuppressive, and secretory functions (Dahlman, 1990, 1991). Observations (> 5 days) showed that a few eggs failed to hatch (three in total), and even those were not encapsulated. While when oviposited into *H. zea*, parasitoid eggs were observed to become encapsulated four days after parasitism, and all *H. zea* dissected 11 days after parasitism contained numerous dead first-instar parasitoid larvae encapsulated by hemocytes (Fig. 3.8 C & D) and no live larvae, except for one individual. This individual contained both encapsulated and live larvae. All encapsulated larvae appeared rather small and shrivelled (Fig. 3.8 D), which suggests that they had died shortly after hatching, while live larvae were much bigger. These observations show that parasitoids laid eggs even within caterpillars that did not support their development. In all lepidopteran larvae that contained parasitoid larvae, always a great number of them (about half of all observed parasitoid larvae in a host) had been cut in half in a transverse plane, usually about half way down their body (Fig. 3.8 E). This was indicative that sororicide occured. Larval sororicide was later further evidenced by direct observation of the act (Fig. 3.8 F).

3.4.3. Testing if *C. vanessae* causes premature death of Lepidoptera larvae

Results obtained for *Spodoptera frugiperda* showed that parasitism can cause the premature death of the host. All *S. frugiperda* parasitized died of premature death while all non-parasitized *S. frugiperda* (used as control) survived. These two outcomes were significantly different ($\chi^2_1 = 70$, $n_1 = 35$, $n_2 = 35$, $P < 0.001$). Larvae from the parasitized group matured to their last instar, but never pupated. Last-instar larvae slowly bloated until their integuments couldn't inflate anymore. At this point they stopped feeding, dark areas eventually appeared on their cuticle and spread over time until larvae were entirely black. During this darkening process the larvae started to shrink, likely because of water loss. Eventually larvae were entirely dark and motionless, at which point they appeared dead, but were still responsive to touch. They were touched daily with forceps and assumed dead when unresponsive. The time taken for parasitized individuals to die was significantly longer than that required for non-parasitized individuals to metamorphose to adults (Welsh's approximate two-sample *t*-test, $t = -7.45$, $df = 40.35$, $P < 0.001$). Dissection of dead larvae showed that their hemocoel was nearly depleted of haemolymph and that their gut was highly bloated and occupied nearly the entire hemocoel. Dissection of the gut showed that it was filled with a gelatinous orange substance similar to the artificial diet, which it was assumed to be (Fig. 3.9). Observation of their haemolymph revealed dead encapsulated first instar parasitoids, which, considering their small size, had died shortly after hatching and long before the death of the host. In these parasitized *S. frugiperda* larvae, as in *H. zea*, and a number of *Mythimna unipuncta* (> 10, dissected for unrelated reasons), encapsulated parasitoids were observed but none of them was also melanised.

Although the relationship between premature death and parasitism was documented only in *S. frugiperda*, similar observations were made in a number of species. For example, time to premature death for *A. ipsilon* larvae (*n* = 20), once parasitized, averaged 98.5 ± 3.07 days (range: 74 to 120), about twice the duration observed for *S. frugiperda*; while individuals that metamorphosed to adults (*n* = 4) did so in 46.5 ± 4.5 days of parasitism. For *A. trifolii* (*n* = 40), larvae died only after 20.9 ± 2.2 days (range: 3 to 30) of parasitism.

3.5. Discussion

The overall results show that *C. vanessae* has a broad fundamental host range within Noctuidae and may potentially become an important parasitoid of key pest species in North America. Hosts in the subfamily Plusiinae were among those that displayed the highest percent of successful parasitism, produced the largest broods, the heaviest individuals, and the fastest-developing parasitoids. In Canada, *C. vanessae* has only been recovered in the wild from Plusiinae; i.e., *Chrysodeixis chalcites*, *Trichoplusia ni*, and *Autographa californica* (Hervet et al., 2014), confirming that Plusiinae are natural hosts. However, it has been demonstrated with other parasitoid species, including with *Cotesia sesamiae* (Cameron) (Branca et al., 2011), that conspecific parasitoids from different geographic regions do not display a similar specificity on the same host species (Dubuffet et al., 2006). Because *C. vanessae* from a single source population was used in the current experiment, it may be that *C. vanessae* from other populations have different host specificities.

Nymphalini appear to be the primary hosts of *Cotesia vanessae* in the southern-western part of its Palearctic range where temperatures are warm enough for parasitoids to refrain from diapause (Stefanescu et al., 2012), but in the northern part of its range *C. vanessae* must necessarily overwinter within an overwintering host larva. Nymphalini commonly either overwinter as adults or migrate to southern latitudes for the winter (Dvořák et al., 2002; Scott, 1979); thus, parasitoids of northern latitudes must find other hosts for the winter period. Noctuidae parasitized by *C. vanessae* have been collected in October (Italy) and May (England), which suggests that *C. vanessae* overwinters within overwintering noctuid larvae in the northern part of its range (Nixon, 1974). This would explain the affinity of *C. vanessae* to Noctuidae, which may be stronger in overwintering northern populations.

In the current study, parasitoids were observed to develop slower on overwintering hosts, thus supporting the previous claim that they overwinter within overwintering hosts. Individuals of univoltine lepidopteran species that overwinter as larvae necessarily are at the larval stage during the fall, and do not occur in the larval stage any other time of the year. Such species were exposed to *C. vanessae* in the fall while they were diapausing or soon to do so, although they remained at 20 °C in experimental conditions throughout their entire development. In Fig. 3.4, the species between (and including) *Apamea devastator* and *Cryptocala acadiensis* (Bethune) (Lepidoptera: Noctuidae) both overwinter as larvae and are univoltine throughout their range, except for *Euxoa tristicula* (Morrison), a species that can be multivoltine. However, personal observations and previous studies (Jacobson, 1969) show that *E. tristicula* must necessarily go through a diapause at the larval stage, which lasts at least two months at 20 °C. Parasitoids took the longest time to develop within these diapausing hosts. This indicates that development within overwintering hosts likely triggered parasitoids to initiate overwintering, but after a short diapause (from a few days, up to two months in *Cryptocala acadiensis* (Fig. 3.4)) parasitoids eventually resumed their development, likely because of conditions non-optimal for overwintering (including too high temperature, too long light cycle, and too strong light intensity). Regarding the other species in Fig. 3.4, only *Abagrotis* spp., *Agrotis vancouverensis*, and *Anaplectoides prasina* also overwinter as larvae and are likely to be obligately univoltine throughout their range. The reason why parasitoids did not attempt to diapause within these hosts is unknown.

Putting aside overwintering hosts, parasitoids usually developed faster in host species that also produced larger broods and heavier individual parasitoids. This observation supports previous findings that fast development can be associated with higher fitness (Doyon et al., 2005; Gao et al., 2016), and that parasitoids may display different fitness when using different host species (Greenblatt et al., 1981). Host species in which parasitoids required longer development period were also usually those that were associated with longer parasitoid cocoon development time. There were two exceptions to the trend that univoltine species that overwinter as larvae had longer development time in both the host and the cocoon: *Apamea lignicolora* and *Cryptocala acadiensis*. Both species produced parasitoids that had amongst the longest development time in the host and amongst the shortest development time in the cocoon. On the other hand, brood size and parasitoid mass did not appear to always be associated with host size. This effect has also been observed in other species, and may be partly explained by the suitability of host species to the parasitoid species, and by how adequate the diet is for these hosts (Greenblatt et al., 1981).

Comparison of genetic relatedness allows for tentative predictions regarding what additional pest species in North American might be hosts for *C. vanessae*. Results indicate that many species in the subfamily Plusiinae, in the tribes Noctuini, and Hadenini, and in the genera *Apamea* and *Mythimna* are likely to be hosts. Hosts were found in the four subfamilies of Noctuidae tested. This indicates that other subfamilies of Noctuidae likely also contain hosts. Most of the main pest species of Noctuidae in North America were tested in this experiment, the majority of which supported the development of *C. vanessae*. Other important pests that were not tested that are likely hosts include: the cotton bollworm, *Helicoverpa armigera*, the pale western cutworm, *Agrotis orthogonia*, and the granulate cutworm, *Feltia subterranea* (Fabricius). Any species in the tribe Nymphalini are also likely to be hosts. On the other hand, species in the family Erebidae and in the genera *Lacinipolia* and *Spodoptera* are not likely to be hosts.

This experiment did not test 30 species previously reported to be hosts of *C. vanessae*: *Aglais io* (L.), *Aglais urticae* L., *Aglais urticae turcica* (Staudinger), *Nymphalis polychloros* (L.), *Vanessa atalanta* (L.), *Vanessa cardui* (L.) (Nymphalidae: Nymphalinae), *Limenitis camilla* (L.) (Nymphalidae: Limenitidinae), *Argynnis aglaja* (L.) (Nymphalidae: Argynninae), *Autographa gamma* (L.), *Chrysodeixis chalcites* (Esper), *Cornutiplusia circumflexa* (L.), *Macdunnoughia confusa* (Stephens) (Noctuidae: Plusiinae), *Actebia praecox* (L.), *Actebia fugax* (Noctuidae: Noctuinae), *Acontia lucida* (Hufnagel) (Noctuidae: Acontiinae), *Lacanobia oleracea* (L.), *Mamestra brassicae* (L.), *Mythimna litoralis* (Curtis) (Noctuidae: Hadeninae), *Helicoverpa armigera* (Hübner), *Heliothis viriplaca* (Hufnagel) (Noctuidae: Heliothinae), *Nycteola revayana* (Scopoli) (Noctuidae: Sarrothripinae), *Calophasia opalina* (Esper) (Noctuidae: Cuculliinae), *Simyra dentinosa* Freyer (Noctuidae: Acronictinae), *Cerura vinula* (L.), *Notodonta ziczac* (Notodontidae), *Cnaemidophorus rhododactylus* (Denis & Schiffermüller) (Pterophoridae: Pterophorinae), *Lasiocampa trifolii* Denis & Schiffermüller, *Malacosoma neustria* (L.) (Lasiocampidae: Lasiocampinae), *Loxostege sticticalis* (L.) (Pyralidae: Pyraustinae), *Thaumetopoea processionea* (L.) (Thaumetopoeidae) (Aubert, 1966; Aue, 1938; Doğanlar, 1982; Fahringer, 1936; Hervet et al., 2014; Karimpour et al., 2001; Meyer, 1927; Morley, 1906; Obregón et al., 2015; Özbek et al., 2012; Papp, 1988, 2011-2012; Schwarz et al., 2000; Shaw et al., 2009; Telenga, 1955; Tobias, 1954, 1971, 1976, 1986; Yu et al., 2016; Zhumanov, 1980). *Spodoptera exigua* (Hübner) has been reported as a host of *C. vanessae* (Tobias, 1971), although it didn’t support the development of *C. vanessae* in the current study.

Results indicate that testing a lepidopteran species multiple times may yield different outcomes (as illustrated by the tests using *Anarta trifolii* and *Helicoverpa zea*). This shows that results from only one set of parasitized caterpillars should be viewed with caution, especially for species listed as non-hosts. Thus, failing to confirm the host status of *S. exigua* does not invalidate this previous report. However, previous reports of parasitoid-host association do not often provide strong evidence of accurate identification of species, either for field-collected host larvae or emerged parasitoids (Nixon, 1974). Thus, some of these reports may be considered doubtful (Hervet et al., 2014). In light of the results and other reliable sources (Shaw et al., 2009; Stefanescu et al., 2012), the records considered to be most reliable, or at least to be the most common hosts, identify species of Noctuidae and Nymphalini. But, given its broad host range in these taxa, host species in other Lepidoptera families seem probable.

Many *Cotesia* species are known to be generalists. During the current study *C. vanessae* was observed to insert its ovipositor and assumed the putative oviposition posture on all exposed lepidopteran species (except *Spilosoma virginica* (Fabricius) (Lepidoptera: Erebidae)), which belonged to 11 families; i.e., Drepanidae, Erebidae, Hesperidae, Lasiocampidae, Noctuidae, Nymphalidae, Papilionidae, Pieridae, Plutellidae, Sphingidae, and Tortricidae. Many of these species were not previously mentioned because they didn’t produce parasitoids and the number of exposed larvae was low (< 6 per species). Hence, no conclusion could be drawn regarding their possible non-host status, but this illustrates how opportunistic *C. vanessae* is. A review of the *Cotesia* species in Taxapad (Yu et al., 2012) (*n* = 266) showed that for those that have at least one known host species (*n* = 214), over one third of them (*n* = 88) have been reported from Noctuidae. This illustrates that there is an affinity of *Cotesia* species for members of this family. For the *Cotesia* species that had at least two known host species (*n* = 135), over half of them (*n* = 75) were associated with hosts in different families, which often included micro- and macrolepidopterans, as well as moths and butterflies. Some *Cotesia* species of particular economic importance have been extensively studied, and for some of them this has yielded the discovery of a high number of host species; e.g., *Cotesia ruficrus* (Haliday): 83 host species in 15 families, *Cotesia marginiventris* (Cresson): 42 host species in 6 families, *Cotesia flavipes* Cameron: 37 host species in 6 families, and *Cotesia congregata* (Say): 28 host species in 5 families (Yu et al., 2016). This illustrates the number and diversity of hosts associated with these parasitoids, although these numbers should be taken with caution as many reports likely stem from misidentifications (Nixon, 1974).

Although *C. vanessae* accepted nearly all the Lepidoptera species in the current experiment, it was sometimes necessary to wait for parasitoids to get closer to the end of their life for them to accept certain host species. Plusiinae were always readily accepted regardless of parasitoid age, and to a lesser extent other Noctuidae too, but Lepidoptera in other families were generally not accepted by young adult parasitoids. This effect has been described as an aspect of “dynamic state variable theory”, known as “partial preference”, which states that the acceptance of less desirable species depends on to the parasitoid egg load, fat reserves, and age (Clark et al., 2000; Mangel, 1989). This means that the host range of a parasitoid species is likely to change over time, with older parasitoids accepting an increasing number of species as hosts.

The plant species that the hosts feed on are a crucial aspect of parasitoid-host specificity. Some *Cotesia* species, although they have been reared from taxonomically distant hosts, appear to be associated with few plant species. For example, *Cotesia ferruginea* (Marshall) has been reared only from three host species, each of them belonging to a different family: *Archanara geminipuncta* (Haworth) (Lepidoptera: Noctuidae), *Phragmataecia castanea* (Hübner) (Lepidoptera: Cossidae), and *Chilo phragmitella* (Hübner) (Lepidoptera: Pyralidae) (Wilkinson, 1945). The two first species feed solely on *Phragmites* spp. and the third one feeds both on *Phragmites* and *Glyceria maxima* (Hartm.) Holmb.

(Poaceae). This suggests that *C. ferruginea* is rather associated with specific plant species than specific host species. The hypothesis that some parasitoid species are associated with plant species rather than host species, and to this effect parasitize taxonomically distantly related species simply because they feed on the same plant species, has previously been formulated (Cushman, 1926). To test this hypothesis, Tawfik (1957) exposed *Cotesia glomerata*, a known parasitoid of *Pieris* spp. (Lepidoptera: Pieridae) associated with *Brassica* spp. (Brassicaceae), to a non-host species, *Mamestra brassicae*, which also feeds on *Brassica* spp. In the laboratory, *C. glomerata* oviposited within *M. brassicae*. Although the eggs didn't hatch within this species because they became encapsulated, this showed a key biology aspect of the parasitoid for selecting hosts. Similarly, the solitary parasitoid *Cotesia marginiventris* and the gregarious parasitoid *Cotesia congregata*, which were thought to be specific on Noctuidae and Sphingidae respectively, were exposed to the other species' natural host in the laboratory; i.e., *C. marginiventris* to *Manduca sexta* (L.) (Lepidoptera: Sphingidae) and *C. congregata* to *T. ni* (Noctuidae). Wasps of both species were found to readily parasitize these larvae, and *C. marginiventris* even did so in the presence of its natural host *S. exigua*. Surprisingly, individuals of both lepidopteran species produced parasitoids, some of which successfully matured to adults (Beckage et al., 2003; Beckage et al., 2002). These results show that *Cotesia* parasitoids are not as specific as they seem to be to particular host species, and that distantly related lepidopteran species may be suitable hosts. However, species found to be hosts in the laboratory may never be hosts in natural settings. A number of steps are necessary to lead to successful parasitism (i.e., host habitat location, host location, host acceptance, and host suitability), which are each essential to complete parasitism (Vinson, 1980). The current study focuses on host suitability. Therefore, resuts reflect the fundamental, but not ecological, host range of *C. vanessae*.

To understand the ecological host range of *C. vanessae* it is essential to at least know the plant species it is associated with. Plant species from which lepidopteran larvae parasitized by *C. vanessae* have been collected include: thistles (tribe Cynareae, family Asteraceae), common mallow, *Malva sylvestris* (L.) (Malvaceae) (Stefanescu et al., 2012), tomatoes, *Solanum lycopersicum* Linnaeus (Solanaceae), cucumbers, *Cucumis sativus* Linnaeus (Cucurbitaceae), cabbages, *Brassica* spp. (Hervet et al., 2014), and *Chenopodiastrum murale* (L.) S. Fuentes, Uotila & Borsch (Amaranthaceae) (Papp, 2011-2012). Known host plants of previously reported host species may also indicate an affinity of *C. vanessae* for: nettles, *Urtica* spp., beet, *Beta vulgaris* Linnaeus (Amaranthaceae), poplars, *Populus* spp., and willows, *Salix* spp. (Salicaceae) (Yu et al., 2016).

The affinity of *C. vanessae* for both Nymphalinae and Noctuidae may have originated in the common food source of its hosts. Species of Noctuidae have been reported from plant species also used by larvae of *V. atalanta*, *V. cardui*, and *A. urticae* (DBIF, 2016; Shaw et al., 1990; Stefanescu et al., 2012). Serendipitous encounter of parasitoids with prospect hosts on plants may have led to the extension of the host range of *C. vanessae* from Nymphalidae to Noctuidae, or from Noctuidae to Nymphalidae.

Results also identified the occurrence of both scramble and contest competition among parasitoid larvae of the same brood. In the current study, certain host species produced particularly large broods considering the sizes of both parasitoid and host (Le Masurier, 1987), some of which displayed a trade-off between parasitoid brood size and individual mass, which is typical of scramble competition (Den Berg et al., 2006; Gu et al., 2003; Harvey et al., 2013). The little mass variation between individuals of the same brood suggests that available resources are divided somewhat equally among individuals of that brood. Contest competition among sibling parasitoids of the same brood is known to occur in other gregarious species (Giron et al., 2004). However, some of these observations were made when immature parasitoids were artificially injected into hosts containing sibling broods, so it is unclear whether this behaviour also occurs in ‘natural broods’ (i.e., without artificial injection of immature parasitoids into hosts) (Grbić et al., 1992; Giron et al. 2007). To our knowledge, larval siblicide has not previously been reported in gregarious Braconidae.

Contest competition was evidenced by direct observation of larval siblicide. Solitary parasitoids, including within Braconidae, are well known to exhibit siblicidal behaviour, and it is considered a necessary behaviour because the relatively small size of their hosts usually only allows for the development of a single parasitoid (Balduf, 1926; Mayhew et al., 1999; Pemberton et al., 1918; Pexton et al., 2002; Salt, 1961; Willard, 1920; Yu et al., 2008). These species are known to either puncture the integument of their rivals and leave them draining of their haemolymph if they have piercing mandibles, or to messily chew any part of their rivals' bodies if they have chewing mandibles (Salt, 1941; Yu et al., 2008). However, larvae of *C. vanessae*, which have chewing mandibles, were observed to always be cut in a straight-across section, suggesting an acquired efficiency. Sibling rivalry seems to take place only to reduce the number of competitors within the host, as cannibalism didn't appear to occur. Gregarious *Cotesia* are known to display interspecific competition through physical and physiological competition, and intraspecific competition via scramble competition only (Fidgen et al., 2000; Reitz et al., 1995; Takagi, 1986; Waage et al., 1984). Theoretical work suggests that gregariousness has evolved multiple times from solitary ancestors within the genus *Cotesia* and their relatives, and that for this evolution to be possible, individuals must lose either their antagonistic behaviour toward their siblings or their motility (Mayhew, 1998; Mayhew et al., 1999; Michel-Salzat et al., 2004; Pexton et al., 2009; Pexton et al., 2002, 2004, 2005; Pexton et al., 2003). Observations here showed that *C. vanessae* is siblicidal and likely also has some motility in order to reach and kill siblings in the host's hemocoel, yet it is gregarious.

It has been hypothesized that spines may aid the locomotion of larvae between host tissues (Pinheiro et al., 2010). This hypothesis is supported by observations that show that first and last instar *Cotesia flavipes* and *C. marginiventris* have both functional mandibles and cuticular spines, but their second instar have neither (Boling et al., 1970; Pinheiro et al., 2010; Yu et al., 2008). The same observations were made with *C. vanessae* (for the last instar, a single row of small spines was observed on the middle of each thoracic and abdominal segment dorsally, and what looked like pairs of pseudopods ventrally). Both first and last instars need locomotion, to reach rivals and to egress from the host, respectively. In addition, observations showed that first instar *C. vanessae* did not appear to have a caudal appendage to aid their mobility. Thus, their mobility within the host is likely limited. The transition from first to second instar being accompanied with a loss of both physical antagonism and cuticular spines could suggest that spines also provide first instars with some protection against physical attacks from siblings. As previously noted, another defense against other parasitoids is to quickly kill surrounding contestants before being killed by them (Salt, 1941).

Larvae of *C. vanessae* displayed protective mechanisms to survive attacks from the host's immune system and possibly also from their siblings. To acquire protection against encapsulation they produce teratocytes. These are macrocells released into the host's hemocoel that serve various functions, including production of *Bracovirus* that target and destroy the host's hemocytes (Dahlman, 1990, 1991). Observation from dissected *H. zea* showed that in some hosts, parasitoids from the same brood survived encapsulation while others did not. It is unknown what caused this in the current study. It has previously been observed that given certain host species and rearing temperatures, a few parasitoids from a brood could successfully hatch from encapsulated eggs (Blumberg, 1977; Blumberg et al., 1991-1992; Blumberg et al., 2001). Other studies found that the rate of encapsulated individuals decreased as clutch size (i.e., number of eggs a parasitoid laid within a host) increased (Kapranas et al., 2012; Kitano et al., 1978). This suggests that clutch size and the timing of egg hatching for some individuals may have prevented them from succumbing to encapsulation.

Cotesia vanessae are very fertile. As previously stated, adult female *C. vanessae* can parasitize many hosts in a short amount of time, and appear to remain very fertile throughout their adult life. Considering the high number of larvae killed through sororicide and yet the large size of broods produced by hosts raised the question of whether this species was polyembryonic or if it carried a large egg load. The absence of males in the laboratory colony suggested that polyembryony was a reasonable hypothesis, but it was invalidated by observation of single, non-dividing, eggs within hosts, from the time they were laid until they hatched. The hypothesis of large egg load was validated by the observation of large number of eggs within the ovaries of a female. Although, the large number of eggs combined with their small size and their tendency to stick to each other and to the pin used for dissection didn’t allow for a count.

Unlike some other parasitoid species (Charnov et al., 1984; Hasan et al., 2010; Zaviezo et al., 2000), female *C. vanessae* did not appear to adjust clutch size according to host size, and that a supernumerary number (i.e., an excess number) of eggs is almost always laid within hosts. Supernumerary larvae are then destroyed by their siblings through contest competition, thus self-adjusting the size of their brood. This adjustment appears imperfect as dissected hosts that contained clutches small enough that all individuals would likely have had sufficient resources to mature also contained a large number of larvae cut in half. Nonetheless, it is hypothesized that this self-regulation of brood size by the larvae has the advantage of maximizing the use of all available resources within a host, and to better compete with other parasitoid species. This gives the ability of *C. vanessae* to develop within hosts of a wide range of sizes. The egression of few, sometimes even single, parasitoids from small hosts was observed (these parasitoids developed slowly, and hosts were parasitized at an early stage). This suggests the ability of *C. vanessae* to undergo facultative gregarious development. In solitary species, the laying of supernumerary eggs within hosts has been attributed to be an adaptation for efficiently competing in multiparasitized hosts (i.e., hosts parasitized by more than one parasitoid species). In this case, all parasitoid larvae compete until only one survives. For these, a larger number of offspring within a host means a greater chance that one of them survives (Pexton et al., 2005). In the western Mediterranean area, *Cotesia vanessae* must compete with a number of other parasitoid species, particularly throughout the winter as hosts become scarce (Stefanescu et al., 2012). In such an environment, the presence of supernumerary siblings within a host would likely provide a competitive advantage. A negative consequence of this evolutionary strategy is that females must dedicate resources to producing supernumerary eggs, whose fate is to die. Other *Cotesia* species that have been reported to display both gregarious and solitary development and to develop in both micro- and macrolepidoptera, such as *C. jucunda* (Marshall) (Yu et al., 2016), may display similar behavioural adaptations.

