Reproductive behaviour of Western bean cutworm, Striacosta ablicosta (Lepidoptera: Noctuidae), females under different abiotic and biotic conditions

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Graduate Program in Biology

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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REPRODUCTIVE BEHAVIOUR OF WESTERN BEAN CUTOWRM, STRIACOSTA ABLICOSTA (LEPIDOPTERA: NOCTUIDAE), FEMALES UNDER DIFFERENT ABIOTIC AND BIOTIC CONDITIONS

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by

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ABSTRACT

The Western bean cutworm (WBC) recently expanded its range eastward from the western United States into the Great Lakes region. Little is known about the reproductive biology of this potential pest, therefore I studied the calling behaviour (the emission of the sex pheromone by females) under different biotic (age, mating status) and abiotic (temperature, relative humidity) conditions. Sexual maturation, which occurred several days after emergence, indicating that WBC is a migratory species, was not directly affected by temperature but rather by the difference between day and night temperatures. Calling behaviour was influenced by female age, but not the abiotic factors tested. Titers of the sex pheromone did not change with female age suggesting older females that call for longer, may be more attractive than younger conspecifics. Females resumed calling within 48h of mating, and did so earlier than virgin ones, which may explain the high incidence of polyandry in WBC.

Keywords

Striacosta albicosta, calling behaviour, sexual maturation, sex pheromones, mating status, refractory period, temperature, relative humidity, age
CO-AUTHORSHIP STATEMENT

Dr. Jeremy N. McNeil will be a co-author of all manuscripts published from the contents of this thesis as he co-designed the experiments and edited all the manuscripts.

Dr. Mark Bernards will be a second author on the manuscript published from the data presented in Chapter 3, as he assisted with gas chromatography analysis.
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LIST OF ABBREVIATIONS

12:Ac- dodecyl acetate  
ANOVA- analysis of variance  
Bt- Bacillus thuringiensis  
GC- gas chromatography  
FID- flame ionizing detector  
JH- juvenile hormone  
MANOVA- multivariate analysis of variance  
MOTC- mean onset time of calling  
MTSC- mean time spent calling  
ON- Ontario  
OSN- olfactory sensory neurons  
PCP- pre-calling period  
RH- relative humidity  
RP- refractory period  
UV- ultraviolet  
WBC – Western bean cutworm  
Z5-12:Ac- Z-5-dodecenyl acetate  
Z7-12:Ac- Z-7-dodecenyl acetate  
Z11-12:Ac- Z-11-dodecenyl acetate
CHAPTER 1

General Introduction

1.1 Reproduction

Individual reproductive success is assured by passing genes on to the next generation. Asexually reproducing organisms incur low reproductive costs and can usually proliferate rapidly to produce genetically identical offspring that are well adapted to their local environment. On the other hand, although more costly, sexual reproduction gives rise to genetically diverse offspring through gene recombination, with more variable responses to changing environmental conditions (Smith and Smith, 2003). While there are hermaphroditic organisms that possess both male and female organs, the most common form of sexual reproduction involves separate sexes that must combine their gametes to complete fertilization (Cockburn, 1991). As no active search for a reproductive partner is possible in sessile organisms (e.g. plants and invertebrates like mussels), male gametes are released in great numbers to maximise the probability of randomly fertilizing female ones (e.g. through passive dispersal by wind/water currents or animal pollination), a broadcast also used by some mobile organisms. Conversely, many mobile organisms are faced with a unique challenge of locating a conspecific of an opposite sex. Moreover, since there are great costs associated with sexual reproduction (both in terms of energy and time), it is important not only to find potential mates, but also to select the best one(s) to produce high quality offspring.