Another biological aspect of some populations of *C. vanessae* is thelytoky (Hervet et al., 2014; Stefanescu et al., 2012). Symbiotic bacteria in the genus *Wolbachia* are known to induce thelytoky in some insects (Czarnetzki et al., 2004; Dong et al., 2006; Kumm et al., 2008; Louis et al., 1993). These bacteria have previously been detected within *Cotesia* species (Rattan et al., 2011). Using primers for the *Wolbachia*-specific *wsp* gene and methods reported in previous studies (e.g., Floate et al., 2006, Li et al. 2015), the presence of *Wolbachia* within our laboratory *C. vanessae* was tested, but not detected. Thelytoky can be induced by other effects, including other symbiotic bacteria (Zchori-Fein et al., 2001). Elucidating the origin of thelytoky in this species was not the goal of this study, so was not investigated further. Thelytokous populations of *C. vanessae* are known to occur in the Mediterranean region, but male-female populations are known from other regions of Europe (Stefanescu et al., 2012). This indicates that the North American population may have originated from the southwestern Palearctic or Afrotropic regions, perhaps accidentally introduced with *Chrysodeixis chalcites*, a species also endemic to these regions whose appearance in southern Ontario coincides with that of *C. vanessae* (Hervet et al., 2014; Murillo et al., 2013).

An interesting observation was the premature death of Lepidoptera larvae caused by *C. vanessae*, to such extent that all parasitized *S. frugiperda* died without producing parasitoids. Such extreme cases of mutually antagonistic interactions are rarely reported. There were indications that within *S. frugiperda*, parasitoid larvae were killed by the caterpillar's immune system shortly after hatching. These caterpillars continued to develop apparently normally until they reached their final instar, but failed to pupate. Their digestive system eventually stopped functioning, which was evidenced in some species by the anus of individuals not fully closing and partly expelled droppings remained attached and dried there (particularly observed in *T. ni*), and food accumulation within their gut (particularly observed in *S. frugiperda*). These larvae remained in their final instar much longer than non-parasitized individuals (Fig. 3.9). If parasitism prevented the pupation of some or all individuals in some species, it didn't in other species. Some species even seemed to be only partly affected. This was particularly evident with *Agrotis ipsilon,* for which most individuals had a prolonged larval development time and eventually died, or turned into larval-pupal intermediates that died following pupation. Other *A. ipsilon* died as larvae producing parasitoids, or produced normal pupae that matured to adults. These individuals didn't seem to display a delay of development time. This shows that parasitism can yield a wide range of possible outcomes, even amongst individuals of the same species. It also shows that this parasitoid species appears more adapted for development using certain host species compared to others. Previous authors found that wasp calyx fluid, alone or combined with venom, polydnaviruses, and teratocytes alone, can induce some or all of these effects. For example, it has been showed that *Chrysodeixis includens* larvae injected with 0.01 to 0.10 wasp equivalent calyx fluid from *Microplitis demilotor* Wilkinson (Hymenoptera: Braconidae) developed into non-viable larval-pupal intermediates; while *Heliothis virescens* was relatively unaffected by injections of any amount of *M. demilotor* calyx fluid (Strand et al., 1991). It has also been shown that a combination of venom and calyx fluid injected by female *Cardiochiles nigriceps* (Viereck) (Hymenoptera: Braconidae) during oviposition suppress the ability of the host's prothoracic glands to produce ecdysteroid, thus inhibiting the ability of the host to pupate (Tanaka et al., 1991). It has also been shown that a polydnavirus (*Bracovirus*) released by parasitoids can inhibit the feeding and gut contractions of their hosts, possibly to prevent the host from predating on emerging parasitoid larvae (Beckage, 2012; Cooper et al., 2010; Pruijssers et al., 2009), and arrest development of the host at the prepupal stage (Lanzrein et al., 2012; Soller et al., 1996). Finally, it has also been shown that the presence of braconid teratocytes in *H. virescens*' hemocoel could prolong the larval stage of this noctuid, followed by either its pupation or its death, and the pupation resulted in either a normal pupa or a non-viable larval-pupal intermediate (Vinson, 1970; Zhang et al., 1989). The type of response was associated with the number of teratocytes injected, the age of these teratocytes, and the maturity of the host (Zhang et al., 1989).

Results show that the North American population of *Cotesia vanessae* displays adaptations that should enhance survival in different environments, including those with low host availability and high interspecific competition; i.e., 1) Adult females are opportunistic in their selection of hosts and are able to develop within numerous host species. They carry large egg loads, they lay supernumerary eggs within hosts, and their larvae display an antagonistic behaviour, which allows them to: 2) maximize the available resources to produce large broods whenever possible, 3) develop within hosts of a wide range of sizes, 4) display high intra- and inter-specific competitiveness. In addition, their ability to facultative diapause allows them to survive in environments with hot and cold winters. These ecological adaptations, and the known geographic range of this species (Hervet et al., 2014), are indications that *C. vanessae* should be able to colonize and thrive in many parts of North America, and that it may become an important parasitoid species for the natural biological control of noctuid pests of certain crops. However, field studies would be needed to elucidate this. The results show that *Cotesia vanessae* has a broad fundamental host range on Noctuidae species, but to uncover its ecological host range it would be necessary to investigate the plant species that are most attractive to this parasitoid, and more generally the host finding and selection processes, and how the diet of the host influences parasitoid development and fitness (Haye et al., 2005).

Table 3.1. Identity of the species studied ("-" = "none").

Scientific name	**Common name**	**Status**	**Suitability for *C. vanessae***	**Winter diapause (North America)**	**Collection stage**	**Collection method**	**Collection location**
Hymenoptera							
Cotesia vanessae (Reinhard)	-	Beneficial		In host	In host	Hands	Essex and Chatham-Kent counties (ON)
Lepidoptera							
Abagrotis sp.	-	Pest	Suitable	Larva	Adult	Hands	Agassiz (BC)
Abagrotis reedi Buckett	-	Pest	Suitable	Larva	Adult	Hands	Agassiz (BC)
Actebia balanitis (Grote)	-	Not a pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Aglais milberti (Godart)	Milbert's tortoiseshell	Not a pest	Suitable	Adult	Larva	Hands	Lethbridge (AB)
Agrotis ipsilon (Hufnagel)	black cutworm	Pest	Suitable	Pupa	Egg	Purchased	Benzon Research (PA)
Agrotis vancouverensis Grote	Vancouver dart	Not a pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Amphipyra tragopoginis (Clerck)	mouse moth	Not a pest	Suitable	*unknown*	Larva	Hands	Lethbridge (AB)
Anagrapha falcifera (Kirby)	celery looper	Pest	Suitable	Larva	Adult	Sweep net	Lethbridge (AB)
Anaplectoides prasina (Denis & Schiffermüller)	green arches moth	Not a pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Anarta trifolii (Hufnagel)	clover cutworm	Pest	Suitable	Pupa	Adult	UV trap	Lethbridge (AB)
Anticarsia gemmatalis Hübner	velvetbean caterpillar	Pest	Not suitable	-	Egg	Purchased	Benzon Research (PA)
Apamea devastator (Brace)	glassy cutworm	Pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Apamea lignicolora (Guenée)	wood-coloured quaker	Not a pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Apamea sordens (Hufnagel)	rustic shoulder-knot	Pest	Suitable	Larva	Larva	Hands	Lethbridge (AB)
Autographa californica (Speyer)	alfalfa looper	Pest	Suitable	Pupa	Larva	Sweep net	Lethbridge (AB)
Caradrina Morpheus (Hufnagel)	mottled rustic	Not a pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Chrysodeixis includens (Walker)	soybean looper	Pest	Suitable	Pupa	Egg	Purchased	Benzon Research (PA)
Cryptocala acadiensis (Bethune)	catocaline dart	Not a pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Dargida diffusa (Walker)	wheat head armyworm	Pest	Suitable	Pupa	Larva	Hands	Brooks (AB)

Eurois occulta (Linnaeus)	great brocade	Pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Euxoa auxiliaris (Grote)	army cutworm	Pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Euxoa messoria (Harris)	darksided cutworm	Pest	Suitable	Egg	Adult	UV trap	Lethbridge (AB)
Euxoa ochrogaster (Guenée)	redbacked cutworm	Pest	Suitable	Egg	Adult	UV trap	Lethbridge (AB)
Euxoa satis (Harvey)	-	Not a pest	Suitable	Egg	Adult	UV trap	Lethbridge (AB)
Euxoa tristicula (Morrison)	early cutworm	Pest	Suitable	Egg	Adult	UV trap	Lethbridge (AB)
Feltia herilis (Grote)	master’s dart	Pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Feltia jaculifera (Guenée)	dingy cutworm	Pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Habrosyne scripta (Gosse)	scribe moth	Not a pest	Not suitable	Pupa	Adult	UV trap	Lethbridge (AB)
Helicoverpa zea (Boddie)	corn earworm	Pest	Suitable	-	Egg	Purchased	Benzon Research (PA)
Heliothis virescens (Fabricius)	tobacco budworm	Pest	Suitable	Pupa	Egg	Purchased	Benzon Research (PA)
Hyles euphorbiae (Linnaeus)	spurge hawk-moth	Not a pest	Not suitable	Pupa	Larva	Hands	Lethbridge (AB)
Lacanobia grandis (Guenée)	grand arches moth	Not a pest	Suitable	Pupa	Adult	UV trap	Lethbridge (AB)
Lacinipolia renigera (Stephens)	bristly cutworm	Pest	Not suitable	Egg	Adult	UV trap	Lethbridge (AB)
Lacinipolia sareta (Smith)	-	Not a pest	Not suitable	Larva	Adult	UV trap	Lethbridge (AB)
Mamestra configurata Walker	Bertha armyworm	Pest	Suitable	Pupa	Adult	UV trap	Lethbridge (AB)
Mythimna unipuncta (Haworth)	true armyworm	Pest	Suitable	Larva	Adult	UV trap	Edmonton (AB)
Noctua pronuba (Linnaeus)	winter cutworm	Pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Pieris rapae (Linnaeus)	small cabbage white	Pest	Not suitable	Pupa	Larva	Hands	Lethbridge (AB)
Sideridis rosea (Harvey)	rosewing moth	Not a pest	Not suitable	Pupa	Adult	UV trap	Lethbridge (AB)
Spaelotis clandestina (Harris)	w-marked cutworm	Pest	Suitable	Larva	Adult	UV trap	Lethbridge (AB)
Spilosoma virginica (Fabricius)	yellow woolly bear	Not a pest	Not suitable	Pupa	Adult	UV trap	Lethbridge (AB)
Spodoptera eridania (Stoll)	southern armyworm	Pest	Not suitable	-	Egg	Purchased	Benzon Research (PA)
Spodoptera exigua (Hübner)	beet armyworm	Pest	Not suitable	-	Egg	Purchased	Benzon Research (PA)
Spodoptera frugiperda (J. E. Smith)	fall armyworm	Pest	Not suitable	Pupa	Egg	Purchased	Benzon Research (PA)
Trichoplusia ni (Hübner)	cabbage looper	Pest	Suitable	-	Egg	Purchased	Benzon Research (ON)
Trichordestra lilacina (Harvey)	aster cutworm	Not a pest	Not suitable	Pupa	Egg	Hands	Lethbridge (AB)
Xestia c-nigrum (Linnaeus)	spotted cutworm	Pest	Not suitable	Larva	Adult	UV trap	Lethbridge (AB)

Figure 3.1. *Cotesia vanessae* parasitizing an early 4th instar *Trichoplusia ni*. The ovipositor is inserted and the wings, middle and hind legs are placed upward in a putative oviposition position.

Table 3.2. Lepidoptera pecies that didn’t produce *C. vanessae*.

Lepioptera species	**Number of larvae that:**	
	Survived	**Died**
Anticarsia gemmatalis	39	6
Habrosyne scripta	27	3
Hyles euphorbiae	12	5
Lacinipolia renigera	22	3
Lacinipolia sareta	31	3
Pieris rapae	12	27
Sideridis rosea	26	9
Spilosoma virginica	30	0
Spodoptera eridania	19	3
Spodoptera exigua	27	2
Spodoptera frugiperda	5	13
Trichordestra lilacina	19	39
Xestia c-nigrum	17	16

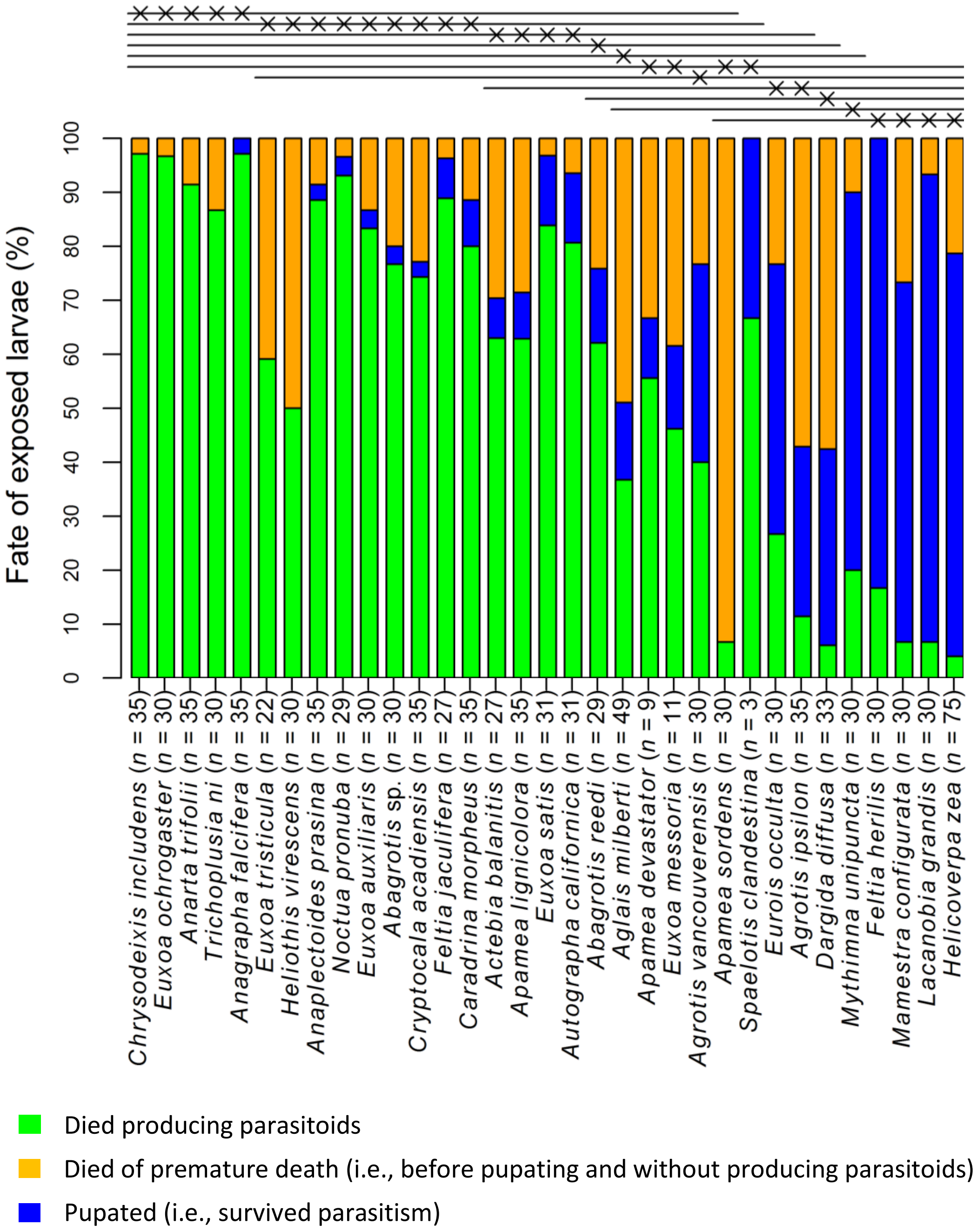


Figure 3.2. Fate of Lepidoptera larvae of species that produced *C. vanessae*. All individual larvae were parasitized, with the exception of *S. virginica* that could not be stung, likely because of its pubescence. Parasitism rate (comparing the number of larvae that “died producing parasitoids” with the number of larvae that “pupated”) of each host species was analyzed with Fisher’s exact tests comparing pairs of species (α = 0.05, corrected according to Bonferroni). Significance is indicated by horizontal lines above the boxes. Each “x” refers to a particular species, and the line it stands on indicates the species that didn’t have a significantly different parasitism rates from the species represented by the “x”.

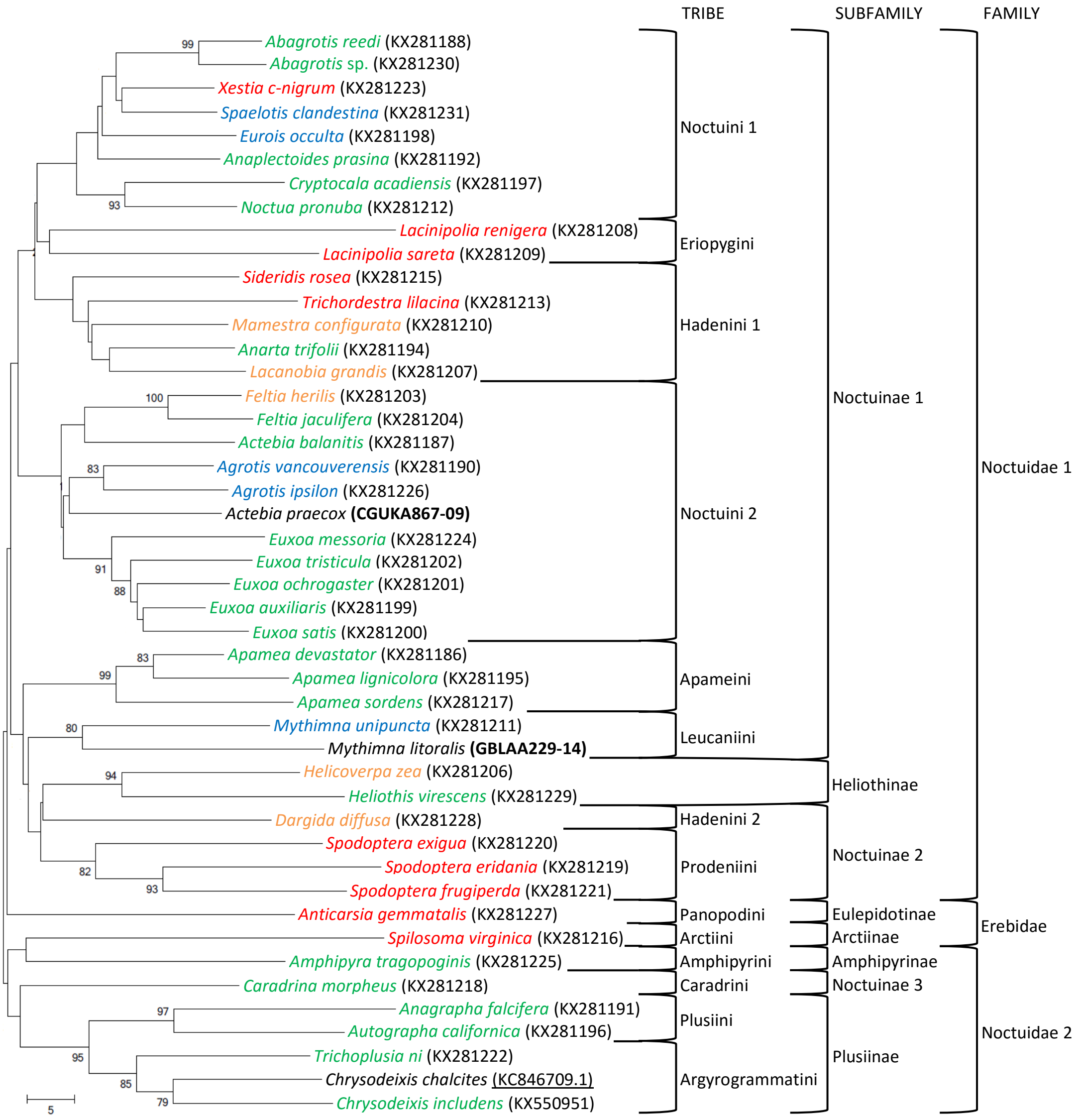


Figure 3.3. Neighbour-joining tree (Kimura 2-parameter distance) for CO1 sequences of known hosts of *C. vanessae* and other tested species within Noctuoidea, generated using Mega 5.2 (allowing for visual representation of relative interspecific divergence, but not a robust phylogenetic analysis). The scale bar represents the percent divergence of the Kimura 2-parameter. Bootstrap values indicate the percentage of times, out of 5000, that clades were paired with one another. Bootstrap values < 70 are not shown are these are considered too low to be meaningful. Accession numbers underlined and in bold font refer to sequences obtained from GenBank and BOLD, respectively. Other accession numbers refer to sequences generated from specimens from this study and deposited in GenBank. Colour of species represents *C. vanessae* parasitism success; i.e., green: excellent host (81-100% parasitism); blue: intermediate host (21-80% parasitism); orange: poor hosts (1-20% parasitism); red: not detected as a host; black: species that were considered reliably reported as hosts but were not tested.

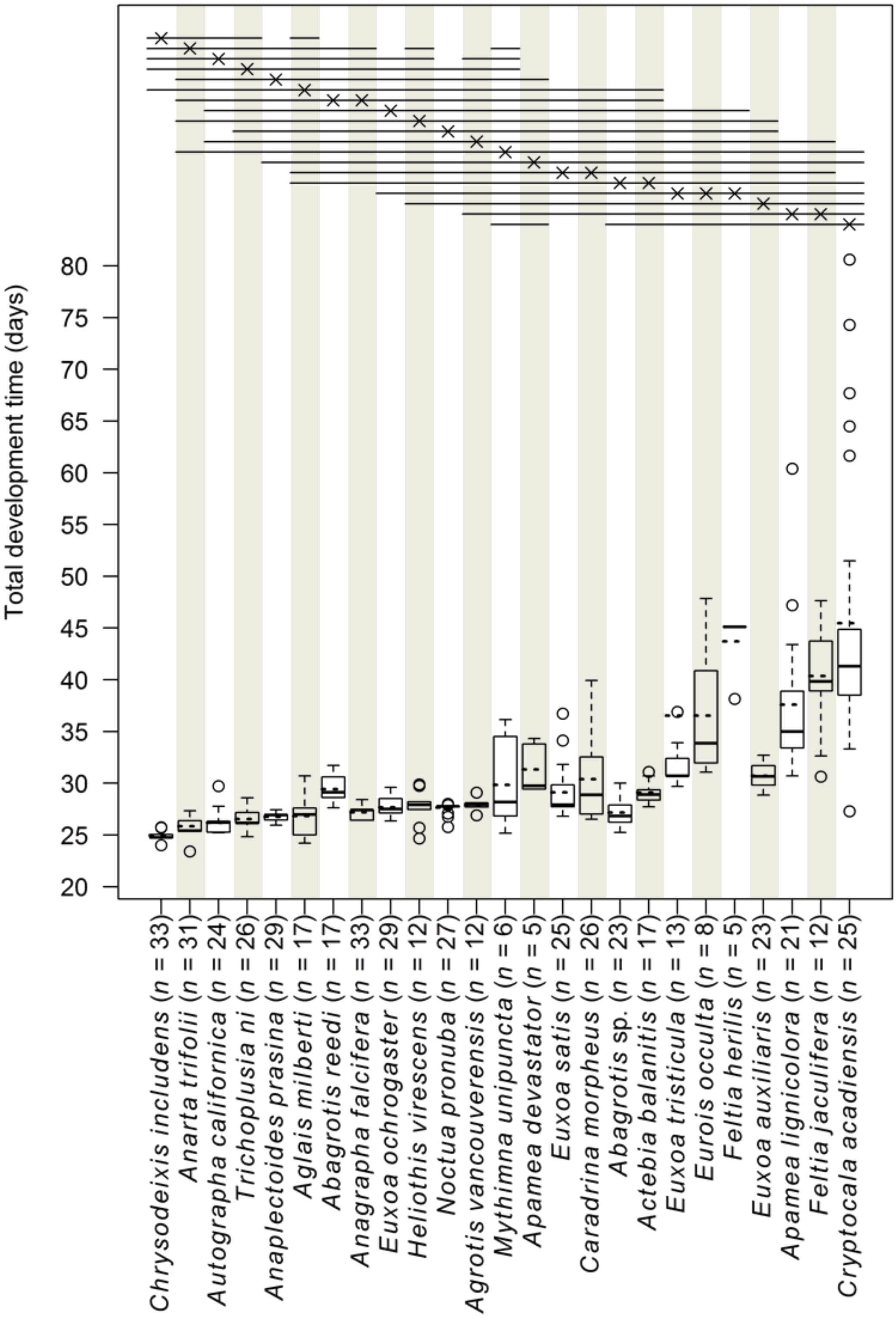


Figure 3.4. *Cotesia vanessae* total development time on each host species. Because of low statistical power, species represented by fewer than 5 individuals were excluded from analyses. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. Medians and means are represented by solid lines and dotted lines, respectively. Significance (Kruskal-Wallis test followed by post hoc Dunn test, α = 0.05 corrected according to Bonferroni) is indicated by horizontal lines above the boxes. Each species is represented by an "x" standing on a line that indicates other species with similar parasitism rates.

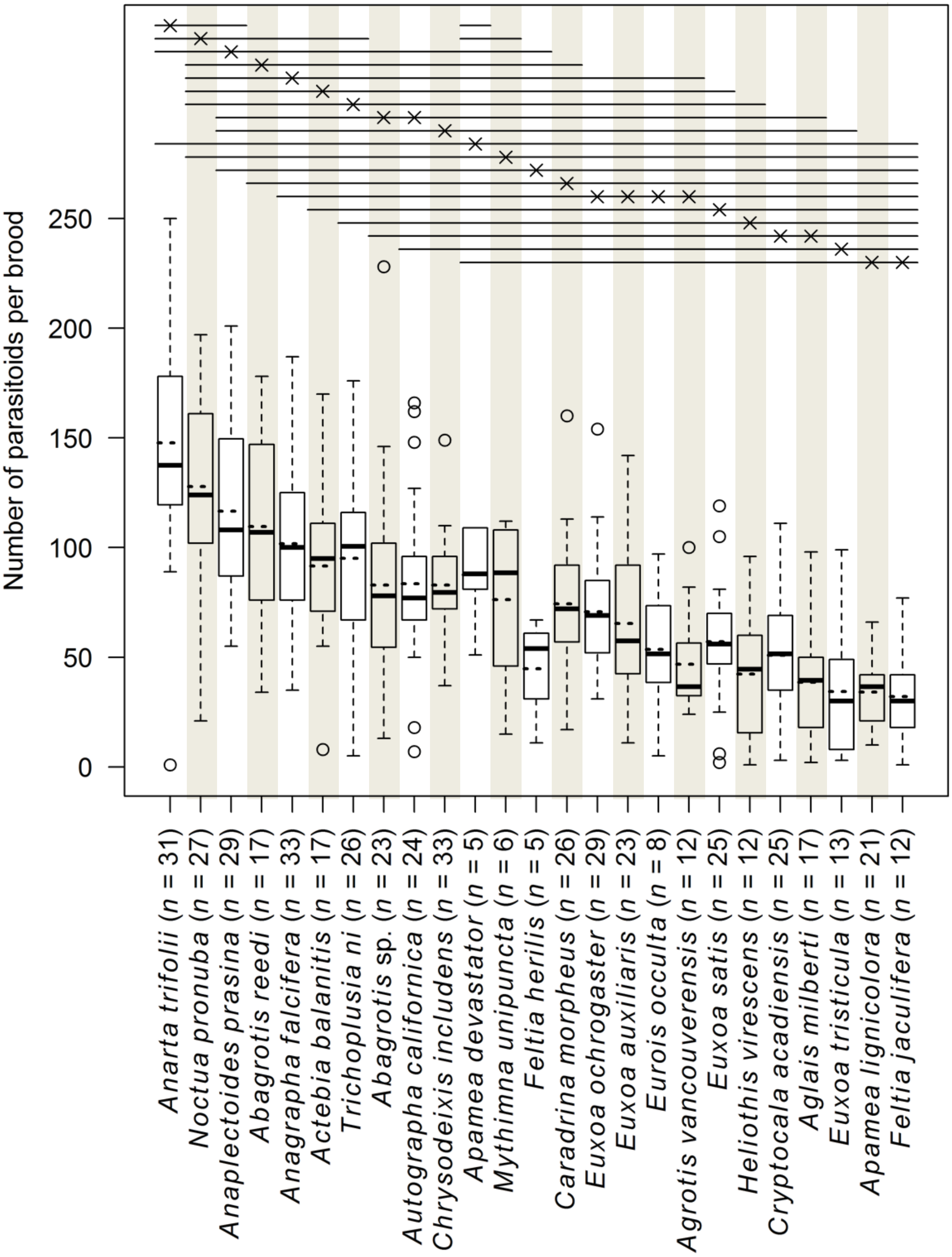


Figure 3.5. *Cotesia vanessae* brood sizes on each host species. Because of low statistical power, species represented by fewer than 5 individuals were excluded from analyses. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. Medians and means are represented by solid lines and dotted lines, respectively. Significance (one-way ANOVA followed by post hoc Tukey's HSD, α = 0.05) is indicated by horizontal lines above the boxes. Each species is represented by an "x" standing on a line that indicates other species with similar brood sizes.

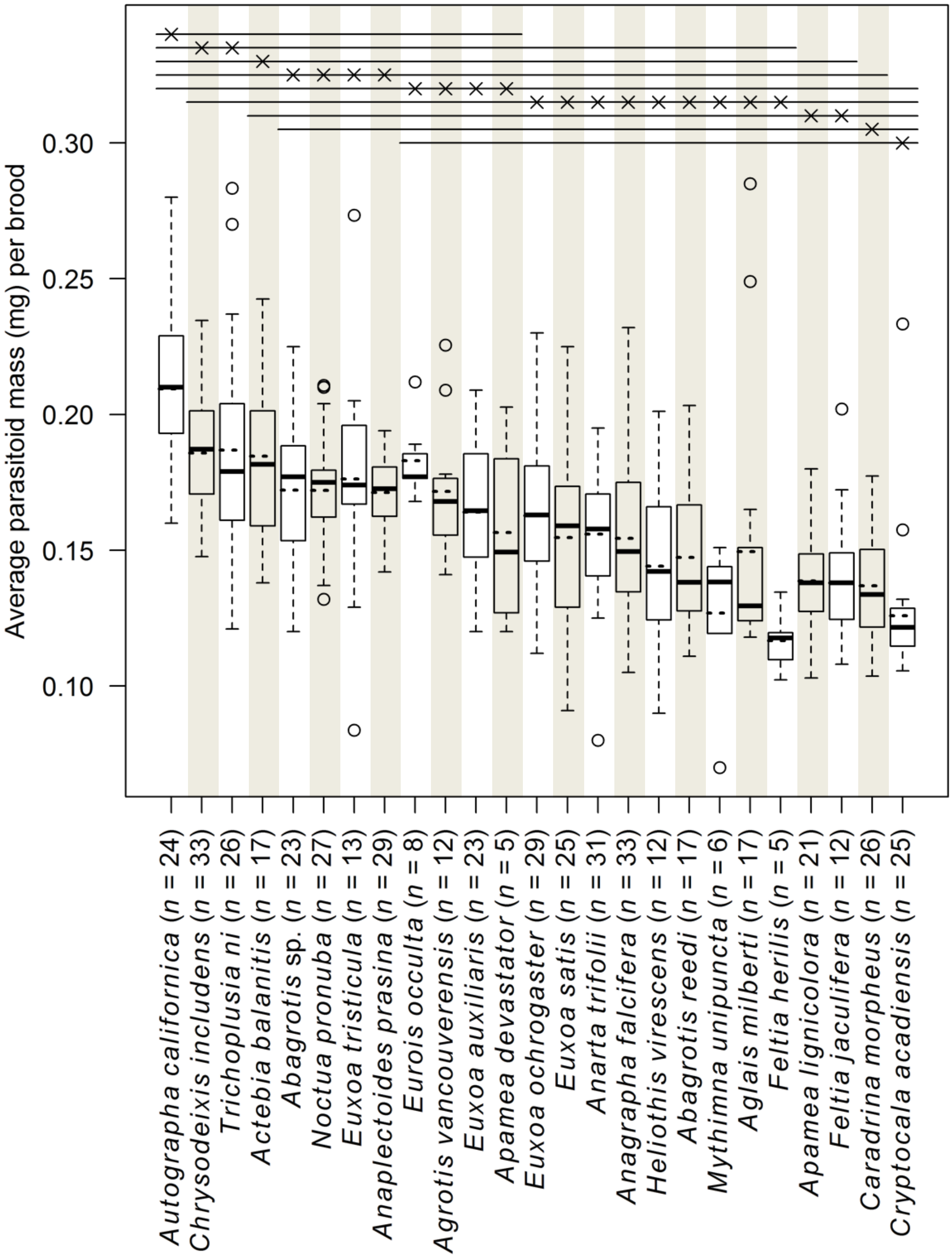


Figure 3.6. *Cotesia vanessae* average adult individual dry mass per brood (average of 30 or fewer wasps per brood) on each host species. Because of low statistical power, species represented by fewer than 5 individuals were excluded from analyses. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. Medians and means are represented by solid lines and dotted lines, respectively. Significance (one-way ANOVA followed by post hoc Tukey's HSD, α = 0.05) is indicated by horizontal lines above the boxes. Each species is represented by a "x" standing on a line that indicates other species with similar individual parasitoid mass.

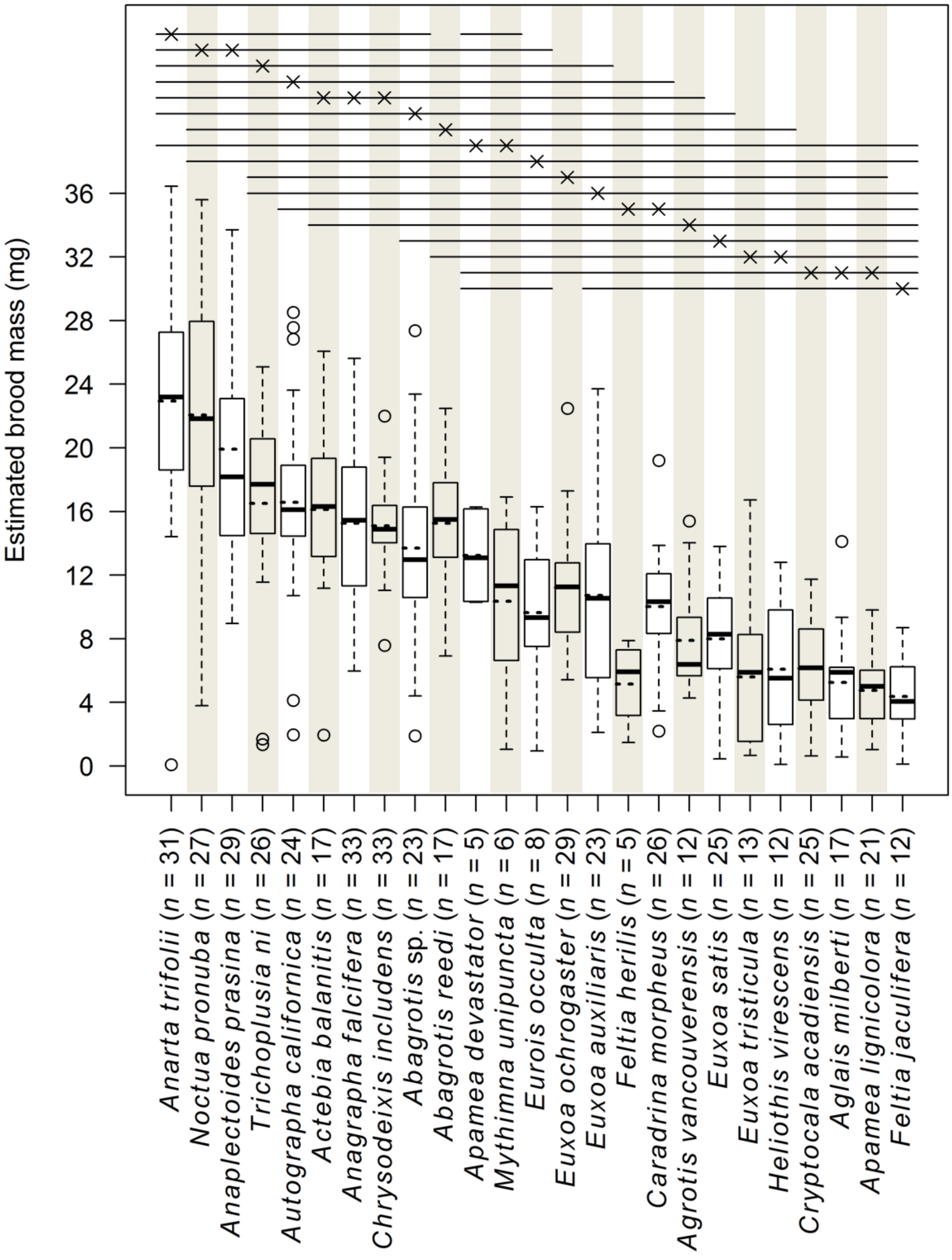


Figure 3.7. *Cotesia vanessae* estimated brood mass per host (average mass of 30 or fewer wasps per brood x brood size) on each host species. Because of low statistical power, species represented by fewer than 5 individuals were excluded from analyses. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. Medians and means are represented by solid lines and dotted lines, respectively. Significance (Kruskal-Wallis test followed by post hoc Dunn tests, α = 0.05 corrected according to Bonferroni) is indicated by horizontal lines above the boxes. Each species is represented by a “x” standing on a line that indicates other species with similar individual parasitoid mass.

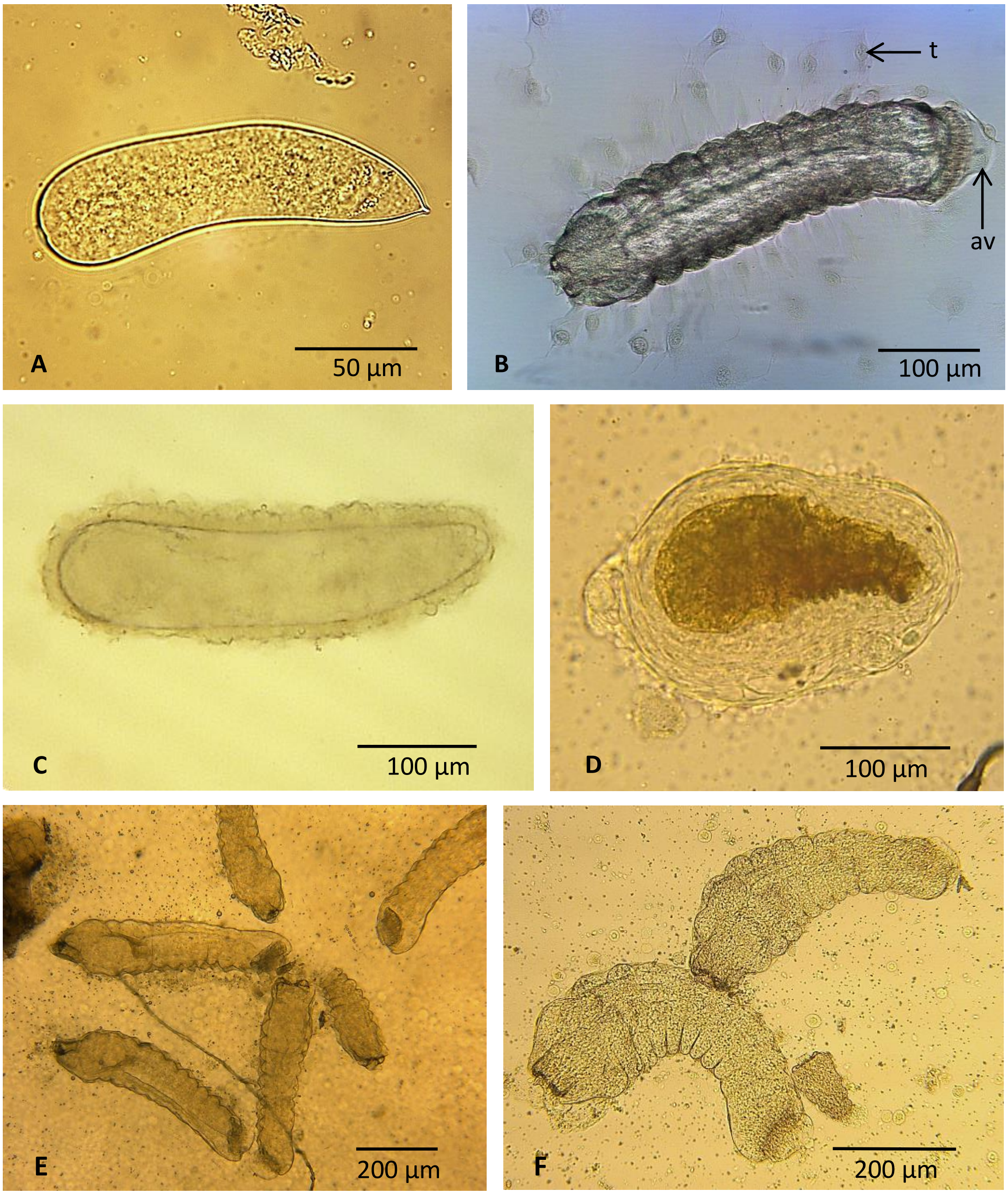


Figure 3.8. Immature *Cotesia vanessae* **A.** Egg, two days after parasitism within *Trichoplusia ni*; **B.** Neonate larva producing teratocytes, five days after parasitism within *Trichoplusia ni*. Head on the left, anal vesicle on the right, thoracic and first 7 abdominal segments each partly surrounded on their dorsal and lateral sides by a row of cuticular spines projecting backward, t: teratocyte, av: anal vesicle (photo courtesy of Stephanie Harris, Agriculture and Agri-Food Canada, Saskatoon, SK); **C.** Egg becoming encapsulated by hemocytes, four days after parasitism within *Helicoverpa zea*; **D.** First instar larva encapsulated, eleven days after parasitism within *Helicoverpa zea* larva; **E.** First instar larvae, seven days after parasitism within *Mythimna unipuncta*. One larva with open mouth (centre), and the fore half of a killed larva on its right; **F.** Larva biting a sibling, and a fore half of a younger larva on the bottom right, seven days after parasitism within *Mythimna unipuncta*.

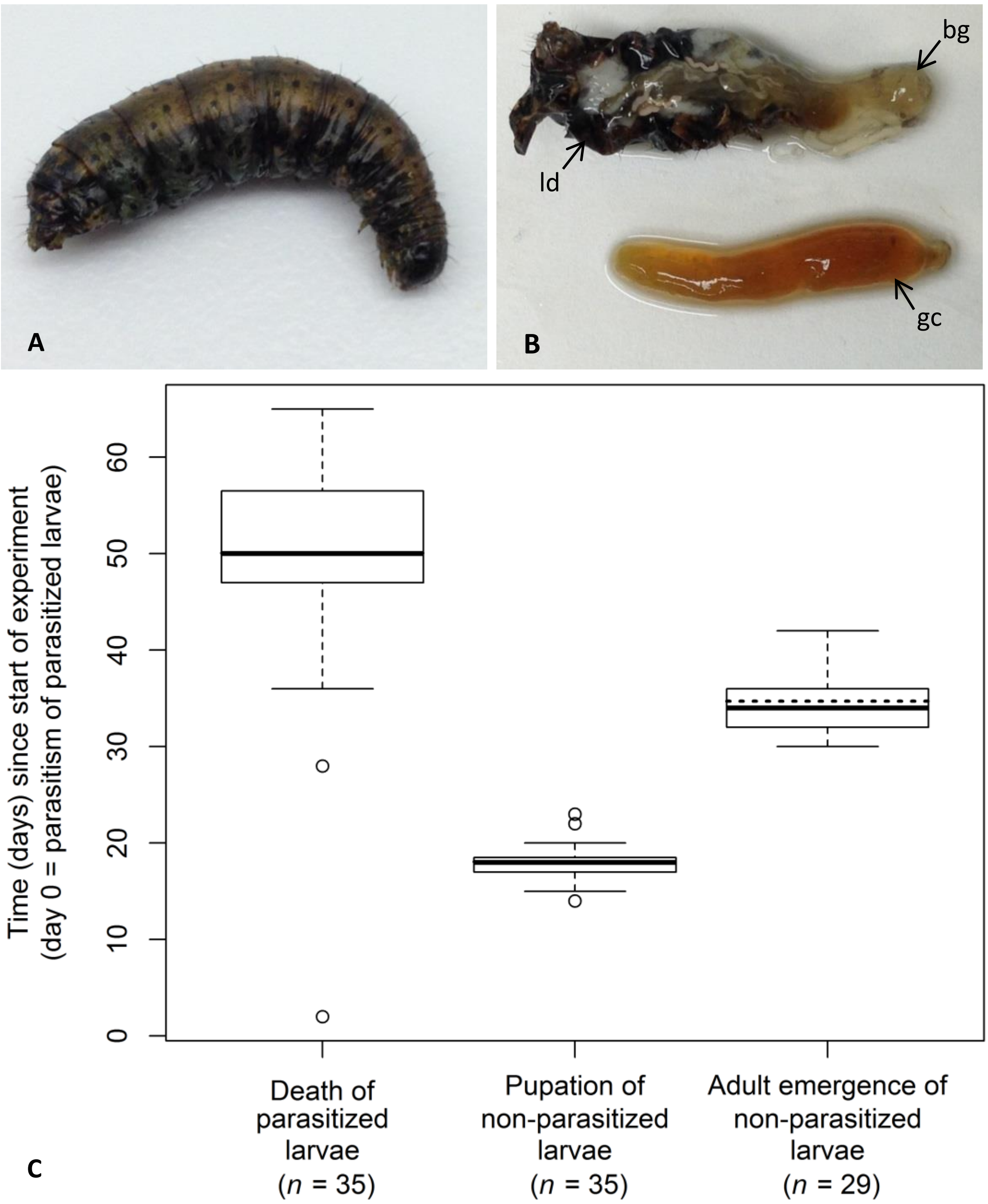


Figure 3.9. *Spodoptera frugiperda* **A.** Live larva 50 days after parasitism; **B.** Same larva dissected (ld), with bloated gut dissected (bg), and gut content extracted (gc); **C.** Boxplot showing time to death of parasitized *S. frugiperda* larvae compared to time to pupation and to adult emergence of non-parasitized individuals (six pupae died in the non-parasitized group). The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. Medians and means are represented by solid lines and dotted lines, respectively (non-visible mean lines are superposed with median lines).

CHAPTER 4: A REVIEW OF THE MCMORRAN DIET FOR REARING LEPIDOPTERA SPECIES WITH ADDITION OF A FURTHER 39 SPECIES

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4.1. Abstract

Research on cutworms led to the exploration the use of the McMorran diet to rear lepidopteran species, mainly Noctuidae, under laboratory conditions. This chapter reports the development of 104 lepidopteran species, including 39 species not previously reported in the literature, on this diet. Given its low cost, ease of preparation, and wide species' acceptance, this diet provides a powerful tool for facilitating Lepidoptera and other insects rearing and research in the laboratory.

4.2. Introduction

Laboratory colonies of herbivorous insects are commonly reared on artificial diets to reduce the labor, time, space, and associated costs of growing their host plants. These diets also simplify the synchronization of insect development with the availability of food and can be optimized to increase insect fitness above that of insects reared on natural foods (McMorran, 1965). Furthermore, the nutritional quality of these diets can be manipulated to facilitate research on topics including insect development, entomopathogens, insecticides, and plant resistance factors (George et al., 1960).

The McMorran diet was used to rear primarily pestiferous species of Noctuidae. This diet has been used as a suitable media to rear at least 154 species of insects, mainly Lepidoptera (Table 4.1). Adkisson et al. (1960) were the first to use wheat germ as an ingredient in an artificial diet they developed to rear the pink bollworm, *Pectinophora* *gossypiella* (Sauders) (Gelechiidae). This recipe was modified by Vanderzant et al. (1962) to rear the corn earworm, *Helicoverpa zea* (Boddie), and further modified by Berger (1963) to rear the corn earworm and the tobacco budworm, *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae). McMorran (1965) modified this later recipe to rear the spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae). Grisdale (1973) subsequently added linseed oil as an ingredient to this latter recipe to reduce the incidence of wing deformities observed in other Lepidoptera species.

There are publications that provide artificial diet recipes with rearing methods of insects and their relatives and it is not the objective here to review these articles. However, it is important to note the existence of key publications that summarize diet recipes and rearing procedures, such as Smith (1966), Singh (1977), Singh and Moore (1985), and Wong (1972).

This chapter provides a list of Lepidoptera that have been reared (i.e., larvae were able to develop) on the McMorran diet in previous studies and in the current study. Comments indicating the suitability of the diet for rearing success are indicated for each species (Table 4.1).

During four years of research on Lepidoptera, an increasing number of species that successfully develop on the McMorran diet was serendipitously discovered. However, assessing rearing success of insect species on this diet was not an objective so no data on specific fitness of each species reared are displayed. Nonetheless, the information presented here should greatly ease rearing of Lepidoptera larvae for many studies. The research conducted included rearing of field collected Lepidoptera larvae until either parasitoid egression or until they metamorphosed to adults for moths and parasitoids identification, and investigation of the host range of a parasitoid. The use of McMorran diet was ideal for these investigations. It allowed for a rapid handling of high numbers of field-collected caterpillars of various species. Use of the McMorran diet saved tremendous time, space, and money because it eliminated the need to grow plants and to develop species-based rearing knowledge as in most cases Noctuidae would develop on this diet. It also facilitated insect maintenance as the food remained good for at least 2 weeks at 4 °C and 5-7 days at room temperature (as per rearing conditions described under Materials and Methods section below), and the rearing cages and cups remained relatively clean until insects completed their development.