1.2 Mate choice

The importance of mate choice in animals varies depending on the mating system (i.e. monogamy vs. polygamy with single and multiple mating partners, respectively) and between sexes, due to different selection pressures related to factors including relative investment in gamete production and parental care (Cockburn, 1991). When males cannot be certain about the paternity of the offspring (e.g. because of female cryptic choice or sperm competition), they will mate with several females to increase their fitness. Therefore, males with traits that make them more attractive to the females (intersexual
selection) or provide them with a competitive advantage over other males (intrasexual sexual selection) can potentially acquire more mates, thus increasing their fitness (Cockburn, 1991; Smith and Smith, 2003). In contrast, females mate less frequently, and are generally choosier when selecting mates as they invest more resources in their offspring than males (Trivers, 1972; Cockburn, 1991).

1.3 Importance of communication in mate choice

The first step towards reproduction is mate attraction and location. Upon sexual maturation, reproduction becomes the ultimate goal with efforts allocated to reproductive behaviours related to mate attraction or location, courtship, and copulation. When a certain behaviour (e.g. mating) requires cooperation of individuals, communication between them must exist. The original function of the cues that serve as signals was likely different, but was enhanced over time to better indicate quality in novel environments (Bro-Jorgensen, 2009), and to elicit behavioural responses beneficial to both the signaler and the receiver. Messages of sexual receptivity and mate quality likely evolved from informative cues with other functions. For example, hormones and metabolites released in water by fish upon sexual maturation could have evolved to serve as sex pheromones, while compounds from injured animals evolved into alarm pheromones (Wyatt, 2003). The individuals able to associate an identified cue with a physiological status of a conspecific would have increased fitness as they would be more effective at locating and securing a mate faster and likely had more mating opportunities. Since the signal is beneficial for both the emitter (avoid harassment while in non-reproductive stage) and the receiver (minimize search costs) (Johansson and Jones, 2007), over time those cues evolved into signals that allowed for advertisement of sexual receptivity for mate attraction. However, there is a possibility that the signaler may attempt to manipulate the receiver of a signal, which leads to a potential conflict and raises questions about the honesty of the signal between the signaler and the receiver (Johnstone, 1997; Backwell et al., 2000). If the signal is costly to produce or leads to a “handicap” in the signaler, honesty is expected, because signaling would only be possible if the signaler was able to support the associated costs (Zahavi, 1975).
1.4 Sensory modalities for communication

Communication between individuals can occur through different sensory modalities (visual, auditory, tactile, and chemical) that are either used separately, or in combination, to acquire and send information. The modality used depends on the proximity of the communicating individuals (through contact or remote senses), and the associated trade-offs in terms of time and energy investment, as well as risk of predation and disease (Bradbury and Vehrencamp, 1998). Once the signal has been generated or emitted, it needs to be transmitted through a medium to a receiver which will in turn make a decision about an appropriate response to that signal, either behaviourally or physiologically (Endler, 1993). There are advantages and disadvantages associated with each of the modalities (Table 1.1.), and the sensory mode primarily used in signaling is the one that allows for efficient transmission of the message in the local environment (Wiley and Richards, 1982), thus maximizing the signal-to-noise ratio, quality, and the effectiveness of the transmitted signal (Endler, 1992; Romer and Lewald, 1992). This “sensory drive” hypothesis (Endler and McLellan, 1988; Endler, 1992) is used to explain time and place as well as microhabitat preferences of the signalers (Bradbury and Vehrencamp, 1998; Boughman, 2002).

Table 1.1 Signal properties of four different types of communication (visual, auditory, tactile, chemical) used by animals (adapted from Endler, 1993).

<table>
<thead>
<tr>
<th>Type of communication</th>
<th>Range</th>
<th>Rate of change</th>
<th>Information content</th>
<th>Production cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual</td>
<td>Moderate</td>
<td>Fast</td>
<td>High</td>
<td>High or low</td>
</tr>
<tr>
<td>Auditory</td>
<td>Long</td>
<td>Fast</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Tactile</td>
<td>Short</td>
<td>Fast</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Chemical</td>
<td>Long</td>
<td>Slow</td>
<td>Low</td>
<td>Low</td>
</tr>
</tbody>
</table>