4.3. Materials and Methods

To compile the list of Lepidoptera previously reported to have been reared on the McMorran diet, research articles reported in the Web of Science, Scopus, Google, Google Scholar citing “McMorran 1965” and “Grisdale 1973” were examined (Table 4.1).

For laboratory use laboratory, McMorran diet ingredients were purchased from the Insect Production Services (IPS) (Great Lakes Forestry Centre, Canadian Forest Service, Sault Ste. Marie, ON, Canada). Ingredients were preserved for up to 6 months in air-sealed packages (excluding linseed oil and potassium hydroxide (KOH), which were kept in bottles) at 2.5 °C. Fresh diet was prepared on a weekly basis in our laboratory. This diet was held for up to 2 weeks at 4 °C before use.

The diet was prepared as per the recipe provided to us by Insect Production Services, which corresponds to that of Grisdale’s (1973) modification of the recipe developed by McMorran (1965). Ingredients for 2-liter diet: 1,720 ml distilled water, 34.72 g agar, 70 g casein, 70 g sucrose, 61.39 g toasted wheat germ, 20 g Wesson’s salt, 10 ml 4 M KOH, 10 ml linseed oil, 10 ml alphacel, 8 g ascorbic acid, 4.2 g aureomycin, 3 g methyl paraben, 2 g choline chloride, 1ml 37% formalin, 20 ml vitamin solution. Ingredients for 2 l vitamin solution: 2 l water, 2 g nicotinic acid, 2 g calcium pantothenate, 2 g riboflavin, 0.5 g thiamine hydrochloride, 0.5 g pyridoxine hydrochloride, 0.5 g folic acid, 0.04 g biotin, and 0.004 g vitamin B12. To better preserve the vitamins, 200 ml (for 2 l of diet) of water were replaced by 200 g of crushed ice, which was blended with the hot mixture in a blender for one minute just before adding the vitamin solution. This was implemented in order to reduce temperature of the mixture. When the diet was ready, the head foam was scraped away, and the diet was poured into 22 ml plastic cups. When the diet was set (~30 min after pouring), these cups were refrigerated until use. Considering that the ingredients were purchased already pre-divided for 2-liter batches, it took only one hour to prepare one batch including washing of equipment.

Most lepidopteran species were purchased as eggs or recovered from adults collected from UV light traps. Commercial sources were Insect Production Services (Sault Ste. Marie, ON, Canada) and Benzon Research Inc. (Carlisle, PA). Importation of species from the United States was done with import permits issued by the Canadian Food Inspection Agency (Permit No. P-2011-04397, P-2013-02134, and P-2014-02394). Light traps were operated in the city of Lethbridge, Alberta, Canada, during the summer and fall of 2012-2014. Additional species were collected as larvae or eggs on various plants in and near Lethbridge, and near Magrath (Cardston county), Alberta.

Eggs were recovered from field-collected adults by placing female moths in Plexiglass cages (40 cm by 34 cm by 30 cm [length x width x height]). A screened window (10 cm in diameter) on each side of the cage provided ventilation. The front of the cage had an opening (15 cm in diameter) with an attached cloth sleeve to provide access into the cage. Females were provided with sucrose crystals, water, and a choice of oviposition materials; i.e., cheese cloth, paraffin film ("Parafilm"), brown paper, paper towel, and fine sand. Eggs on the oviposition substrate were placed into new cages of the same design in which the hatched caterpillars were reared until pupation or until used in experiments. Usually about 50 caterpillars were reared per cage (or 30 for Plusiinae). Eggs obtained commercially were similarly handled. For species maintained for more than one generation, pupae were placed in clean cages of this same design for emergence, mating, and oviposition.

In the laboratory, insects were reared in growth cabinets at 20 °C, 70-80% humidity, and 12:12 (L:D) hours cycle under low incandescent and fluorescent lighting. Caterpillars were provided with blocks of diet (20 cm^3) positioned mainly near the sides and corners of the cage. Small pieces of paraffin film (~9cm^2) placed beneath and on top of each diet block facilitated its removal, reduced desiccation, and allowed caterpillars to feed while standing on the underside of the upper piece of the paraffin film (Fig. 4.1) as they would under a plant leaf in natural conditions. Fresh diet was added about every 5-7 days when the previous one became too old or dry. The diet had to be replaced about four times to sustain complete larval development of most species reared. For cages with first- to third-instar larvae, and any instar of Plusiinae (i.e., “loopers”), fresh diet blocks were placed as close as possible to the old blocks to facilitate the movement of caterpillars onto the fresh diet as these caterpillars have difficulty moving on the floor of the cage. Blocks of old diet were removed from cages after about 10 days.

Variously sized pieces of brown paper (laid flat or slightly crumpled) were placed in the cages of Noctuidae species (except Plusiinae) to provide sheltered areas. This noticeably reduced diurnal activity of the caterpillars and biting of each other. Cannibalism of aggressive species (e.g., bertha armyworm and corn earworm) was further reduced by placing an increasing amount of crumpled brown paper, using bigger cages, having fewer caterpillars in a cage, and adding of a few centimeters of vermiculite on the bottom of the cage (Fig. 4.2).

Lepidoptera larvae were also reared individually in translucent plastic (Polar Plastic Ltd.) 8 oz (240 ml) containers (Fig. 4.3). These included caterpillars hatched from eggs in the laboratory or collected outside (same species as these reared in cages). A non-threaded sewing machine was used to ease piercing of holes (0.5 mm in diameter) in the bottom and lid of containers to prevent the accumulation of condensation within. This reduced the incidence of mold on the diet and prevented early-instar larvae from becoming trapped and dying in water droplets. Each cup contained a piece of filter paper (55 mm in diameter) onto which a block of diet was placed (6.7 or 10 cm^3, depending upon the size of the larva), with a piece of paraffin film beneath and on top as described previously. Rearing conditions were as described for caterpillars reared in cages. The caterpillars pupated either in their cages or individual cups. They remained this way under the conditions described above until moth emergence.

4.4. Results and Discussion

The results of the literature search and laboratory studies are reported in Tables 4.1 and 4.2. Many studies do not report if the diet used contained linseed oil as per Grisdale's modification (1973) or any other modification. Table 4.1 reports Lepidoptera species successfully reared on the McMorran diet, whereas Table 4.2 reports species that failed to develop on this diet. Grisdale (1973) reports successfully rearing grasshoppers, *Melanoplus* spp. (Orthoptera: Acrididae) on the McMorran diet for at least one generation.

Rearing successive generations of Lepidoptera on nutritionally unbalanced diets can cause wing deformities (Morris 1967; personal observation), reductions in insect weight, fecundity, longevity, and increased mortality of all life stages (Morris, 1967). The suitability of the McMorran diet for rearing successive generations of the species listed in Table 4.1 typically was not assessed. Some species were unwilling or unable to feed on McMorran diet as early instars but fed on the diet in later instars to complete their development; i.e., *Hyles euphorbiae* (L.) (Sphingidae) (*n* = ~17; most adults had deformed wings) and *Polychrysia esmeralda* (Oberthür) (Noctuidae) (*n* = ~60; no deformed wings observed). These are indications that further modification of the McMorran diet is needed to maintain laboratory colonies of these species.

Minor modifications to the McMorran diet can enhance rearing success. For example, Syme and Green (1972) reared larvae of European pine shoot moth, *Rhyacionia buoliana* (Denis & Schiffermüller) (Tortricidae) with the addition of 1 ml soya bean oil per 100 ml of McMorran diet. Gardiner (1970) reared to adults larvae of 49 identified species (and additional unidentified species) of Cerambycidae (Coleoptera), 17 of them from the egg, by adding 5 ml of dried pulverized host plant material to 100 ml McMorran diet. Similarly, Trudel successfully reared immature stages of the white pine weevil, *Pissodes strobi* Peck (Coleoptera: Curculionidae), and maintained adults, by adding 5% pulverized white pine bark to the McMorran diet.

Other diets similar in composition to the McMorran diet include that of Adkisson et al. (1960), Vanderzant et al. (1962), and the "Vanderzant-Adkisson special wheat germ diet" (marketed by MP Biomedicals, headquartered in Santa Ana, CA), which have also been used to rear a wide range of Lepidoptera and some Coleoptera species. This ability has been attributed to their wheat germ base (Cohen, 2004).

Research that requires maintenance of laboratory colonies over multiple generations or aiming to study species performance should try to determine suitability of the McMorran diet for specific species, which may require adjustments of the recipe and further investigation into insect performance, to ensure maintenance of healthy colonies. Nonetheless, it is shown here that the McMorran diet can be used to facilitate the laboratory rearing of numerous species, both in cages and in 240 ml containers, and can likely be used to rear many more species than those reported here.

Table 4.1. Species of Lepidoptera reared (i.e., were able to grow) on McMorran diet as reported in the literature and from the current study.

Species	**Reference**	**Comments**
Erebidae (Arctiidae)		
Estigmene acrea (Drury) [Saltmarsh caterpillar]	(Barber et al., 1993); This study	Reared from egg to at least 3rd instar (Barber 1993); Partly developed larvae reared to adults (*n* = 3) (this study)
Hyphantria cunea (Drury) [Fall webworm]	(Morris, 1967)	Reared over one generation and a partial second generation, severe wing deformities, degeneration
Lymantria monacha (Linnaeus) [Nun moth]	(Grijpma et al., 1987)	Reared for at least one full generation
Orgyia antiqua (Linnaeus) [Rusty tussock moth]	(Grant, 1977)	Likely at least one full generation
Orgyia cana Edwards	(Grant, 1977)	Likely at least one full generation
Orgyia definita Packard [Definite tussock moth]	(Grant, 1977)	Likely at least one full generation
Orgyia leucostigma (J.E. Smith) [Whitemarked tussock moth]	(Grisdale, 1973; Percy et al., 1971)	Reared over multiple generations
Orgyia pseudotsugata (McDunnough) [Douglas-fir tussock moth]	Non-modified (Grisdale, 1973; Morris, 1970) and modified recipe (Lyon et al., 1966)	Reared over multiple generations
Spilosoma congrua Walker [Agreeable tiger moth]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Spilosoma virginica (Fabricius) [Yellow woollybear]	(Barber et al., 1993); This study	Reared from egg to adults (*n* = ca. 200)
Gelechiidae		
Filatima obscuroocelella (Chambers)	This study	Partly developed larvae reared to adults (*n* = 7)
Geometridae		
Lambdina fiscellaria fiscellaria (Guenée) [hemlock looper]	(Otvos et al., 1973)	Reared for at least one full generation

Operophtera bruceata (Hulst) [Bruce spanworm]	(Ives et al., 1980)	Reared from egg to pupa
Operophtera brumata (Linnaeus) [Winter moth]	(Feeny, 1968)	Reared at least from eggs to pupae; modified recipe
Lasiocampidae		
Malacosoma disstria Hübner [Forest tent caterpillar]	(Grisdale, 1973; Stairs, 1965)	Reared at least for one full generation
Noctuidae		
Abagrotis sp.[1]	This study	2nd instars reared to adults (*n* = ca. 80).
Abagrotis reedi Buckett	This study	Reared from egg to adult (*n* = ca. 50)
Actebias balanitis (Grote)	This study	Reared from egg to adult (*n* = ca. 20)
Actebia fennica (Tauscher) [Black army cutworm]	?	Mentioned on the IPS website (when visited in 2016)
Agrotis ipsilon (Hufnagel) [Black cutworm]	(Barber et al., 1993); This study	Reared for two generations (*n* = ca. 100)
Agrotis vancouverensis (Grote) [Vancouver dart]	This study	Reared from egg to adult (*n* = ca. 100)
Anagrapha falcifera (Kirby) [Celery looper]	(Barber et al., 1993); This study	Reared from egg to adult (*n* = ca. 80)
Anaplectoides prasina (Denis & Shciffermüller) [Green arches moth]	This study	Reared from egg to adult (*n* = ca. 20)
Anaplectoides pressus (Grote) [Dappled dart]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Anarta (Hadula) trifolii (Hufnagel) [Clover cutworm]	This study	Reared from egg to adult (*n* = ca. 100); few specimens with deformed wings
Anticarsia gemmatalis (Hübner) [Velvetbean caterpillar]	This study	Reared from egg to adult (*n* = ca. 70)
Apamea devastator (Brace) [Glassy cutworm]	This study	Reared from egg to adult (*n* = ca. 2)

Apamea lignicolora (Guenée) [Wood-colored Apamea]	This study	Reared from egg to adult (*n* = ca. 20)
Apamea sordens (Hufnagel) [Rustic shoulder-knot]	This study	Partly developed larvae reared to adults (*n* = 5)
Autographa bimaculata (Stephens) [Two-spotted looper]	This study	Last instar larvae reared to adults (*n* = 2)
Autographa californica (Speyer) [Alfalfa looper]	This study	Reared from egg to adults (*n* = ca. 200)
Autographa flagellum (Walker) [Silver whip]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Autographa precationis (Guenée) [Common looper]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Caradrina morpheus (Hufnagel) [Mottled rustic moth]	This study	Reared from egg to adults (*n* = ca. 100)
Caradrina multifera (Walker) [Speckled rustic moth]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Cerastis salicarum (Walker)[2] [Willow dart]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Chrysodeixis includens (Walker) [Soybean looper]	(Grisdale, 1973); This study	Reared from egg to adults (*n* = ca. 60)
Crocigrapha normani (Grote) [Climbing cherry cutworm]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Cryptocala acadiensis (Bethune) [Catocaline dart]	(Barber et al., 1993); This study	Reared from egg to adults (*n* = ca. 30)
Cucullia intermedia Speyer [Goldenrod cutworm]	This study	Three last instars reared to adults (*n* = 1); the younger instars didn't feed very willingly on the diet
Dargida diffusa (Walker) [Wheat head armyworm]	This study	Partly developed larvae reared to adults (*n* = ca. 20)
Diachrysia aereoides (Grote) [Dark-spotted looper]	This study	Partly developed larvae reared to adults (*n* = 5)

Diarsia rubifera (Grote) [Red dart]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Diarsia jucunda (Walker) [Smaller pinkish dart]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Eueretagrotis attenta (Grote) [Attentive dart]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Eupsilia tristigmata (Grote) [Brown fruitworm]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Eurois occulta (Linnaeus) [Great brocade]	This study	Reared from egg to adults (*n* = ca. 60)
Euxoa auxiliaris (Grote) [Army cutworm]	This study	Reared from egg to adults (*n* = ca. 50)
Euxoa messoria (Harris) [Darksided cutworm]	This study	Reared for two generations (*n* = ca. 150)
Euxoa ochrogaster (Guenée) [Redbacked cutworm]	This study	Reared for three generations (*n* = ca. 300); ca. 5% adults had deformed wings
Euxoa satis (Harvey)	This study	Reared from egg to adult (*n* = at least 1)
Euxoa tristicula (Morrison) [Early cutworm]	This study	Reared for five generations (*n* = ca. 600)
Feltia herilis (Grote) [Herald dart]	This study	Reared from egg to adult (*n* = ca. 30)
Feltia jaculifera (Guenée) [Dingy cutworm]	This study	Reared for two generations (*n* = ca. 60)
Fishia illocata (Walker) [Wandering brocade]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Helicoverpa zea (Boddie) [Corn earworm]	(Grisdale, 1973); This study	Reared for two generations (*n* = ca. 80)
Heliothis virescens (Fabricius) [Tobacco budworm]	This study	Reared for two generations (*n* = ca. 80)

Homorthodes furfurata (Grtote) [Northern scurfy quacker moth]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Hyppa xylinoides (Guenée) [Cranberry cutworm]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Lacanobia grandis (Guenée) [Grand arches moth]	(Barber et al., 1993); This study	Reared from egg to adult (n = ca. 40)
Lacanobia radix (Walker) [Garden arches moth]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Lacinipolia renigera (Stephens) [Bristly cutworm]	This study	Reared from egg to adult (n = ca. 40)
Lacinipolia vicina (Grote)	This study	Reared from egg to adult (n = ca. 50)
Leucania multilinea Walker [Many-lined Wainscot moth]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Mamestra configurata Walker [Bertha armyworm]	(Grisdale, 1973);This study	Reared from egg to adult (n = ca. 50); last instar was highly cannibalistic
Melanchra picta (Harris) [Zebra caterpillar]	This study	Partly developed larvae reared to last instar (n = 3); two of them died from parasitoids, the last one from disease.
Melanchra pulverulenta (Smith)	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Morrisonia latex (Guenée) [Fluid arches moth]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Mythimna unipuncta (Haworth) [True armyworm]	(Barber et al., 1993); This study	Reared for over five generations (n = ca. 1000)
Noctua pronuba (Linnaeus) [Large yellow underwing]	This study	Reared from egg to adult (n = ca. 20); few specimens with deformed wings
Orthosia revicta (Morrison) [Rusty whitesided caterpillar]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Peridroma saucia (Hübner) [Variegated cutworm]	(Birch et al., 1976)	Reared for four generations
Phlogophora iris Guenée [Olive angle shades moth]	(Barber et al., 1993)	Reared from egg to at least 3rd instar

Plusia putnami Grote [Lempke's gold spot]	This study	Last instars reared to adults (*n* = 2)
Pyrrhia exprimens (Walker) [Purple-lined sallow moth]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Sideridis rosea (Harvey) [Rosewing moth]	This study	Reared from egg to adult (*n* = ca. 20); most adults with deformed wings
Spaelotis clandestina (Harris) [W-marked cutworm]	This study	Reared from egg to adult (*n* = ca. 20)
Spodoptera eridania (Stoll) [Southern armyworm]	This study	Reared for three generations (*n* = ca. 200)
Spodoptera exigua (Hübner) [Beet armyworm]	This study	Reared for two generations (*n* = ca. 200)
Spodoptera frugiperda (J.E. Smith) [Fall armyworm]	This study	Reared for two generations (*n* = ca. 100)
Trichoplusia ni (Hübner) [Cabbage looper]	(Grisdale, 1973); This study	Reared for at least three generations, multiple times (*n* >10,000)
Trichordestra lilacina (Harvey) [Aster cutworm]	This study	Reared from egg to adult (*n* = 12)
Xestia c-nigrum (Linnaeus) [Setaceous Hebrew character]	(Barber et al., 1993); This study	Reared from egg to adult (*n* = ca. 40)
Xestia smithii (Snellen) [Smith's dart]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Xylena nupera (Lintner) [American swordgrass moth]	This study	Partly developed larva reared to adults (*n* = 1); wings of adult deformed
Zanclognatha pedipilalis (Guenée) [Grayish zanclognatha]	(Barber et al., 1993)	Reared from egg to at least 3rd instar
Pieridae		
Pieris napi (Linnaeus) [Green-veined white]	(Barber et al., 1993)	Reared from egg to at least 3rd instar

Pyralidae		
Dioryctria reniculelloides Mutuura & Munroe [Spruce coneworm]	(Hamel, 1977)	Field collected larvae to?
Dioryctria abietivorella (Grote) [Fir coneworm]	(Trudel et al., 1995)	Fourth instars to adults
Ostrinia nubilalis (Hübner) [European corn borer]	(Grisdale, 1973)	Reared for at least one full generation
Saturnidae		
Hyalophora cecropia (Linnaeus) [Cecropia moth]	(Grisdale, 1973)	Reared for at least one full generation
Sphingidae		
Daphnis nerii (Linnaeus) [Oleander hawk-moth]	(Retnakaran et al., 1985)	Reared over 30 generations
Hyles galii (Rottemburg) [Bedstraw hawk-moth]	This study	Penultimate instar to pupae (*n* = 5)
Manduca sexta (Linnaeus) [Tobacco hornworm]	?	Mentioned on the IPS website (when visited in 2016)
Tortricidae		
Acleris gloveranus (Walsingham) [W*estern* blackheaded budworm]	(Gray et al., 1993)	Field collected caterpillars reared until parasitoid emergence
Acleris variana (Fernald)[3] [Eastern blackheaded budworm]	?	Mentioned on the IPS website (when visited in 2016)
Archips cerasivorana (Fitch) [Uglynest caterpillar]	(Grisdale, 1973)	Reared for at least one full generation
Choristoneura conflictana (Walker) [Large aspen tortrix]	(Grisdale, 1973)	Reared for at least one full generation
Choristoneura fumiferana (Clemens) [Spruce budworm]	(Grisdale, 1973; McMorran, 1965)	Reared over multiple generations

Choristoneura occidentalis Freeman [Western spruce budworm]	(Clancy, 1991)	Reared over multiple generations; modified ingredients
Choristoneura pinus pinus Freeman [Jack pine budworm]	(Allen et al., 1958)	Reared for multiple generations, degeneration observed
Choristoneura rosaceana (Harris) [Obliquebanded leafroller]	(Barber et al., 1993)	Reared to at least 3rd instar

[1] Species called "*Abagrotis orbis* (Grote)" in Hervet et al. (2016b). Further investigations revealed that it could be another species. A CO1 sequence of this species was deposited into GenBank (GenBank accession number: KX281230) (http://www.ncbi.nlm.nih.gov/genbank/) for later reference.

[2] Species misspelled as "*Cerastsis salicarum*" in Hervet et al. (2016b)

[3] Species not listed in Hervet et al. (2016b)

Table 4.2. Lepidoptera species that did not successfully develop on McMorran diet in the present study.

Species	Comments
Drepanidae	
Habrosyne scripta (Gosse) [Lettered Habrosyne]	First instars (*n* = ca. 60)
Gelechiidae	
Syncopacma sp.	Partly developed larvae collected on *Lupinus argenteus* Pursh (Fabaceae) (*n* = ca. 10)
Hesperiidae	
Epargyreus clarus (Cramer) [silverspotted skipper]	Partly developed larvae (*n* = 2)
Nymphalidae	
Aglais milberti (Godart) [Milbert's tortoiseshell]	Partly developed larvae (*n* = ca. 60)
Coenonympha tullia (Müller) [Common ringlet]	First instars (*n* = ca. 15)
Limenitis arthemis (Drury) [White admiral]	Partly developed larvae (*n* = 3)
Papilionidae	
Papilio Canadensis (Rothschild & Jordan) [Canadian tiger swallowtail]	First instars (*n* = 1)
Papilio multicaudata Kirby [Two-tailed swallowtail]	First instars (*n* = 1)
Pieridae	
Colias philodice Godart [Clouded sulphur]	Partly developed larvae (*n* = ca. 20)
Pieris rapae (Linnaeus) [Small white]	Partly developed larvae (*n* = ca. 50)
Saturniidae	
Hemileuca eglanterina (Boisduval) [Sheep moth]	Partly developed larvae (*n* = 2)

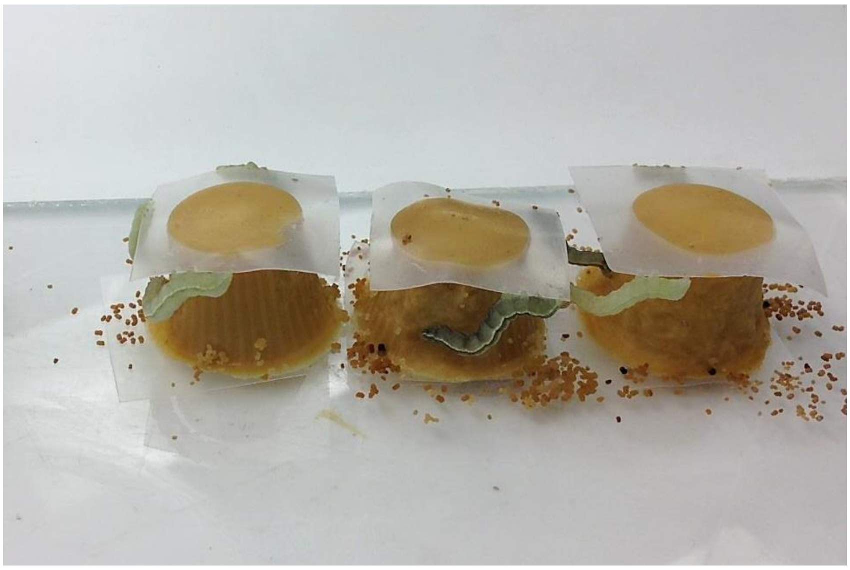

Figure 4.1. Set up used to rear caterpillars in cages on the McMorran diet (on this picture: *Trichoplusia ni*).

Figure 4.2. Set up for rearing cannibalistic species in cages. This picture shows a corner of a 60 x 47 x 44 cm Plexiglas cage used to rear 50 *Mamestra configurata* larvae (pupa visible in vermiculite). Three caterpillars died of disease, the others pupated, but none were cannibalized.

Figure 4.3. Polar Plastic Ltd. cup (240 ml) used to rear individual caterpillars (here a *Euxoa auxiliaris*).