Each form of communication can be used separately or in combination to convey various messages relating to mating. Active or passive displays of body parts or patterns and coloration by males are common in visual communication, in organisms such as fish (Pomiankowski and Iwasa, 1993) and birds (Chaine and Lyon, 2008), to attract conspecific females. Some amphibians (Gerhardt, 1994; Amezquita et al., 2006) or birds
(Hauber et al., 2010) rely primarily on acoustic signals for mate attraction. Visual communication can be also combined with auditory vocalizations, acoustic body part clicking indicating fighting ability, or seismic signaling indicative of immune function (Wiley, 1973; Hebets, 2005; Bro-Jorgensen and Dabelsteen, 2008). Antennal tapping and substrate-borne vibrations have also been documented in many vertebrates and arthropods (Hill, 2008; Ryan and Sakaluk, 2009). Chemical communication is common in many animals including invertebrates, fish, lizards, turtles, mice, and primates (Stacey, 2003; Poschadel et al., 2006; Scordato and Drea, 2007; Thompson et al., 2008; Hurst, 2009), and is regarded as the most widespread and oldest form of communication (Bradbury and Vehrencamp, 1998; Wyatt, 2003). Miscommunication can lead to reproductive interference between species through misdirected courtship, heterospecific mating attempts or rivalry (Groening and Hochkirch, 2008), which ultimately negatively affects the fitness of at least one species involved. Effective communication is therefore essential for mate selection and successful reproduction.

1.5 Communication in insects

Similar to other organisms, insects may communicate using one or several of the four sensory modalities mentioned above to attract conspecific mates. Visual communication is important for species recognition in both diurnal and nocturnal insects. Most insects are UV light sensitive (Briscoe and Chittka, 2001) and UV reflectance is used for mate quality assessment in some butterflies (Lepidoptera), for example Pieris rapae (Obara et al., 2008). Although no ambient light is present at night, fireflies (Coleoptera) produce their own light through bioluminescence and communicate through flashes and flickers in a courtship dialogue (Lewis and Cratsley, 2008). Auditory communication in insects can also be performed in a duet (Bailey, 2003), and the sound is usually produced by drumming, stridulation (rubbing body parts against each other), tremulation (body vibration), or tymbal bucking, but can differ between sexes as shown in crickets (Hoy, 1991) and cicadas (Sueur et al., 2010). Conspecific recognition by antennal tapping occurs in crickets (Ryan and Sakaluk, 2009), and communication through substrate-borne vibrations is common in leaf and plant hoppers (Hemiptera) (Hoch and Wessel, 2006; Miranda, 2006) with the vibrations sensed through subgenual
organs inside insect legs (Cokl, 1983; Michel et al., 1983; Cokl and Doberlet, 2003). The use of intraspecific chemical signals (pheromones) for mate attraction and assessment of mate quality is widespread among insects and is a primary form of communication in nocturnal Lepidoptera (moths). As insect lineages radiated, and exploited different habitats, different sensory modalities were preferred to maximize the message content and transmission. Communication through those modalities evolved into spatially and temporally separated functional systems of mate attraction (Endler, 1992).

1.6 Insect sex pheromones: evolution and diversity

Pheromones are species specific chemical signals that cause a physiological (primer pheromone) or behavioural (releaser pheromone) response in a receiver (Karlson and Luscher, 1959). There exists an astounding diversity in sex pheromones across species, with small differences in composition, ratios or isomers being easily detected by the receivers. During the course of evolutionary time, a great diversity of pheromones arose in different insect species. However, small changes in signals used for mate attraction should theoretically be selected against, since small deviation from the signal could mean reduced attractiveness (Symonds and Elgar, 2008). Evolution of new pheromone blends can happen in one of two ways: through small changes in pheromone structure (Roelofs and Brown, 1982) or ‘saltational’ shifts (Baker, 2002). Pheromones of many closely related species are similar in composition, so it was proposed that there were gradual changes (through addition or reduction) or shifts in relative proportions of compounds over time (Roelofs and Brown, 1982; Symonds and Elgar, 2008). More recently, it was suggested that pheromones evolved rapidly resulting in diverse signals in closely related species (Baker, 2002; Roelofs et al., 2002), as proposed for the ermine moths (Lofstedt et al., 1991). Regardless of how the sex pheromone system evolves, there is a greater selection pressure on the receiver’s sensitivity and response to the signal, rather than on a signalling capacity of the signal emitter (Svensson, 1996; Johansson and Jones, 2007). This view is supported by the observation that males of some insect species are sensitive to a wider spectrum of pheromones than is emitted by their conspecific females (Lofstedt, 1990) while maintaining reproductive isolation and minimizing interference with other species.
Although sex pheromones differ between insect lepidopteran species, they are made up of similar components synthesized through shared biosynthetic pathways (Tillman et al., 1999; Symonds and Elgar, 2008). Two major groups of sex pheromones are unsaturated fatty alcohols and their derivatives (Type I, C_{10}-C_{18}), and polyunsaturated hydrocarbons and their epoxies (Type II, C_{17}-C_{23}) (Ando and Yamakawa, 2011) (Table 1.2). Only few genes encoding oxidase (chain shortening), desaturase (addition of double bond), and reductase (additions of oxygen functional group) enzymes (Bjostad et al., 1987; Jurenka and Roelofs, 1993; Tillman et al., 1999; Roelofs and Rooney, 2003) control the pheromone blends composition in Lepidoptera. Consequently, the same hydrocarbon chains can be modified in many ways by changing the number of carbons, adding different functional groups and double bonds in different configurations and positions along the chain (Ando and Yamakawa, 2011). For example, the synthesis of very different pheromone components from the same fatty acid precursor occurs in closely related Asian (Ostrinia furnicalis) and European (Ostrinia nubilalis) corn borers (Roelofs et al., 2002).

Table 1.2 Major pheromone groups of Lepidoptera (1Kou et al., 1992, 2Chen et al., 2006, 3Kalinova et al., 2012, 4Ando et al., 1997, 5Wakamura et al., 2001, 6Yamamoto et al., 2007).

<table>
<thead>
<tr>
<th>Pheromone type</th>
<th>Percent of Lepidoptera</th>
<th>Main functional groups</th>
<th>Pheromone example</th>
<th>Insect example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>75</td>
<td>Primary alcohols</td>
<td>Z11-16:OH</td>
<td>Pseudaletia separata¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetates</td>
<td>E3-14:Ac</td>
<td>Cossus insularis²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aldehydes</td>
<td>Z11-16:Ald</td>
<td>Diatraea flavipennella³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ascotis selenaria⁴</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Polyunsaturated</td>
<td>Z3,Z6,Z9-19:H</td>
<td>Orgyia postica⁵</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hydrocarbons</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epoxies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type II</td>
<td>15</td>
<td>Secondary alcohols</td>
<td>Z6,Z9,t-epo11-21:H</td>
<td>Lyclene dharma⁶</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ketones</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Esters</td>
<td></td>
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</tbody>
</table>

Symonds and Elgar (2008) suggested that the O. furnicalis pheromone originated from that of O. nubilalis through saltational shift in one of the desaturase genes, since some European corn borer males respond to the pheromone blend of O. furnicalis (Roelofs et al., 2002). Genes encoding desaturase enzymes play a very important role in
pheromone biosynthesis, and this gene family underwent several gene duplication events (Roelofs et al., 2002, Roelofs and Rooney, 2003; Nei and Rooney, 2005) with ∆11-desaturase being present only in Lepidoptera and used exclusively in pheromone production (Lienard et al., 2008). Lepidopteran sex pheromones are highly specialized evolved signals that convey species specific messages, and are hence indispensable for communication, mate choice, and successful reproduction.