CHAPTER 5: MULTI-TROPHIC EFFECTS OF PROTEIN-RESTRICTED DIET ON THE DEVELOPMENT AND FITNESS OF THE CABBAGE LOOPER, *TRICHOPLUSIA NI* (LEPIDOPTERA: NOCTUIDAE), AND ITS PARASITOID *COTESIA VANESSAE* (HYMENOPTERA: BRACONIDAE)

> "*It's a kind of scientific integrity, a principle of scientific thought that corresponds to a kind of utter honesty–a kind of leaning over backwards. For example, if you're doing an experiment, you should report everything that you think might make it invalid–not only what you think is right about it: other causes that could possibly explain your results* […]*. Details that could throw doubt on your interpretation should be given, if you know them.* […] *If you make a theory, for example, and advertise it, or put it out, then you must also put down all the facts that disagree with it, as well as those that agree with it.* […] *The idea is to try to give* all *of the information to help others to judge the value of your contribution; not just the information that leads to judgment in one particular direction or another.*"
> Richard P. Feynman (1999). Cargo Cult Science.

5.1. Abstract

Understanding the trophic effects of unbalanced diets on the parasitoids of crop-pest insects can improve our understanding of parasitism in the field. In this study, casein levels in an artificial diet were manipulated to test the effects of a diet primary metabolite: protein, on a Lepidoptera larva, the cabbage looper *Trichoplusia ni*, and on its parasitoid, *Cotesia vanessae*. Host larvae reared on diets with less than 25.5 g/L of protein exhibited delayed growth and lighter pupae. Similarly, parasitoids emerging from hosts reared on diets containing less than 25.5 g/L of protein exhibited delayed growth in the host and smaller broods. This shows that host development is linked to the protein content of its diet. Parasitoid fitness is likely linked to its host's diet protein content through variation of host quality. However, the percentage of hosts successfully parasitized and the mass of individual parasitoids were not affected by diet quality. An increase in cabbage looper death was observed only for individuals being reared on the most protein-poor diet (8.1 g/L of protein), which suggests that cabbage loopers can tolerate high variation in protein availability. As a compensatory mechanism, cabbage loopers increasing their food consumption on diets of lower protein content. Food consumption of cabbage loopers was also higher in parasitized individuals (only on high-

protein diets), likely to compensate for nutrients lost to parasitoids. No parental effect of hosts' diet quality on parasitoids was found: offspring of parasitoids that developed on caterpillars reared on high-protein diet and offspring of parasitoids that developed on caterpillars reared on the most protein-poor diet showed no difference of development time, brood size, individual parasitoid mass, and brood mass (offspring were reared on caterpillars fed the same high-protein diet). The results also show that *C. vanessae* can produce a large variety of brood sizes and individual parasitoid masses, and that average individual parasitoid mass is not affected by the quality of the food of its host. These aspects could contribute to the parasitoid success in different environments.

5.2. Introduction

The quality of diet for herbivores affects not only their fitness, but also the fitness of members of higher trophic levels. Most previous studies have investigated the effect of secondary metabolites; e.g., glucosinolates, terpenes, and toxins, in the food of insect herbivores on higher trophic levels (Hopkins et al., 2009; Katsanis et al., 2016; Ponsard et al., 2002; Price et al., 1980), but few studies have investigated aspects of nutritional balance by primary metabolites in tri-trophic contexts involving parasitoids (Bloem et al., 1990; Pimentel, 1966).

A key component of insect diet is nitrogen, predominantly from proteins. In theory, any nutrient can limit growth and fitness, but certain nutrients may never be limiting because of their excess in natural foods (Harrison et al., 2012). Proteins are often limiting in the natural diets of herbivores (Mattson Jr., 1980; Scriber, 1984; White, 1993). For example, small increases of protein in artificial diets, relative to that of natural foods, often increase the fitness of herbivorous insects (Huberty et al., 2006; McMorran, 1965; White, 1993). It has been hypothesized that if nitrogen is truly a limiting factor in the food of herbivores, natural selection should result in the adoption of strategies to maximize their intake of nitrogen-rich food sources (Mattson Jr., 1980; Milton, 1978). Indeed, “early-season feeders” are species that evolved to synchronize their main feeding stages with the occurrence of nitrogen-rich sprouts and seedlings (Baker, 1972; McNeil et al., 1978; Schwertzer, 1979). The preference of early-season feeding Noctuidae (e.g., cutworms) for seedlings and buds makes them particularly damaging as pests of crops. The effect of fertilizer to elevate levels of nitrogen in plants and subsequently the fitness of herbivorous insects is well known (Waring et al., 1992). However, an enhanced understanding of the ecology of crop-pest insects also requires knowledge of how variation in nitrogen content in the food of herbivorous insects affects the fitness of their natural enemies.

A number of studies have measured the fitness of insects and of their parasitoids by looking at development time, survival rates, length of body or of body part, body mass, female egg load, adult longevity, mating success, number of hosts parasitized, time spent searching for hosts, efficiency in exploiting host patches, etc. Body size (as measured by its length or mass) is the most common variable used by researchers to assess the fitness of parasitoids (Roitberg et al., 2001). It has been shown that body size strongly relates with other metrics of fitness, such as longevity and fecundity of parasitoids and other organisms (Bennett et al., 1998; Bloem et al., 1990; Denno et al., 1985; Gao et al., 2016; Godfray, 1994; Karowe et al., 1989; Kazmer et al., 1995; López et al., 2009; Nicol et al., 1999; Pimentel, 1966; Roff, 1986; Salt, 1941; Vet et al., 1994). In the current study, it was assumed that insects of larger body size had higher fitness, but a number of other fitness parameters were also considered.

Here, the fitness consequence of nitrogen-poor diets of an herbivorous insect on its parasitoid was examined in a laboratory experiment. Specifically, I manipulated the protein content of an artificial diet, the McMorran diet (McMorran, 1965), on which I reared cabbage loopers, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), to serve as hosts for the parasitoid *Cotesia vanessae* (Reinhard) (Hyenoptera: Braconidae). Diets containing different amounts of protein were used to test the following hypotheses: **1)** Because protein-restricted diets lead to reduced fitness for herbivores, decreasing proteins in McMorran diet should lead to a decrease in fitness and an increase of larval development time for *T. ni*; **2)** Because parasitoid larvae necessarily derive all of their nutrients from their hosts, parasitoids should have highest fitness and quickest developmental time when developing in hosts reared on high-quality diets; **3)** Because the resources provided by a host are finite and because parasitoid larvae egress in synchrony from the host, parasitoid larvae developing within the same host are expected to share among themselves all available resources thus should exhibit scramble competition; **4)** The metabolism of parasitods is highly efficient (i.e., a high proportion of the food ingested by the larva is transformed into parasitoid tissues, as illustrated by the small amount of meconium expelled by the parasitoid prior to pupating (Fig. 2.4), which is the only waste accumulated during larval development (Marian et al. 1982; Yu et al. 2008)), therefore, if there is indeed scramble competition, then the total mass of parasitoids produced by a host (= mass of the brood) should remain relatively constant and not be correlated with the number of parasitoids in the host (= size of the brood), when hosts develop on diets of similar quality. **5)** Considering the tolerance to low-quality diets observed in other polyphagous Noctuidae (Bloem et al., 1990; Karowe et al., 1989), the decrease of fitness of *T. ni* and that of *C. vanessae* are expected to be low or negligible on diets with small protein-restrictions, but significant on diets with high protein-restriction; **6)** Because insects in cocoon/pupae do not feed, the development time of *T. ni* and *C. vanessae* at these stages should not be affected by diet quality; **7)** Because *T. ni* likely needs to ingest a minimum amount of protein in order to complete its development, *T. ni* should increases its food consumption on protein-restricted diets; **8)** Because parasitoids consume nutrients from their host, to compensate for this loss it is expected that parasitized *T. ni* larvae would consume more food than non-parasitized larvae; **9)** As nutrients used to produce eggs are ingested by the mother, there could be an inter-generational effect in which the fitness of the progeny of parasitoids from hosts that developed on low-quality diets is lower than these from hosts that developed on higher quality diets, as has been found in previous studies (Bonduriansky et al., 2007; Einum et al., 1999; Gould, 1988; Hafer et al., 2011; Marshall et al., 2006; Wellington, 1976, 1977).

5.3. Materials and methods

5.3.1. Studied insects

A thelytokous strain of the gregarious larval koinobiont endoparasitoid *Cotesia vanessae* was used in the experiment. These individuals descended from parasitoids originally collected in 2009 in southern Ontario, Canada (Hervet et al., 2014). Females lay several eggs in the body of their hosts in a single oviposition event. The eggs hatch into larvae that feed on their host's haemolymph, and likely on fat tissues too, as do other *Cotesia* species (Harvey et al., 2008). When larvae reach maturity, they egress in synchrony from the sides of their host. If the host reaches larval maturity before the parasitoids do, the host ceases any activity until parasitoid egression, and never pupates. Immediately upon egression, larvae spin cocoons that collectively form a cocoon mass surrounded by frothy silk. In most cases, the host regains some movement immediately upon parasitoid egression, often crawls a few centimeters and dies within a day. Adult parasitoids usually emerge in synchrony from the cocoon mass. The hosts of *C. vanessae* are Lepidoptera larvae in the family Noctuidae, in tribe Nymphalini (Nymphalidae), and potentially in other taxa as well (Hervet et al., 2014).

The host used in this experiment is the cabbage looper *Trichoplusia ni*, a polyphagous pest of vegetables in North America (Capinera, 2001). Cabbage looper eggs were obtained from Insect Production Services (IPS), Natural Resources Canada, Sault Ste. Marie, Ontario, Canada. To facilitate rearing and to allow for protein manipulation in their diet, cabbage loopers were reared on artificial diet.

5.3.2. Diet preparation

The artificial diet was prepared as indicated in Hervet et al. (2016b), as per McMorran's recipe (1965) and Grisdale's modification (1973). Diet ingredients were purchased from IPS. In this artificial diet, the two main sources of nitrogen were casein and wheat germ. As casein is a pure protein while wheat germ contains other nutrients (USDA, 2015), the amount of casein but not that of wheat germ was modified. Diets with six different amounts of protein (Table 5.1) were used, with diet #1 being considered the control as it contains the original amount of casein (i.e., 35 g/L) as per McMorran's recipe (McMorran, 1965). Because dry wheat germ contains about 26.07 g protein /100 g (USDA, 2015), and because there is 30.695 g/L wheat germ in McMorran diet, each of the six diets contain about 8 g/L protein from wheat germ (Table 5.1). Diet #1 (containing 43 g/L of protein) and diet #6 (containing 8.1 g/L of protein) respectively contain greater and lower amounts of protein than that of the natural foods of cabbage looper. All other diets (diets #2 to #5) contain similar amounts of protein to known host-plants; e.g., Brussel sprouts, broccoli, cauliflower, as well as leaves of cabbages, spinach, lettuce, watercress, and dandelion (Capinera, 2001; USDA, 2015).

Because potassium hydroxide (KOH) is used in the McMorran diet to dissolve the casein, correspondingly reduced amounts of KOH were used in the preparation of diets with reduced amounts of casein (Table 5.1). To avoid artificially increasing the concentration of all other diet components, the same mass of extracted casein + KOH was replaced by pure cellulose (purchased as Alphacel (or Alpha-Cel^{TM}) from MP Biomedicals, Santa Ana, California, USA). Cellulose is already an ingredient in the McMorran diet and is the main structural component of the cell walls of plants, thus a natural component of *T. ni* 's natural diet; e.g., leaves of the cilion cabbage, *Brassica oleracea* L. variety *capitata* L. (Brassicaceae), which is a host plant of *T. ni*, are about 8% cellulose (Komolka et al., 2012). Because caterpillars cannot digest cellulose (Martin, 1983; Martin, 1991), increasing its amount in the diet was an appropriate way of maintaining the concentration of the non-casein components. Fresh diet blocks were kept in sealed plastic bags in a Styrofoam cooler in a 4 °C refrigerator until needed.

5.3.3. Insect handling

Upon receipt, *Trichoplusia ni* eggs were placed at room temperature and hatching was monitored. On the first day of egg hatching, neonate caterpillars were placed individually in translucent plastic containers (240 ml, Polar Plastic Ltd.) (Fig. 4.3 (Chapter 4)). Filter paper (55 mm in diameter) was placed at the bottom of each container, on top of which was placed a 6.7 cm^3 block of diet. On top and beneath each diet block, squares (9 cm^2) of paraffin film (Parafilm®) were placed to slow evaporation and allow the caterpillars to stand beneath the upper film while feeding, as they would under a leaf in natural conditions. The blocks of diet were replaced every ~5 days as they became too old (turned from yellow to orange) or dry. The containers were placed in shuffled locations in the growth cabinet every time the diet was replaced. Small holes were pierced on the bottom and on the lids of the containers beforehand to reduce the humidity, to avoid the development of mould on the diet and caterpillar frass as well as the formation of droplets within the containers' walls that are death traps for neonate caterpillars. Throughout the experiment the insects were reared in a growth cabinet at 70 to 80% humidity and a 12:12 h light-dark cycle under low incandescent and fluorescent lighting. The temperature of the cabinet was adjusted so that the insides of the rearing containers were at a constant 20 °C, monitored by temperature loggers placed inside of two containers.

Caterpillars were either allowed to complete development to adults or were parasitized by *C. vanessae*. For the latter group, caterpillars that recently molted to their 4th instar were exposed to *C. vanessae* until the parasitoid's ovipositor was inserted once. It was previously determined that this stage showed the best success for *C. vanessae* (higher host survival rate, larger parasitoid brood size, and higher attractiveness to parasitoids compared to earlier stages; and, higher rate of successful parasitism and lower physical resistance to parasitoid attacks of hosts compared to later stages). Each oviposition event lasted approximately 10 seconds. For consistency, caterpillars were not kept if parasitoids inserted their ovipositors more than once, or if there was suspicion that they might have just probed and rejected the host; i.e., if the thrust lasted less than one second, and if the parasitoid didn't put its wings, mid- and hind lend upward, which was considered a sign of oviposition (Fig. 3.1). These two groups (i.e., parasitized and non-parasitized) were themselves subdivided into 6 subgroups, which represented the diets containing 6 different amounts of protein (Table 5.1). On each diet, a target number of 45 caterpillars were allowed to develop to adults and a target number of 45 were parasitized (final *n* values were between 39 and 45, with 10 of the 12 treatment combinations having *n* = 45; Table 5.2). For logistical reasons, 10 and 35 replicates of each treatment were initiated on February 28th and June 17th, 2014, respectively. Supplementary caterpillars were reared on the diet containing 8.1 g/L of protein due to the high rate of "premature death" (i.e., died as larvae and without producing parasitoids) on this particular diet.

5.3.4. Measurements

5.3.4.1. Data inclusion / exclusion

A small number of data points were excluded due to minor errors during data collection. Sample sizes for the same treatments indicated in boxplots may vary between figures because within individuals a missed data point for one variable did not always impede collection of another variable (e.g., failing to record development time of an individual did not hinder the measurement of its mass). No outliers were excluded from the analyses.

5.3.4.2. Measurements for non-parasitized *T. ni*

The effect of diet on *T. ni* was assessed by measuring the development time and survival of larvae, and the mass of pupae (male, female). In order to allow for the pupae exoskeletons to harden before manipulation and to make consistent measurements, pupae were removed from their cocoons the day following pupation. They were then weighed using a 1 mg precision balance and sexed using morphological features (Fig. 5.1). Individuals that did not reach pupation (i.e., died of premature death or produced parasitoids) were neither sexed nor weighed.

5.3.4.3. Variables measured for *C. vanessae*

The effect of host diet on *C. vanessae* was assessed by measuring the development time inside the host (eggs then larvae) and inside the cocoon mass (prepupae then pupae), the number of parasitized hosts from which parasitoid larvae egressed, the number of adult parasitoids produced per host, and the dry mass of both individual parasitoids and parasitoid broods. Brood mass was assessed using an average individual mass calculated from the mass of 30 (or fewer depending on availability) intact adults haphazardly chosen from each brood and weighed simultaneously.

To test for an inter-generational effect of host quality, the fitness of F2 parasitoids whose mothers developed in hosts fed high- and low-quality diets were compared. Specifically, 14 parasitoids (from different mothers) that developed on hosts fed the control diet (containing 43 g/L of protein) and 14 parasitoids that developed on hosts fed the diet containing the lowest amount of casein (containing 8.1 g/L of protein) each inserted their ovipositor once into one early 4th instar *T. ni*. These caterpillars were reared on the control diet in the same conditions as described above. The development and fitness of these F2 parasitoids were measured using the same criteria as for the F1 parasitoids described above.

5.3.4.4. Measurement of food eaten by *T. ni*

The amount of food consumed by caterpillars was also measured to help estimate the expected relative damage of noctuid larvae on high- versus low-quality food sources, and of parasitized versus non-parasitized caterpillars. Knowing the extent of feeding in these different situations would help plan integrated pest management strategies, particularly in regard to the use of *C. vanessae* to control noctuid pests, given that the parasitized pests will not be killed right away and may continue to feed.

To estimate the amount of food consumed by caterpillars, the mass of moist diet blocks was measured just prior to being given to caterpillars using a 1 mg precision scale. After 5 to 7 days the partly eaten diet blocks were removed from the experimental containers and placed in individual 57 x 89 cm paper envelopes, which were placed in a dryer for 12 to 14 months (the samples were likely dry after a few days but the unit was also used for storage) before being weighed again using the same scale. The dry diet was weighed immediately after being removed from the dryer to prevent re-hydration. The mass lost between the two weighing events was of two natures: diet eaten by caterpillars and drying. Because I didn’t know how much mass of each of ingredient would be lost by drying, I measured it experimentally using 90 diet blocks (15 of each of the 6 diet types) of about 22 ml from the same diet batches used to feed the caterpillars in the experiment. These diet blocks were not fed to caterpillars but were manipulated in the same time and in similar ways as the experiment to mimic experimental conditions. The percent mass lost by drying of these blocks was found to be significantly different between diet types ($F_{5, 84} = 82.86$, $P < 0.001$), which corresponded to the mass of KOH used in these diets. The results of this manipulation were used to estimate the dry mass of food consumed by each caterpillar in the experiment.

5.3.4.5. Data analyses

Data for non-parasitized *T. ni* were analyzed using two-way ANOVA tests with diet and sex as explanatory variables and larval development time, pupal mass, and food eaten as response variables. Data for parasitized *T. ni* were analyzed using a one-way ANOVA with diet as the explanatory variable and food eaten as response variable (sex of host was not a variable because *T. ni* were sexed based on pupal morphology, and since *C. vanessae* kills its hosts before they pupate, parasitized individuals were not sexed).

Data for *C. vanessae* were analyzed using one-way ANOVA tests with diet as the explanatory variable and development time in host, development time in cocoon, total development time (i.e., in host + in cocoon), brood size (i.e., number of parasitoids produced per host), average individual parasitoid dry mass, and brood mass (i.e., estimated dry mass of all the parasitoids from a brood) as response variables. ANCOVA tests were used to investigate the effects of diet type and development time within the host on cocoon development time, the effects of diet type and brood size on individual parasitoid mass, the effects of diet type and brood mass on individual parasitoid mass, the effects of diet type and brood size on the mass of broods, the effect of diet type and brood size on development time in the host, and to investigate the effect of diet type and brood size on food consumption. Each of these tests provide evidence to support or not support hypotheses mentioned in the Introduction.

χ^2 tests were used to compare the rates of premature death (i.e., percent *T. ni* larvae that died before pupating and without producing parasitoids) of both non-parasitized and parasitized *T. ni*, and to compare the rate of successful parasitism (i.e., number of *T. ni* that produced parasitoids compared to these that survived to pupation) between diet types.

When an ANOVA test detected a treatment effect, post hoc Tukey’s range tests were performed to identify which treatments differed. When an interaction between the main effects was detected in two-way ANOVA, interaction plots were used to identify the nature of the interaction. When an interaction between the main effects was detected in ANCOVA, scatterplots and post hoc Pearson product-moment correlations with Bonferroni correction for $\alpha = 0.05/6 = 0.0083$ were used to identify the nature of the effects and which treatments differed. When a χ^2 test was significant, comparisons between pairs of treatments using χ^2 with Bonferroni correction for $\alpha = 0.05/(((6x6)-6)/2) = 0.0033$ were performed to identify which treatments differed.

Assumptions of normal distribution and homoscedasticity of samples were verified using residual-by-predicted plots. None of the samples showed strong deviations from these assumptions. All means reported ± SEM. All analyses were conducted in R version 2.14.1 (R Development Core Team, 2011).

5.4. Results

The eventual outcomes of the caterpillars used in the different treatments in this experiment are detailed in Table 5.3. In this section, no to moderate protein reduction refers to 43 to 25.5 g/L of protein (diets #1, #2, and #3), whereas pronounced protein reduction refers to 16.75 to 8.1 g/L of protein (diets #4, #5, and #6).

5.4.1. *Trichoplusia ni*

Lower casein content of diet led to significantly longer development time of *T. ni* larvae (two-way ANOVA, $F_{5, 233} = 462.42$, $P < 0.001$) when the protein reduction was pronounced (Fig. 5.2). There was no significant effect of sex on larval development time (two-way ANOVA, $F_{1, 233} = 3.30$, $P = 0.07$), nor was there an interaction between sex and diet type (two-way ANOVA, $F_{5, 233} = 0.63$, $P = 0.72$).

The mass of male pupae was significantly greater than that of female pupae (two-way ANOVA, $F_{1, 232} = 53.53$, $P < 0.001$). Pronounced protein reduction led to lower pupal mass (two-way ANOVA, $F_{5, 232} = 56.47$, $P < 0.001$) (Fig. 5.3). There was no significant interaction between sex and diet type (two-way ANOVA, $F_{5, 232} = 1.42$, $P = 0.22$).

5.4.2. *Trichoplusia ni* rates of survival and successful parasitism

Rate of premature death of *T. ni* larvae was significantly different between diet types for both non-parasitized ($\chi^2 = 50.27$, $df = 5$, $P < 0.001$) and parasitized ($\chi^2 = 15.05$, $df = 5$, $P = 0.01$) groups (Table 5.3). Pairwise χ^2 comparisons found that premature death was only higher on the 8.1 g/L protein diet compared to each of the other diet types in the non-parasitized group, but found no significant difference in the parasitized group after Bonferroni correction, although it was visibly higher on the 8.1 g/L protein diet (Table 5.3). In the parasitized group this rate was artificially reduced because the number of larvae that died prior to the parasitism event was not recorded.

There was no difference of successful parasitism rate between the different diet types ($\chi^2 = 5.66$, $df = 5$, $P = 0.34$) (Table 5.3).

5.4.3. *Trichoplusia ni* food consumption

For non-parasitized caterpillars, decreasing diet quality (two-way ANOVA, $F_{5, 158} = 334.88$, $P < 0.001$), but not sex (two-way ANOVA, $F_{1, 158} = 0.01$, $P = 0.93$), was associated with increased food consumption. There was no significant interaction between diet quality and sex (two-way ANOVA, $F_{5, 158} = 0.45$, $P = 0.82$). Non-parasitized caterpillars developing on the 8.1 g/L protein diet consumed on average 3.63 times more food than these developing on the control diet (food consumption of 1.1710 ± 0.0397 g and 0.3227 ± 0.0090 g respectively). Parasitized caterpillars also consumed more food with decreasing diet quality (One-way ANOVA, $F_{5, 139} = 100.10$, $P < 0.001$); e.g., parasitized caterpillars that developed on the 8.1 g/L protein diet consumed on average 2.21 times more food than those developing on the 43 g/L protein diet (food consumption of 1.0743 ±0.0360 g and 0.4857 ±0.0134 g respectively) (Fig. 5.4).

There were significant main effects on food consumption of both parasitism status (two-way ANOVA, $F_{5, 330} = 28.15$, $P < 0.001$) and diet quality (two-way ANOVA, $F_{5, 330} = 388.18$, $P < 0.001$), as well as an interaction between these two main effects (two-way ANOVA, $F_{10, 330} = 4.31$, $P < 0.001$) (both sexes combined). On diets containing 43 g/L protein (control) to 11.5 g/L protein, parasitized caterpillars ate more food than their non-parasitized counterparts, but only on the diet containing 8.1 g/L protein (the lowest quality diet) did non-parasitized caterpillars eat more than parasitized ones (Fig. 5.4).

There was a significant interaction between diet type and *C. vanessae* brood size on *T. ni* food consumption (ANCOVA, $F_{5, 127} = 2.46$, $P = 0.04$), as well as a main effect of diet type (ANCOVA, $F_{5, 127} = 44.12$, $P < 0.001$), but no main effect of brood size (ANCOVA, $F_{1, 127} = 1.05$, $P = 0.31$). Scatterplots and Post hoc Pearson-product moment correlation showed a possible correlation between brood size and food consumption only on 8.1 g/L protein diet ($r = 0.55$, $df = 21$, $P = 0.007$).