1.7. Production and reception of sex pheromones in Lepidoptera

In Lepidoptera, the sex pheromone that serves to attract the opposite sex is generally produced in an eversible pheromone gland located on the terminal abdominal segment of the female (Percy-Cunningham and MacDonald, 1987), and is emitted through evaporation from the surface of the exposed gland. Sex pheromone production is controlled by pheromone biosynthesis activating neuropeptide (PBAN) (Raina et al., 1987; Raina et al., 1989) produced in subesophageal ganglion (Jaffe et al. 1986) and depending on species, transported to the pheromone gland either through the hemolymph (Jurenka and Roelofs, 1993; Jurenka, 1996), or ventral nervous system (Teal et al., 1989; Christensen et al., 1991). Juvenile hormone (JH) may also play a role in sex pheromone synthesis and is involved in the timing of sexual maturation, especially in species that migrate in response to habitat deterioration. If the habitat is suitable for reproduction the JH levels are high (Cusson et al., 1994) and appear to ‘prime’ the pheromone gland to respond to PBAN (Fan et al., 1999). On the other hand, in poor habitats there are low levels of JH that prevent ovarian development and pheromone synthesis resulting in migratory flight (McNeil et al., 1996; McNeil et al., 2000).

Once males detect the female sex pheromone they begin upwind flight following the plume. The different components of the pheromone blend are detected by the olfactory sensory neurons (OSN) located on the male antennae, and induce neural stimuli that are transmitted through the CNS via the glomeruli of the antennal lobe (Hansson and Christensen, 1999; de Bruyne and Baker, 2008). Each odour activates a unique pattern of neurons in the glomeruli, with a behavioural response being produced only if the neural firing stimulated by an arriving odour is synchronized (Wyatt, 2003). Since males need to detect and interpret the female odour, male antennae are larger, and their antennal lobes more complex than those of females.
1.8 Influence of abiotic and biotic factors on pheromone communication

The female calling behaviour (extrusion of the pheromone gland from the abdomen) as well as male response to the signal vary depending on abiotic and biotic factors. Environmental factors such as temperature, humidity, photoperiod, light intensity, or wind speed (Sower et al., 1970; Webster and Cardé, 1982; Conner et al., 1985; Delisle and McNeil, 1987; Royer and McNeil, 1991; Delisle and Royer, 1994; Raina, 2003), as well as biotic factors like age (Turgeon and McNeil, 1982; Delisle, 1992) and the availability of suitable host plants (McNeil and Delisle, 1989) can affect the release and reception of sex pheromones in insects.

Female calling behaviour and pheromone synthesis can be affected not only prior to, but also post mating. Transient or permanent loss of sexual receptivity accompanied by loss or reduction of sex pheromones, a process called pheromonstasis, is often observed after mating in many insects (Raina et al., 1986; Gibultowicz et al., 1991). Species that mate multiply tend to resume sexual activity after a refractory period, which varies with male quality and the amount of viable sperm provided by previously mated males (Tayor, 1967; Marcotte et al., 2005).

Insects, like many other animals, have two reproductive strategies to deal with deterioration of the habitat, when reproduction is not possible (Southwood, 1977; Solbreck, 1978). The first is the so-called ‘here later’ strategy where the animals enter a state of arrested development and emerge in the same habitat when conditions improve. The second is the ‘there now’ scenario where adults migrate in search of a habitat that is suitable for reproduction. Johnson (1969) noted that it was immature adults that initiated migratory flight and proposed the ‘oogenesis-flight syndrome’ model, where reproduction and migration are mutually exclusive. In a number of migratory Lepidoptera (e.g. *Autographa gamma*, *Helicoverpa armigera*, *Homoeosoma electellum*) the time of sexual maturation varies as a function of abiotic and biotic factors (McNeil and Delisle, 1989; Riley et al., 1992; Hill and Gatehouse, 1993) but even under ideal conditions for reproduction, adults take several days to become sexually mature. These findings support the hypothesis that the onset of calling behaviour can be used to infer migratory status of an insect species (McNeil, 1986).
1.9 Western bean cutworm (WBC)