5.4.4. *Cotesia vanessae* development time

Moderate protein reduction of the host's diet had no effect on *C. vanessae* development time within the host, but pronounced reduction significantly delayed development time within the host (one-way ANOVA, $F_{5, 183} = 21.78$, $P < 0.001$) (Fig. 5.5). There was a significant effect of the host's diet type on the development time of *C. vanessae* within their cocoons ($F_{5, 186} = 3.76$, $P = 0.003$). However, a post hoc test indicated that the only significant difference was between groups on 43 g/L protein diet and 11.5 g/L protein diet ($P = 0.013$) (Fig. 5.6). Pronounced protein reduction led to a significant increase of *C. vanessae* total development time (one-way ANOVA, $F_{5, 183} = 24.91$, $P < 0.001$) (Fig. 5.7).

There was a significant interaction between development time within the host and diet type on development time in cocoons (ANCOVA, $F_{5, 177} = 3.75$, $P = 0.003$). There was also a significant main effect of diet type (ANCOVA, $F_{5, 177} = 1.64$, $P = 0.002$), but not of development within the host (ANCOVA, $F_{1, 177} = 1.64$, $P = 0.20$). Post hoc Pearson-product moment correlation found a significant negative correlation between development time within the host and within the cocoon for the two highest quality diets (43 g/L protein diet : r = -0.71, n = 28, $P < 0.001$; 34.25 g/L protein diet : r = -0.53, $n = 32$, $P = 0.002$), but no correlations in the other diet types ($|r| \leq 0.14$, $n = 26$~38, $P > 0.05$) (Fig. 5.8). Thus, on the 43 g/L protein and 34.25 g/L protein diets, the parasitoid broods that developed faster within their host subsequently developed slower within their cocoons, and vice versa.

5.4.5. *Cotesia vanessae* numbers and mass

Hosts developing on lower-quality diets produced significantly fewer parasitoids per brood (one-way ANOVA, $F = 15.31$, $P < 0.001$) (Fig. 5.9), and broods of lower masses (one-way ANOVA, $F_{5, 183} = 21.26$, $P < 0.001$). However, there was no effect of diet type on the mass of individual parasitoids (ANOVA, $F_{5, 189} = 1.30$, $P = 0.27$) (Fig. 5.10). The size of broods reflects both the number of eggs laid in hosts and the survival rate of parasitoids.

ANCOVAs showed significant main effects of brood size ($F_{5, 183} = 152.82$, $P < 0.001$) and brood mass ($F_{5, 183} = 152.82$, $P < 0.001$) on parasitoid individual mass. There was no interaction between diet type and brood size ($F_{5, 183} = 1.17$, $P = 0.33$) and between diet type and brood mass ($F_{5, 183} = 0.27$, $P = 0.93$). This indicates that larger and heavier broods correspond with lower mass of individual parasitoids (Fig. 5.11).

There were significant main effects of diet type (ANCOVA, $F_{5, 183} = 165.89$, $P < 0.001$) and number of parasitoids per brood (ANCOVA, $F_{1, 183} = 1357.19$, $P < 0.001$) on the mass of broods. There was also a significant interaction between diet type and number of parasitoids per brood (ANCOVA, $F_{5, 183} = 5.50$, $P < 0.001$). Post hoc Pearson product-moment correlation found significant positive correlations between brood size and brood mass on each diet type ($r \geq 9.1$, $n = 27$ to 38, $P < 0.001$). A scatterplot indicated that, even if diets of reduced protein contents were associated with reduced brood size and mass, the correlation between brood size and mass was similar across diet types (Fig. 5.12).

There were significant main effects of diet type (ANCOVA, $F_{5, 175} = 28.25$, $P < 0.001$) and brood size (ANCOVA, $F_{1, 175} = 3.92$, $P = 0.049$) on *C. vanessae* development time. There was also a significant interaction between diet type and total development time (ANCOVA, $F_{5, 175} = 5.69$, $P < 0.001$). Scatterplots and post hoc Pearson product-moment correlation showed a correlation between brood size and development time only on 25.5 g/L protein diet ($r = -0.71$, $df = 26$, $P < 0.001$), mainly caused by the inexplicably long development time of five small broods (1 to 33 parasitoids).

5.4.6. *Cotesia vanessae* F2 development, numbers, and mass

There was no effect of diet (one-way ANOVAs, $P > 0.05$) on the measured fitness parameters for the F2 progeny of parasitoids emerging from hosts reared on control versus -99.7% casein diet; i.e., developmental time within the host, developmental time within cocoons, number of emergent wasps per host, mean dry individual adult mass per host, and the estimated total dry adult parasitoid mass per host (Fig. 5.13).

5.5. Discussion

Analyses did not detect a decrease of fitness for either *T. ni* and *C. vanessae* for diets containing at least 25.5 g/L of protein, but did detect a decrease of fitness for both *T. ni* and *C. vanessae* associated with a decrease of protein on diets containing less than 25.5 g/L of protein. This indicates that the decrease of fitness of *C. vanessae* is linked with that of its host. It also indicates that *T. ni* can develop on a wide range of protein concentrations in its diet, a tolerance that has also been observed in other polyphagous Noctuidae (Bloem et al., 1990; Karowe et al., 1989). Across all treatments, the number of premature deaths was consistently higher in parasitized groups (Table 5.3) (although not significant). Some of the parasitized *T. ni* that died prematurely appeared constipated, as did caterpillars observed in Chapter 3, an effect attributed to parasitism disabling the host's digestive system.

T. ni larvae appeared to exhibit a compensatory feeding response when reared on diets of reduced quality, which likely contributes to their ability to develop on diets of different quality. On diets containing 43, 34.25 and 25.5 g/L of protein, non-parasitized larvae had similar development time and pupal mass, but their food consumption increased for each reduction of protein. However, on diets containing 16.75, 11.5, and 8.1 g/L protein, although food consumption increased, larval development time increased and pupal mass decreased. Similarly, parasitized *T. ni* ate more food than non-parasitized ones, likely to compensate for nutrients consumed by parasitoids. This effect has been observed with other koinobiont endoparasitoid species (Shi et al., 2002), although the opposite effect has also been documented (Doetzer et al., 1998; Elzinga et al., 2003).

Another physiological effect detected with *C. vanessae* was the trade-off of time spent in the host and in the cocoon when hosts were reared on the two diets having the highest protein concentration; i.e., 43 and 34.25 g/L. This effect might be because larvae develop until they egress in synchrony from the host upon perception of probable egression cues. Thus, the longer larvae spend in the host, the more mature they are, and the less time they need to spend in the cocoon. This effect might not represent actual "egg-larva" development time (from laying of egg to pupation of larva) and "pupa" development time and might simply reflect the time spent in the cocoon as prepupa before pupating. Indeed, a few *C. vanessae* that didn't form a cocoon following their emergence from hosts allowed for observation that they displayed various length of time between their egression and their pupation. For these, pupation occurred between one to at least three days post egression. Thus, there might not be a trade-off between actual egg-larval and pupal development times.

This effect was not detected on lower-quality diets. This was perhaps because of the general increase of *C. vanessae* development time in the host (Fig. 5.5) when hosts were fed low-protein diets. Previous authors noticed that the egg-larval development time of both solitary and gregarious parasitoids was associated with the stage of the host being parasitized, with injection of eggs into earlier stages inducing longer parasitoid egg-larvae development time (Allen et al., 1991; Avilla et al., 1987; Bell et al., 2003; Elzinga et al., 2003; Nakamura et al., 2001; Obrycki et al., 1985; Shi et al., 2002). For the solitary endoparasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), this effect is attributed to the delayed development of the 1st instar parasitoid, which molts to 2nd instar only when the host reaches its 4th instar (Nechols et al., 1977). The closely related *Encarsia tricolor* Förster (Hymenoptera: Aphelinidae) displayed a similar increase in egg-larval development time when earlier stages of the host were parasitized, but in this case the effect is mainly due to a delay in egg hatching (Avilla et al., 1987). In the latter study, only when the pharate adult host is parasitized does the pupation time of the parasitoid increase. Otherwise, none of these studies found a correlation between parasitoid egg-larval and pupal or cocoon stages development time, as did the present study. In the current study, it seems unlikely that host maturity had any effect on parasitoid development time in the host as all the hosts were parasitized at the early 4th instar.

Parasitoid individual mass significantly decreased with increasing brood size and mass. This effect has also been found in previous studies (Fidgen et al., 2000; Reitz et al., 1995; Takagi, 1986; Waage, 1986; Waage et al., 1984). This effect can be explained by the finite resources provided by a host, which translates into scramble competition; i.e., finite resources are split between all individuals present, thus a higher number of parasitoids results in smaller individuals.

However, on all diets, larger broods were also heavier. This contradicts the hypothesis that in an environment of scramble competition all the usable resources are consumed and transformed into parasitoid mass, thus brood mass should not correspond to brood size. A possible explanation could be that larger broods have a longer development time or induced their host to feed more to allow the host to produce more resources (Elzinga et al., 2003). This does not appear to apply here because brood size was neither correlated with parasitoid development time nor host food consumption. Another possible explanation could be that with increasing brood size, parasitoids increasingly feed on less desirable host tissues. But if this was the case then why not always feed on these tissues if it creates a gain of mass with no cost to development time? Perhaps the cost of such behaviour, if it exists, could be due to a fitness variable that was not measure; e.g., longevity and fertility. Perhaps part of the answer can be found in Harvey et al. (2000), who argue that while tissue-feeding parasitoids favor maximization of body mass and usually display a trade-off between development time and body mass, haemolymph-feeding Microgastrinae show more complicated interactions with their host and favour fast development time over large body size. Haemolymph feeders do not consume all the available resources and their development time may not correlate with the amount of available resources (i.e., host size). Because *C. vanessae* larvae are gregarious and exhibit synchronized egression from the host, it could be that the intense pressure to develop quickly reduces the ability of parasitoid larvae to exploit all the resources from their host, perhaps by lack of time to explore all areas of the haemocoel, thus leaving behind haemolymph and fat in the unoccupied areas. Indeed, the concentration of parasitoids was observed to always be higher in the posterior part of the host, and with small broods there are often few individuals emerging from the thorax area of the host. Thus, I predict, although did not test, that body mass of individuals that developed in the posterior part should be lower than these that developed in the anterior part. This situation would explain why even small broods that do not consume all available resources display scramble competition and why brood mass increases with brood size.

If resources are truly limiting in a host, there should be a point at which brood size becomes so large that parasitoids exploit all the resources available and increase of brood size do not correspond to an increase of brood mass anymore. Fig. 5.12 shows that the largest parasitoid brood (from a host that developed on the 43 g/L protein diet) is not the heaviest one. This indicates a possible threshold (approximately 180 parasitoids per brood) at which an increase in brood size does not result in an increase of brood mass, and perhaps even a decrease of brood mass. Another parasitoid brood that contained over 180 parasitoids seemed to confirm this pattern (brood mass of 23.1 g for 201 parasitoids), but it was excluded from the analysis because the host was parasitized twice.

Although at the individual level, parasitoids from small broods have the ability to grow bigger and gain a fitness advantage, the greater number of smaller-size parasitoids produced by larger broods may not necessarily result in a disadvantage from the parents' perspective. The variation of parents' realized fitness due to the number or the size of offspring is dependent on ecological conditions; e.g., number of hosts available, competition with other parasitoids, availability in space and time of hosts (Vos et al., 2003). Thus, the ability of *C. vanessae* to produce a large diversity of brood sizes (Fig. 5.9) with individuals of various masses (Fig. 5.10) and its ability to maintain individual masses on hosts feeding on diets of different qualities (Fig. 5.10) would likely contribute to its success in different environments.

There was no inter-generational effect of poor-quality diet on the fitness of F2 parasitoids. Studies on other organisms from various taxa found that parents that developed in more favorable environments generally produced higher quality offspring (Bonduriansky et al., 2007; Einum et al., 1999; Gould, 1988; Hafer et al., 2011; Marshall et al., 2006; Wellington, 1976, 1977), although not always (Myers et al., 2011). However, differences in offspring quality might only be noticeable if offspring develop in low-quality environments, as compensatory mechanisms can help low-quality offspring to quickly improve from their initial poor condition in high-quality environments (Donelson et al., 2009; Rombough, 1994). In the present study, F2 *C. vanessae* developed in hosts being reared on high-quality diet (43 g/L protein), which is a favorable environment. This could be why offspring quality from high- and low-condition parents was similar.

The similar overall effects of host food quality on the development of *T. ni* and *C. vanessae* indicate that the fitness of the parasitoid is linked with the quality of the diet of its host's food. The fitness of *T. ni* and of *C. vanessae* follow similar patterns. This could indicate that parasitoid fitness is closely associated with host fitness. In this case, the association between host food quality and parasitoid fitness might only exist because of the associations between host food quality and host quality, and between host quality and parasitoid fitness. Because host mass decreased with decreasing food quality, and because parasitoids fully rely upon the availability of host tissues to develop, it may be that host mass is the only true factor influencing parasitoid fitness. In this study, the mass of parasitized hosts was not measured, but because host mass is closely associated with diet quality, we cannot link parasitoid fitness to diet quality independently of host mass. To investigate if parasitoid fitness is an indirect effect of host mass (or size), a future study comparing the fitness of parasitoids produced by hosts of similar mass and size reared on different quality diets would be necessary.

Table 5.1. McMorran diet manipulations. Total protein amount was estimated as to the amount of casein plus 8 g/L of protein from wheat germ.

Diet #	**Total protein amount (g/L)**	**Casein amount (g/L)**	**Casein % removed compared to original recipe**	**4 M KOH amount (ml/L)**	**Cellulose amount (g/L)**
1	43	35	0% (Control)	5	0.5
2	34.25	26.25	25%	3.75	9.25
3	25.5	17.5	50%	2.5	18
4	16.75	8.75	75%	1.25	26.75
5	11.5	3.5	90%	0.5	32
6	8.1	0.1	99.7%	0.15	35.4

Table 5.2. Summary of the different treatments at the start of the experiment, not accounting for replication later discarded due to errors and supplementary replications added to the 8.1 g/L protein diet to compensate for the high rate of premature death of caterpillars developing on this diet.

Diet #	**Protein amount (g/L)**	***n* non-parasitized caterpillars**[1]	***n* parasitized caterpillars**
1	43	45 (16♀, 29♂)	45
2	34.25	45 (22♀, 21♂)	45
3	25.5	39 (23♀,13♂)	42
4	16.75	45 (18♀, 18♂)	45
5	11.5	45 (20♀, 23♂)	45
6	8.1	45 (16♀, 19♂)	45

[1] Individuals that died before pupating were not sexed

Table 5.3. Outcome of *Trichoplusia ni* individuals at the end of the experiment.

	Protein amount (g/L)	**Premature death**	**Produced parasitoids**	**Pupated, pupa died**	**Turned to moth**
Group A: non-parasitized	43 (*n* = 45)	0	NA	0	45
	34.25 (*n* = 43)	0	NA	0	43
	25.5 (*n* = 39)	2	NA	1	36
	16.75 (*n* = 45)	2	NA	1	42
	11.5 (*n* = 45)	0	NA	1 (+1)[1]	43
	8.1 (*n* = 50)	15	NA	1	34
Group B: parasitized	43 (*n* = 37)	1	31	2	3
	34.25 (*n* = 45)	3	35	0	7
	25.5 (*n* = 42)	3	29	4	6
	16.75 (*n* = 45)	2	38	1	4
	11.5 (*n* = 43)	2	37	1	3
	8.1 (*n* = 45)	10	27	0	8

[1] Pupa accidentally killed during manipulation

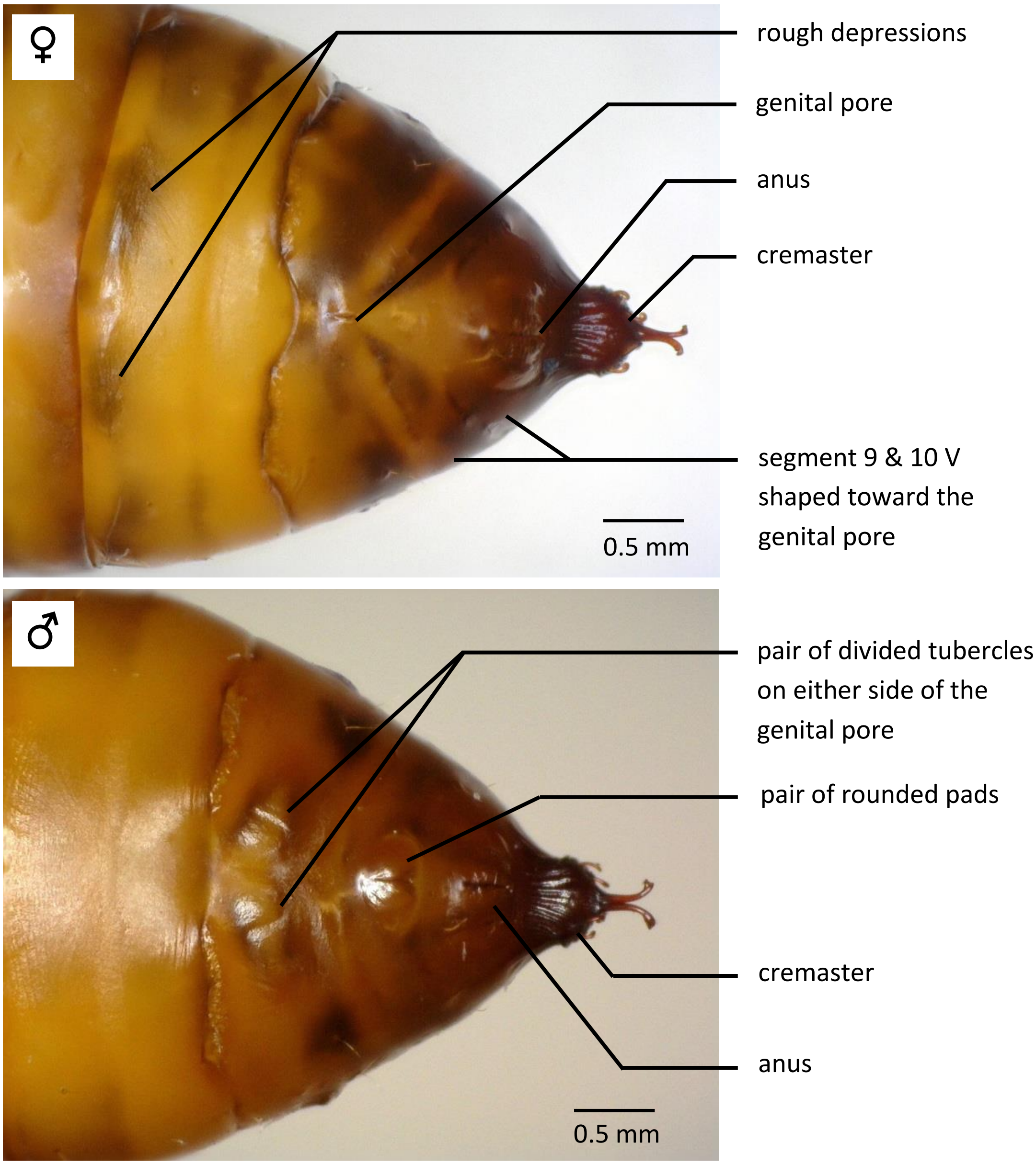

Figure 5.1. *Trichoplusia ni* pupae, female (top) and male (bottom), posterior end, ventral side.

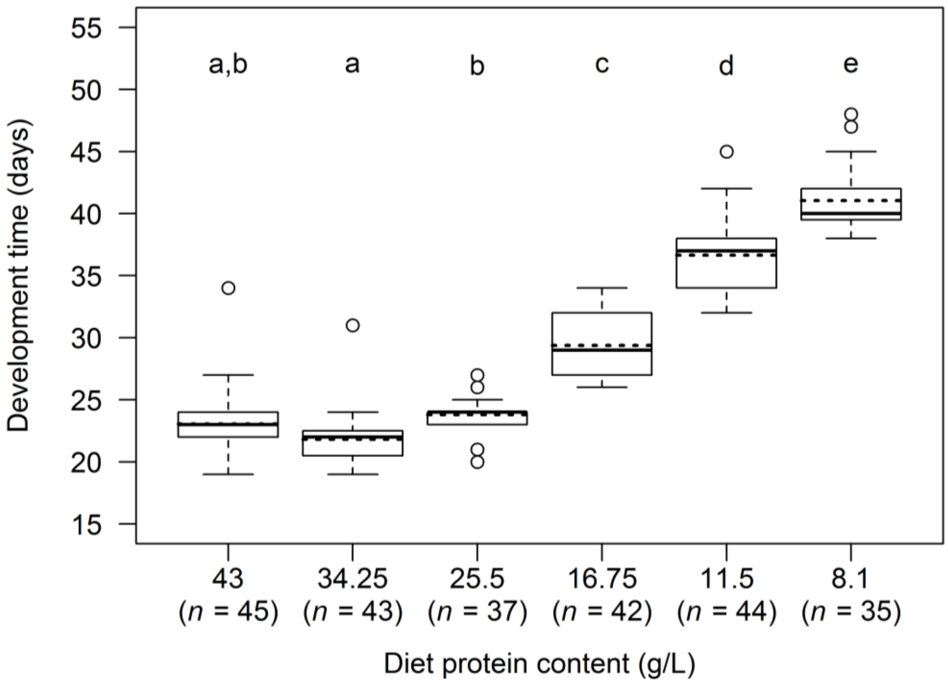

Figure 5.2. *Trichoplusia ni* larvae (males and females combined) development time on diets containing different amounts of protein. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. Medians and means are represented by solid lines and dotted lines, respectively. Significance (Tukey's HSD, α = 0.05, following one-way ANOVA) is indicated by a letter above each box (i.e., boxes with different letters represent significantly different means).

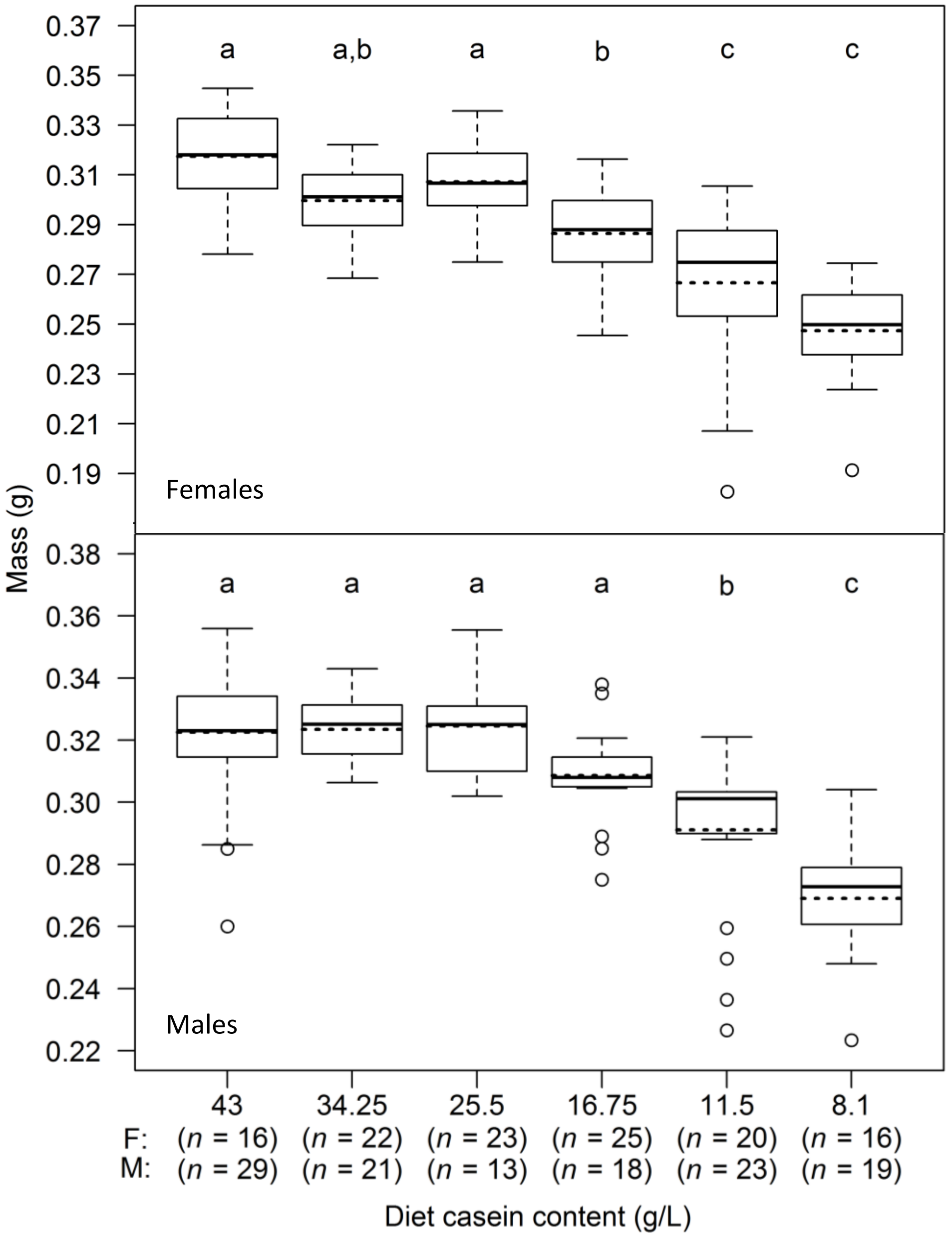

Figure 5.3. *Trichoplusia ni* mass of pupae from larvae that developed on diets containing different amounts of protein. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. The median and mean are represented by a solid line and a dotted line respectively. Significance (Tukey's HSD, α = 0.05, following one-way ANOVAs analyzing males and females separately) is indicated by a letter above each box (i.e., boxes with different letters represent significantly different means). Boxplots were analyzed separately.

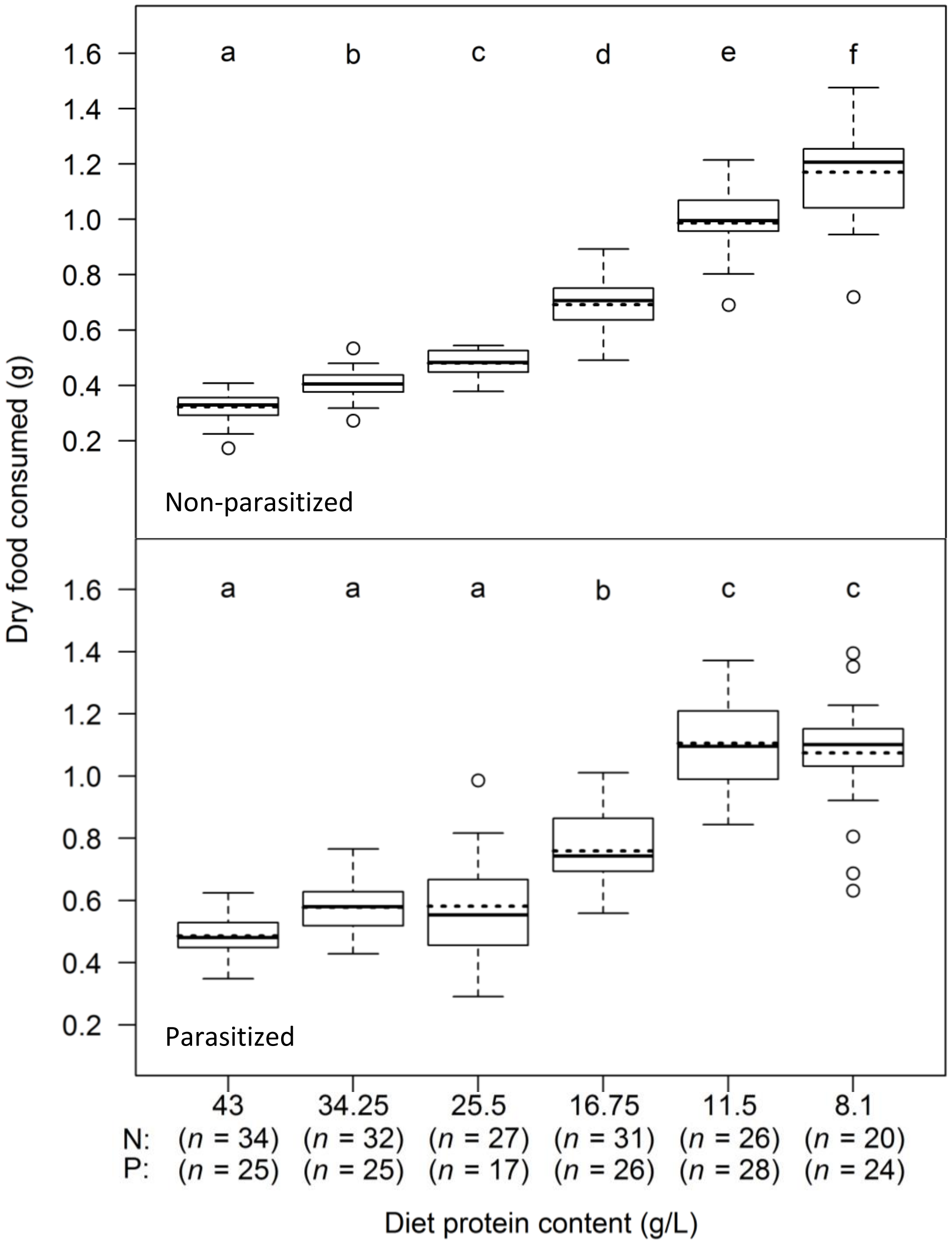

Figure 5.4. Estimated dry mass of food consumed by non-parasitized (top) and parasitized (bottom) *T. ni* larvae developing on diets containing 43 to 8.1 g/L of protein. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. The median and mean are represented by a solid line and a dotted line respectively. Significance (Tukey's HSD, α = 0.05, following one-way ANOVAs analyzing separately non-parasitized and parasitized *T. ni*) is indicated by a letter above each box (i.e., boxes with different letters represent significantly different means). Boxplots were analyzed separately.

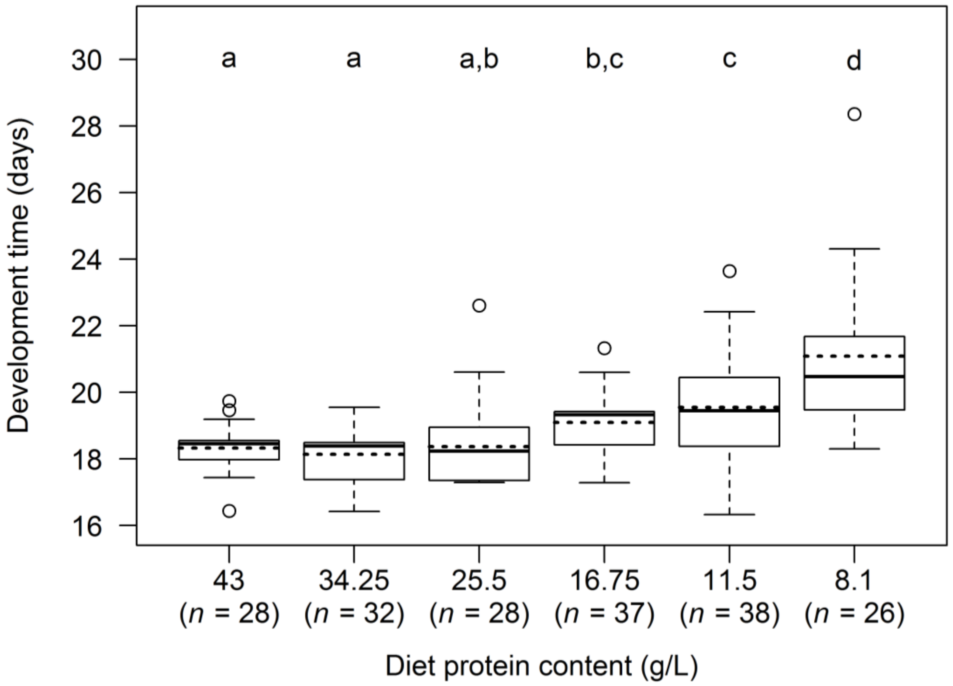

Figure 5.5. *Cotesia vanessae* development time within *T. ni* larvae that developed on diets containing different amounts of protein. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. The median and mean are represented by a solid line and a dotted line respectively. Significance (Tukey's HSD, $\alpha = 0.05$, following one-way ANOVA) is indicated by a letter above each box (i.e., boxes with different letters represent significantly different means).

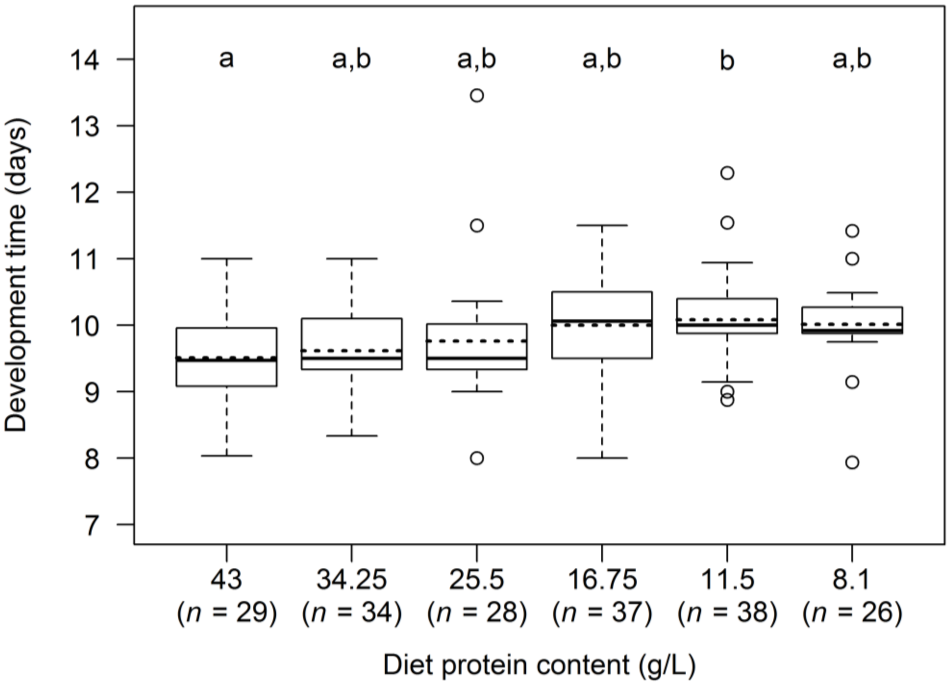

Figure 5.6. *Cotesia vanessae* development time within their cocoons from *T. ni* hosts that developed on diets containing different amounts of protein. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. The median and mean are represented by a solid line and a dotted line respectively. Significance (Tukey's HSD, α = 0.05, following one-way ANOVA) is indicated by a letter above each box (i.e., boxes with different letters represent significantly different means).

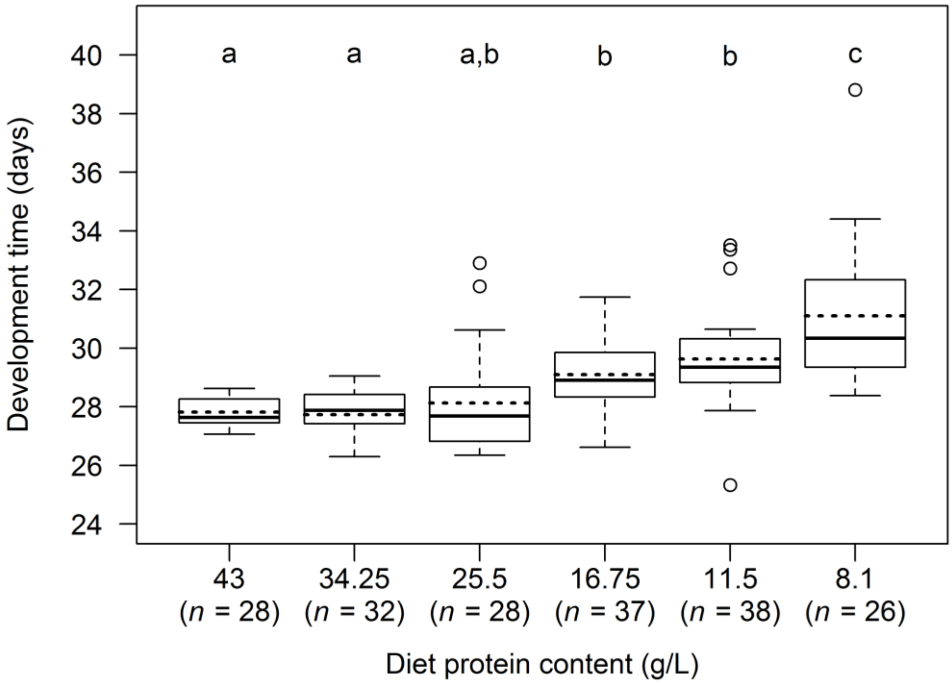

Figure 5.7. *Cotesia vanessae* total development time in *T. ni* hosts that developed on diets containing different amounts of protein. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. The median and mean are represented by a solid line and a dotted line respectively. Significance (Tukey's HSD, α = 0.05, following one-way ANOVA) is indicated by a letter above each box (i.e., boxes with different letters represent significantly different means).

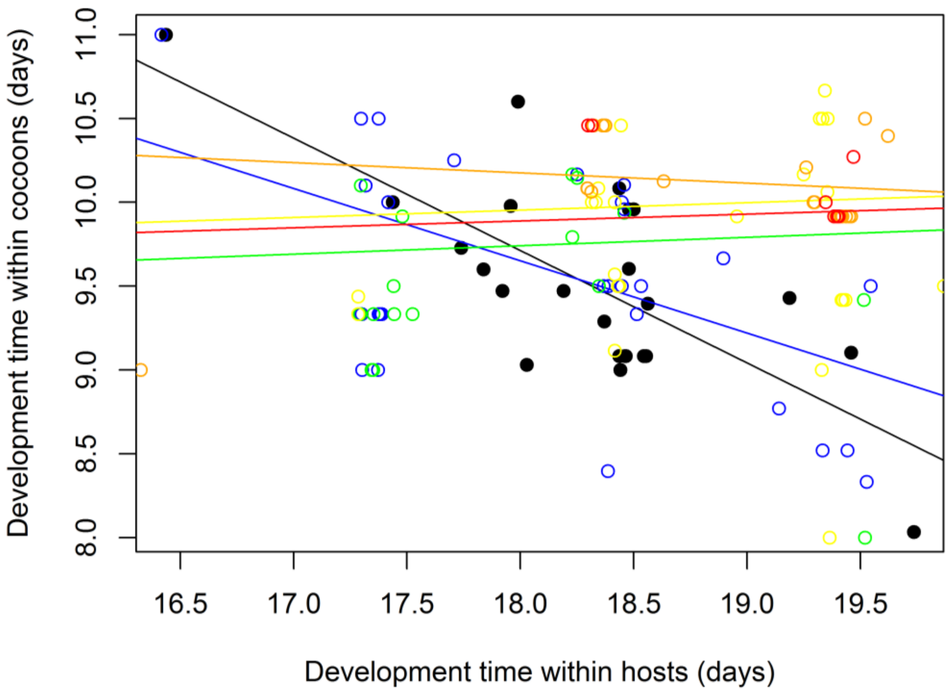

Figure 5.8. Scatterplot with trend lines showing *Cotesia vanessae* development time inside their host in relation to their development time inside cocoons, on each diet type; i.e., 43 g/L (black, slope = -0.67; r = -0.71), 34.25 g/L (blue, slope = -0.43; r = -0.53), 25.5 g/L (green, slope = 0.05, r = 0.07), 16.75 g/L (yellow, slope = 0.04, r = 0.05), 11.5 g/L (orange, slope = -0.06, r = -0.14), 8.1 g/L (red, slope = 0.04, r = 0.14).

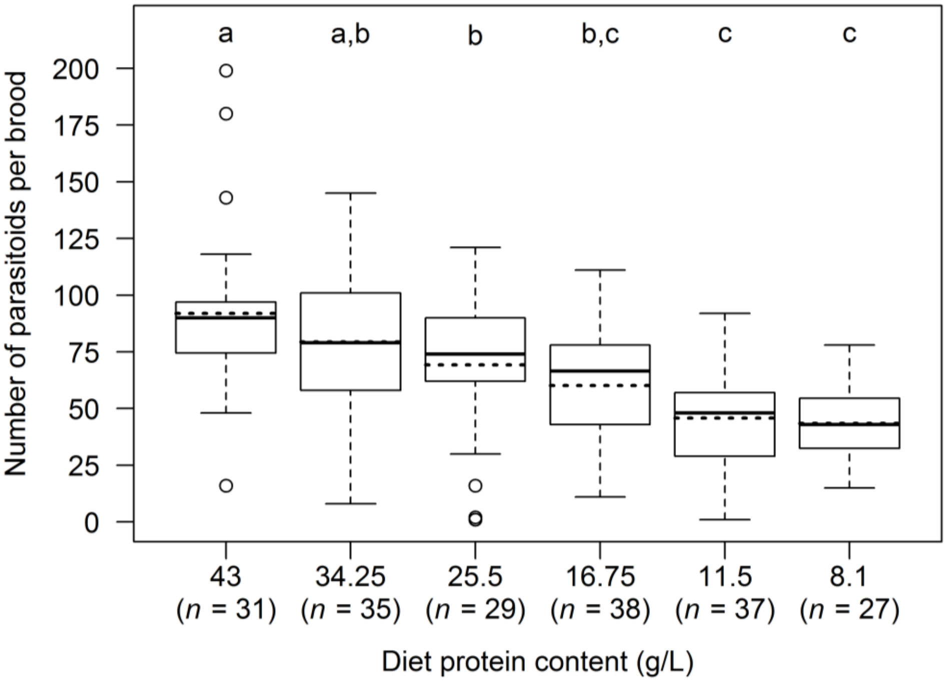

Figure 5.9. *Cotesia vanessae* brood size per type of diet from *T. ni* hosts that developed on diets containing different amounts of protein. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. The median and mean are represented by a solid line and a dotted line respectively. Significance (Tukey's HSD, α = 0.05, following one-way ANOVA) is indicated by a letter above each box (i.e., boxes with different letters represent significantly different means).

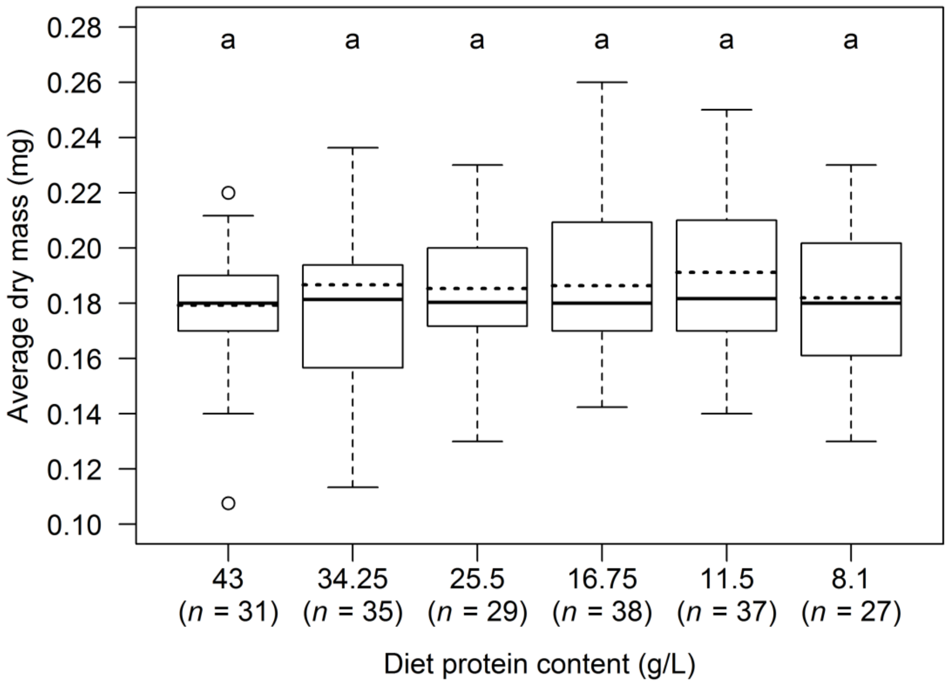

Figure 5.10. Average dry mass of individual adult *Cotesia vanessae* per brood from *T. ni* hosts that developed on diets containing different amounts of protein. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. The median and mean are represented by a solid line and a dotted line respectively. Significance (Tukey's HSD, α = 0.05, following one-way ANOVA) is indicated by a letter above each box. No significant difference was found between any treatment.

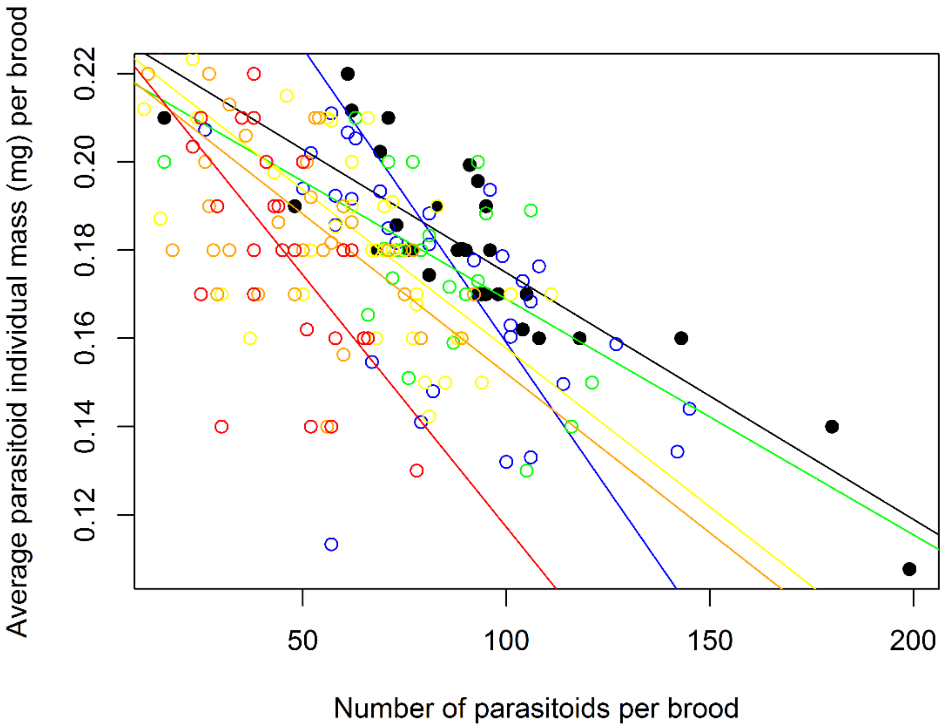

Figure 5.11. Scatterplot with trend lines showing *C. vanessae* brood size in relation to average dry mass of individual parasitoids emerged from *T. ni* fed diets containing different amounts of protein; i.e., 43 g/L (black, slope = -0.005, r = -0.87), 34.25 g/L (blue, slope = -0.0013, r = -0.62), 25.5 g/L (green, slope = -0.0005, r = -0.64), 16.75 g/L (yellow, slope = -0.0007, r = -0.67), 11.5 g/L (orange slope = -0.0007, r = -0.59), 8.1 g/L (red, slope = -0.0011, r = -0.66).

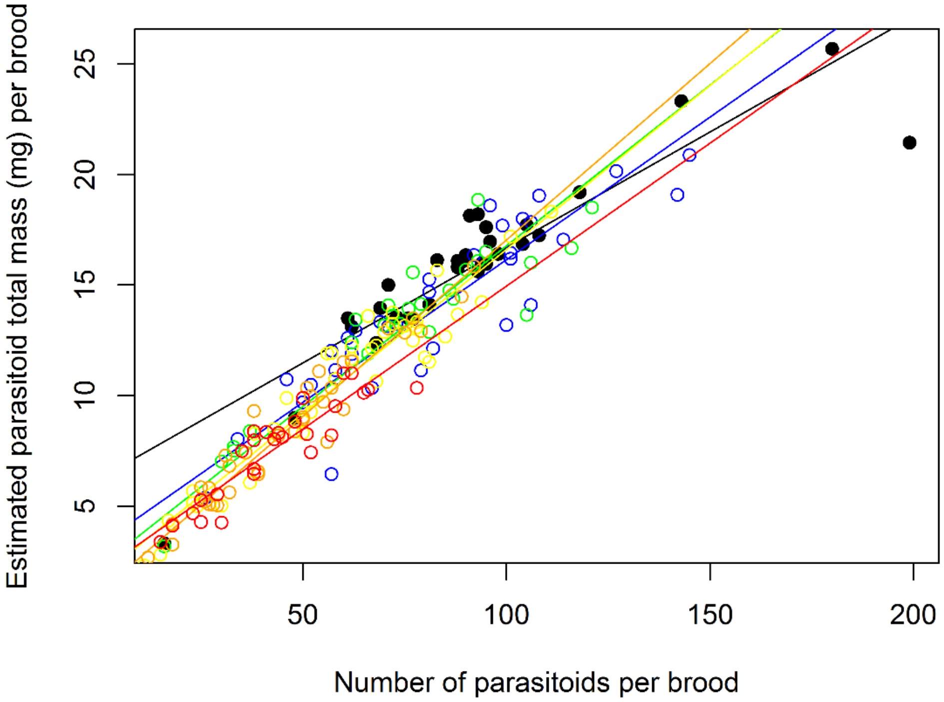

Figure 5.12. Scatterplot with trend lines showing number of *C. vanessae* adult per brood in relation to estimated total wasp mass of these broods, from *T. ni* fed diets containing different amounts of protein; i.e., 43 g/L (black), 34.25 g/L (blue), 25.5 g/L (green), 16.75 g/L (yellow), 11.5 g/L (orange), 8.1 g/L (red). All slopes between 0.10 and 0.16, and r ≥ 9.1.

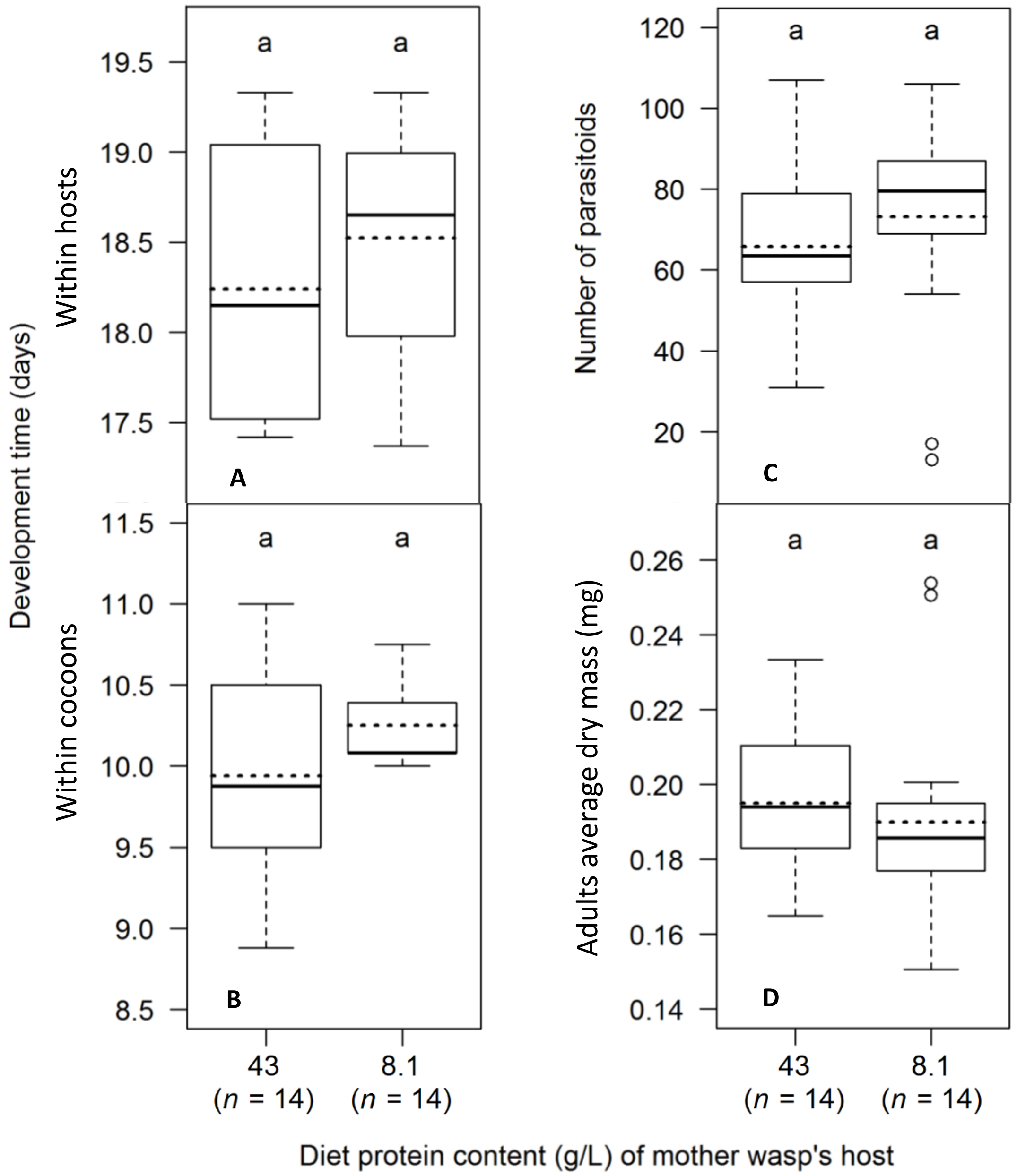

Figure 5.13. F2 *C. vanessae*: A. Development time within host, B. Development time within cocoon, C. Brood size, D. Adults average dry mass per brood; from *T. ni* hosts that developed on diets containing 43 and 8.1 g/L of protein. The boxes are delimited by the 25th and 75th percentiles. The upper and lower whiskers indicate Q3 + 1.5 IQR and Q1 – 1.5 IQR, respectively. The median and mean are represented by a solid line and a dotted line respectively. Significance (Tukey's HSD, α = 0.05, following one-way ANOVA) is indicated by a letter above each box (i.e., boxes with different letters represent significantly different means). Boxplots were analyzed separately.

CHAPTER 6: GENERAL DISCUSSION

> "*The scientist has a lot of experience with ignorance and doubt and uncertainty, and this experience is of very great importance, I think. When a scientist doesn't know the answer to a problem, he is ignorant. When he has a hunch as to what the result is, he is uncertain. And when he is pretty darn sure of what the result is going to be, he is still in some doubt. We have found it of paramount importance that in order to progress we must recognize our ignorance and leave room for doubt. Scientific knowledge is a body of statements of varying degrees of certainty–some most unsure, some nearly sure, but none* absolutely *certain.*"
> Richard P. Feynman (1988). The Value of Science.

Results of this study show that thelytokous *Cotesia vanessae* occur in Canada and have a broad fundamental host range within the family Noctuidae. Within this family, species in the subfamily Plusiinae (the "loopers") appear to be the best hosts. *Cotesia vanessae* can also develop on species of Nymphalini, as previously reported (Stefanescu et al., 2012). In no-choice tests, *C. vanessae* attempted to parasitize larvae of nearly all Lepidoptera species it was exposed to. Thus it may be able to develop on species in other lepidoperan families, as suggested by data in Yu (2016).

The current study shows that parasitoid fitness is affected by host species, and that the fitness of the parasitoid and that of its host are affected by the quality of the host's diet in term of protein content. *Trichoplusia ni* hosts were able to complete development on diet of various nutritional qualities, although development time and individual mass was affected. Diets that affected the host also affected the parasitoid, in terms of development time, brood size and brood mass, but not individual parasitoid mass. The next generation of parasitoids appeared unaffected by host's diet quality.

During this study, lepidopteran individuals of some species emerged with deformed wings (not caused by problems during emergence from the pupa), including individuals never exposed to parasitoids. Based on previous research (Morris, 1967), maladapted diet was considered the likely cause. Results in the Chapters 3 and 5 indicate that the McMorran diet is likely not optimal for all the species reared. This initially provided grounds for investigating parasitoid fitness on hosts fed diets of different qualities (Chapter 5 study), but misshapen wings were observed in both good and poor host species (see Chapter 4). This suggests no strong link with the cause of these deformities and parasitoid fitness.

Cotesia vanessae was found to display scramble competition in certain species (brood size inversely correlated with individual mass), and contest competition in all species dissected (siblicidal first instar). Contest competition through larval siblicide has not previously been observed in gregarious Braconidae species. The parasitoid population studied was gregarious and parthenogenetic, hence, offspring in a brood must be genetically very close to one another. We can only speculate as whether or not larval siblicide also occurs in male-female populations of *C. vanessae*. This siblicidal behaviour seems to contradict previous hypotheses that gregariousness and close relatedness among offspring should be associated with altruistic behaviour (Hamilton, 1963; Segoli et al., 2009; Giron et al. 2004; Godfray, 1994; Pexton et al., 2002; Rosenheim, 1993). This could either indicate that gregariousness in *C. vanessae* is still evolving and altruism among brood siblings might eventually arise, or that siblicidal behaviour is a desirable trait (Tuda et al., 1998). This behaviour appears to be an inefficient strategy because of the costs for the mother of producing extra eggs, the food wasted on larvae that are killed (which do not appear to be consumed by their siblings), and the time spent by larvae searching for siblings and killing them rather than feeding. However, adult females devote little resources for individual egg production because eggs are hydropic, i.e., small and apparently yolkless. Devoting fewer resources for individual eggs allows for the production of a larger number of eggs in the ovaries (Strand, 2000). Laying of extra eggs into hosts may increase the overall fitness of this species by increasing its intra- and interspecific competitiveness in super- and multiparasitized hosts, and in maximizing the number of offspring produced by hosts of wide range of sizes. In addition, the loss of the caudal appendage of *C. vanessae* larvae should be associated with a reduced motility of individuals. This means that contest competition likely only occurs among larvae in close proximity within the host's hemocoel, and is likely a key anatomical feature that permits gregariousness in this species.

High host specificity is a desirable trait when selecting species to use in biological control programs due to reduced risk of parasitism on non-target species (Brodeur, 2012; Hervet et al., 2016a). For example, the use of the non-host specific *Cotesia glomerata* (L.) in a classical biological control effort for reducing populations of the cabbage white, *Pieris rapae* (L.), in North America resulted in the extirpation from part of its range of the native non-pest mustard white butterfly, *Pieris oleracea* Harris (Benson et al., 2003). On the other hand, the wide host-range of *Cotesia marginiventris* (Cresson) within the family Noctuidae did not impede investigation as a candidate for the biological control of these pests in greenhouse, which provided efficient control of *Trichoplusia ni* (Gillespie et al., 1999; Messelink, 2002). Unfortunately, the high costs associated with the production of *C. marginiventris* led to the discontinuity of this biological control program. Like *C. marginiventris*, *C. vanessae* could also be considered for the biological control of *T. ni* and other noctuid pests. Advantageous traits of *C. vanessae* life history may result in it being more economical to produce than *C. marginiventris*: 1) Adult *Cotesia vanessae* in our laboratory commonly lived up to one month, and on some occasions up to a month and half (as per the rearing conditions described in Chapters 3 and 5). This reduces the need to frequently regenerate the laboratory colony and reduces the risk of accidentally losing the colony. In contrast, the longevity of adult female *C. marginiventris* is about 10 to 14 days (Boling et al., 1970; Gillespie et al., 1997a); 2) *Cotesia vanessae* is very fertile throughout its adult life. It is able to parasitize a large number of hosts in a short amount of time; e.g., one individual was observed to parasitize 50 *Euxoa ochrogaster* (Guenée) larvae in 20 minutes; although these larvae were not further observed to determine parasitism success and size of broods. It is a gregarious species that produces relatively large broods per host; i.e., in Chapter 5, parasitized *T. ni* reared on control diet produced an average 92 ± 6.16 (SE) parasitoids (range: 16 to 199) (*n* = 31). *Cotesia* *marginiventris* being solitary, each parasitized larva produces a single parasitoid, thus requiring the rearing of many more hosts to obtain a similar number of parasitoids; 3) Thelytokous *C. vanessae* do not need to find mates, and each individual can lay eggs; whereas *C. marginiventris* populations are bisexual with a male-bias (Gillespie et al., 1997b; Jalali et al., 1987); 4) *Cotesia vanessae* usually parasitize the host upon first encounter and is fertile one day after emergence from the cocoon. *Cotesia marginiventris* starts to lay eggs two days after emergence (Gillespie et al., 1997a). Some unfavorable aspects are that 1) *C. vanessae* likely requires more time to develop (about 17 days to develop in the host and an additional 10 days in the cocoon, at 20 °C) than does *C. marginiventris* (about 7 to 9 days in the host and 6 to 8 days in the cocoon, at 25 °C); 2) *Cotesia vanessae* does not prevent maturation of its hosts prior to killing it, like *C. marginiventris*, thus parasitism does not directly prevent plant damage; 3) parasitized hosts consume more food than non-parasitized individuals. This means that its use in biological control programs can only be used to control pest populations over more than one generation.

To elucidate the ecological host range of *C. vanessae*, it would be essential to investigate its association with plant species and conduct tests in more natural conditions. Its ability to live in warm and cold climates, its high fertility, and its broad fundamental host range within the family Noctuidae indicate that it may become a common species in North America and significantly contribute to the control of noctuid pests, either naturally or through biological control programs.

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APPENDIX 1: SOME OBSERVATIONS OF *COTESIA VANESSAE* NATURAL HISTORY

A.1. Engaging an unlikely host

While large parasitoid hymenopteran species and a few small species that use their ovipositor as a defensive weapon have been reported to sting humans (Lee et al., 2014; Maxwell, 1921; Papini, 2014; Schmidt, 2016), these reports appear non-existent for small parasitoid hymenopteran species that do not use their ovipositor defensively, which are usually considered to have a too weak ovipositor to penetrate human skin. However, once after having handled *Trichoplusia ni* larvae, I introduced my hand into a cage containing a few hundred *Cotesia vanessae*, one of which landed on the dorsal side of my right index finger and immediately inserted its ovipositor into the skin. While doing so, it positioned its wings, mid and hind legs upward as displayed in Figure 3.3 (Chapter 3), as if parasitizing a host. The event lasted about 10 seconds and was followed by the appearance of a small (~1 mm diameter) slightly itchy red swell, which disappeared an hour later. It has been shown that parasitoids can be fooled by artificial hosts, given that they have the right shape, odor, and texture (Dindo et al., 2002; Grenier, 2012; House, 1978). As displayed in Chapter 3, *C. vanessae* is opportunistic, thus could have been misled by a host's smell on a finger. It might also have been close to the end of its life, as such individuals become more likely to engage unlikely hosts (personal observation). Because I have handled tens of thousands of *C. vanessae* the chance to observe unusual behavior was increased and this is certainly not typical of the species.

A.2. Ovipositor attachment

Sometimes parasitoids were observed to struggle to, or even to be unable to, remove their ovipositor from a host. Hosts were sometimes observed to carry dead parasitoids attached by their ovipositor. Attempting to remove stuck individuals with tweezers sometimes resulted in the ripping of their abdomen, leaving behind the ovipositor and abdominal organs attached. *Cotesia vanessae* was observed to possess a barbed spear alongside its ovipositor. A feature shared with other *Cotesia* species (Thomas, 2012), which likely helps them remain firmly attached to their host during oviposition.

A.3. Climbing prior to parasitoid egression

Parasitized hosts usually climb shortly prior to parasitoid egression. This behavior was also observed with *Autographa californica* in canola fields parasitized with another *Cotesia* species (GenBank accession number: KF640233), and with *Aglais milberti* parasitized with yet another *Cotesia* species (unidentified), which egressed from hosts at the top of plants. What causes this behaviour is unknown, but one possible benefit to parasitoids is to ensure that they do not find themselves buried when egressing from a hiding host.

A.4. Parasitoid larvae egression

Cotesia vanessae larvae egress simultaneously from both of its host's sides (as displayed by exit holes in Fig. A.1), although the larvae located toward the posterior of the host always egress slightly prior to anterior ones, and rarely from the dorsal side. In the experiments described in Chapter 3 and Chapter 5, about 10 host larvae were dissected after egression. Dead parasitoid larvae that failed to egress were always seen inside of them. Hosts (*Trichoplusia ni*) from the experiment described in Chapter 5 only contained a few parasitoid larvae (<10), all of which appeared to have reached their full size (Fig. A.2), but hosts (*Euxoa messoria*) from the Chapter 3 experiment contained various numbers of dead parasitoid larvae. One *E. messoria* that died of premature death contained over 300 *C. vanessae* larvae of different stages (from nearly visible to the naked eye to virtually fully mature). In the Chapter 3 experiment the mother wasp remained 48 hours with its host, thus it was able to parasitize it multiple times, whereas in the Chapter 5 experiment each host was parasitized only once, thus reducing parasitoid crowdedness within the host. This should explain the different numbers of dead parasitoid larvae remaining within hosts. However, the total numbers of emerged parasitoid from *T. ni* host were similar in both experiments (Chapter 3 and 5).

Immediately after leaving their host, each parasitoid larvae form individual cocoons. Each cocoon is attached to the surrounding siblings' cocoons by silk, thus forming a cocoon mass. In small cocoon masses, individual cocoons are usually attached to the substrate from their sides, whereas in big cocoon masses (as in Fig. A.3) the cocoons are usually attached to the substrate by their bottom (where the rear end of the parasitoid is located). Hosts usually crawl away after the last parasitoid has egressed from their body, thus allowing parasitoid larvae that egressed from both sides of the host to reunite and form a single cocoon mass. Sometimes the host remained where the parasitoids emerged from its body and died there, and in other rare occasions the hosts traveled during parasitoid larvae egression. Both events usually resulted in parasitoids forming two or more cocoon masses. To avoiding misunderstanding, in the experiments described in Chapters 3 and 5 a "cocoon mass" refers to all the cocoons from a brood of parasitoids that egressed from one caterpillar. An extra ~2 mm of fluffy silk is produced to surrounds the cocoon mass, likely a protection against hyperparasitoids and unfavorable weather. It was observed that parasitoids larvae that happened to be far from other larvae (≥ 2 mm) often did not manage to fully form a cocoon and eventually dried out and died, either as larvae or pupae.

Within cocoon masses the cocoons were always relatively well-aligned and staggered (Figs. A.3 & A.4), in a way that is reminiscent of the structure of nests of colonial wasps (Vespinae and Polistinae) and bees (*Apis* spp.). Parasitoid larvae can probably sense the position of their surrounding siblings and place themselves precisely between them. Even individuals that reunite themselves after having egressed from either side of a host end up well staggered. This organization may provide each adult parasitoid with a fair chance to successfully emerge from the cocoon mass considering that the anterior end of the cocoon is not obstructed by other cocoons, which may not be the case for the *Cotesia* species that do not appear to have any organization in their cocoon mass (personal observations with a *Cotesia* sp. (GenBank accession number: KF640232) egressed from *Autographa californica* larvae collected from alfalfa fields in southern Alberta in 2013 and 2014).

A.5. Larvae color according to host taxa

Surprisingly, the *C. vanessae* larvae that egressed from Noctuidae hosts were always grey, while those that egressed from Nymphalidae were always bright yellow (observed from *Aglais milberti* and *Vanessa cardui* hosts. The latter observation stems from a communication with Mark R. Shaw, National Museums of Scotland). This bright yellow color was also observed in larvae of *Cotesia atalantae* (Packard) reared from *A. milberti* collected in southern Alberta in 2016. These Nymphalidae species were reared on stinging nettle, *Urtica dioica*. In contrast, Noctuidae hosts were reared on McMorran diet, or on Bock choy, *Brassica rapa*, broccoli, *Brassica oleracea*, and canola, *Brassica napus*, between experiments, but never on nettle. Either the host identity or its food source could have induced this color difference.

Figure A.1. Last instar *Trichoplusia ni* larva that died following egression of *Cotesia vanessae* from its body. The dark circles on the side of the caterpillar are the exit holes made by parasitoid larvae while egressing. Near the centre, a parasitoid larva that died without completing egression is visible.

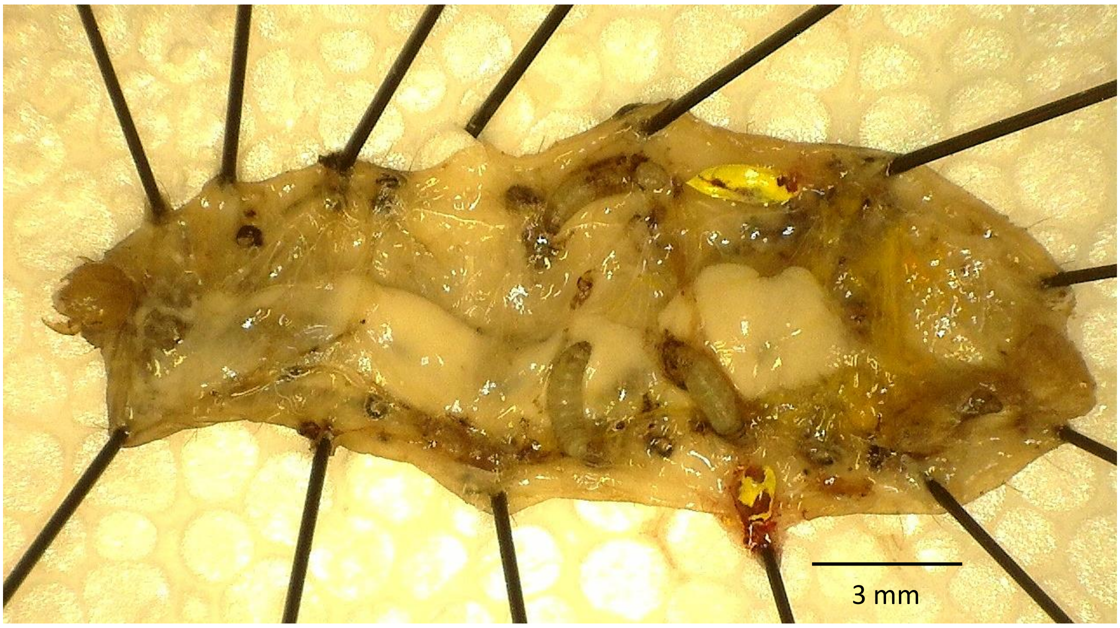

Figure A.2. Same *T. ni* larva as in Fig. A.1 dissected dorsally. Four fully-developed dead parasitoid larvae that failed to egress from the host are visible. The two red bodies surrounded by yellow tissue are the gonads of the host. The thick longitudinal white mass is fat surrounding the digestive system of the caterpillar.

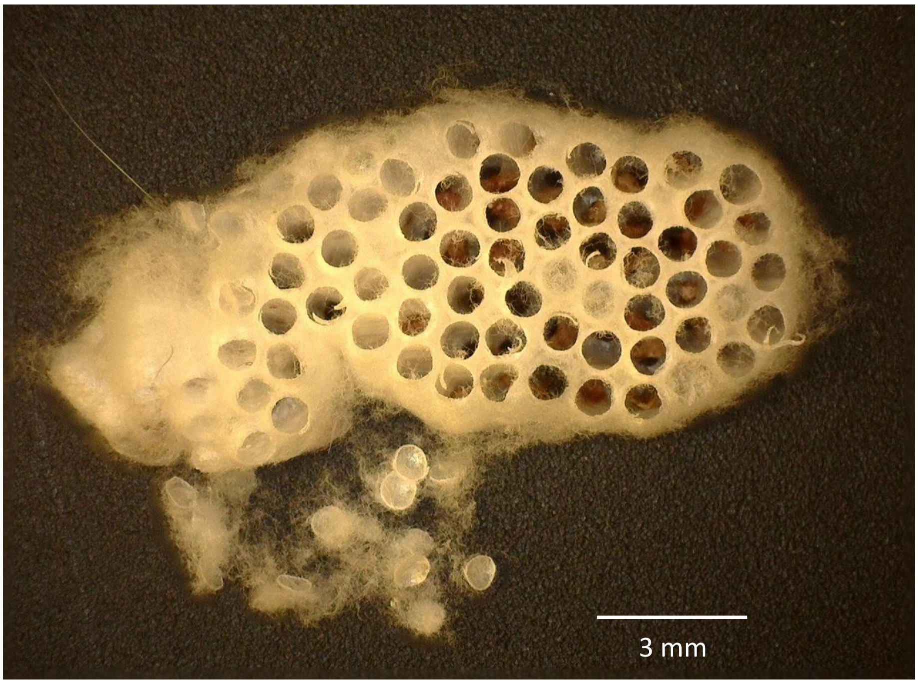

Figure A.3. Top view of a *Cotesia vanessae* cocoon mass, with surrounding silk and open opercula of cocoons removed (some opercula are displayed below the cocoon mass). The few unopened cocoons contain dead parasitoids.

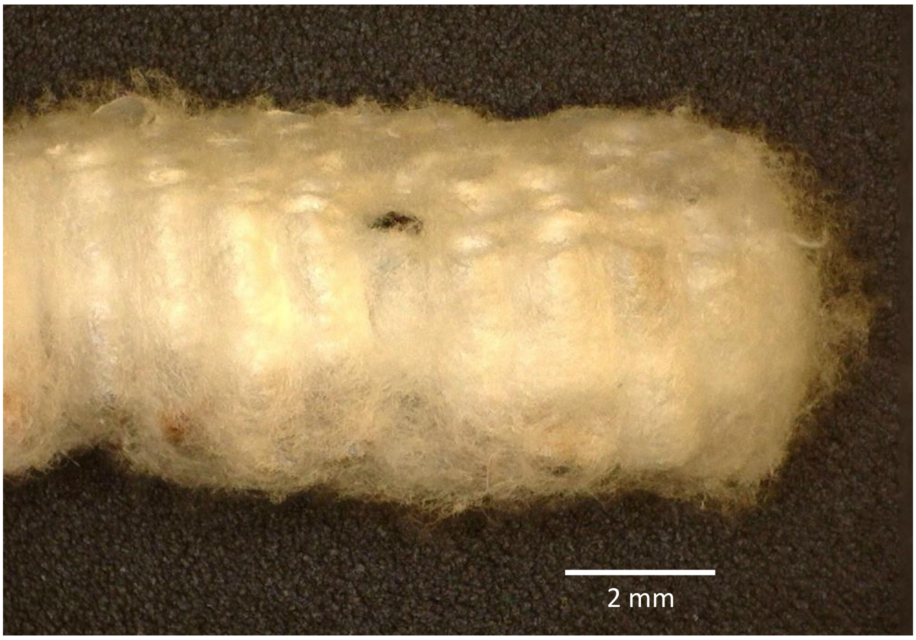

Figure A.4. *Cotesia vanessae* cocoon mass lateral side, with surrounding silk and open opercula of cocoons removed. A dead parasitoid adult that opened its cocoon but failed to come out of it is visible in the middle of the first row.