The Western bean cutworm (WBC) *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), a univoltine (1 generation/year) insect pest of corn (*Zea mays* L.) and dry beans (*Phaseolus vulgaris* L.), causes both direct crop yield loss and quality reduction in areas where it occurs (Fig. 1.1). While larval feeding is concentrated on developing corn ears and bean pods, damaged parts are more susceptible to additional injury by other insects or pathogens, which may further reduce harvested crop quality (Hagen, 1962). Until recently, this North American native species was restricted to the western United States, however in the last decade WBC has expanded its range eastward. There is growing concerns of WBC becoming a major pest in Ontario and Quebec since the first captures were made in 2008 and 2009, respectively (Michel et al., 2010). Preliminary data (McNeil et al., in prep) suggest that current WBC populations in Ontario are comprised of immigrants from the west that arrive in late June to early July (corresponding with strong westerly winds during the flight period; see www.insectforcast.com), and residents that start emerging in late July to early August (with potentially different calling behaviour). With prices of corn and soybeans at $5.25 and $11.75 per bushel respectively, and the estimated loss due to field infestation of one WBC larva per corn ear at 3.7 bu/ac (Appel et al., 1993; McGee, 2011), Ontario growers could face significant economic losses (i.e. roughly $20/acre for corn).

A number of factors could explain the eastward range expansion of WBC, including changes in climatic conditions and farming practices. Warmer winters in the central United States region (Diffenbaugh et al., 2008), as well as reduced tillage for weed management due to use of glyphosate resistant crops (Givens et al., 2009), favour the overwinter survival of the diapausing prepupal stage. Furthermore, the widespread planting of transgenic corn varieties expressing Bt Cry1Ab protein caused a drastic decline in European corn borer (the primary pest of corn) populations (Pilcher et al., 2002) and reduced use of insecticides (Hunt et al., 2007). Since the WBC is minimally affected by transgenic corn varieties (with an exception of those expressing Cry1F protein), the widespread use of Bt corn caused a niche opening as interspecific competition with other species was low, resulting in higher populations and subsequent range expansion of WBC (Catangui and Berg, 2006; Eichenseer et al., 2008; Dorhout and
Rice, 2010). Regardless of the reasons for WBC’s range expansion, these insects are now present in Ontario, and their progression and distribution need to be monitored.

**Figure 1.1** Characteristics of Western bean cutworm (WBC) *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae). (A) Adult WBC with identifying characteristics of the wings: (1) stripe on the edge of the forewing, (2) small pale circle, (3) crescent shape; (B) WBC life cycle; damage inflicted by WBC larvae on dry beans (C) and corn (D). (Adapted from Michel et al., 2010).

1.10 Pheromone-mediated mating of WBC

Although a network of pheromone traps was established in the Great Lakes region to monitor changes in regional distribution and inter-year population fluctuations of WBC, pheromone trap catches are not reliable indicators of subsequent defoliation in either corn or bean crops in Ontario (Baute, personal communication). There are a
number of reasons for the poor performance of pheromone traps to forecast infestation levels, such as competition between feral females and the pheromone lures, as prevailing weather conditions may differentially influence pheromone emission the two potential sources. However, possible explanations will only be found with a good understanding of the pheromone mediated mating system of the WBC. Little is known about reproductive biology of WBC, partly because of difficulty in establishing successful laboratory colony maintained over many generations, as well as lack of interest in studying WBC as, until recently, it was not a primary pest of economically important crops. A study was hence initiated to investigate pheromone-mediated mating of this insect pest to gain basic understanding of its reproductive biology. Consequently, the goal of this thesis is to investigate the calling behaviour and sex pheromone synthesis of WBC female moths under different abiotic (temperature, relative humidity) and biotic (age, mating status) conditions.

1.11 Objectives

Sex pheromone communication is essential for reproduction in WBC, and although this lepidopteran is native to North America, surprisingly little is known about its reproductive biology and pheromone-mediated mating. As females are the choosy sex, knowledge of female calling behaviour and sex pheromone synthesis would improve our understanding of the reproductive biology of WBC. Additionally, the effect of abiotic and biotic factors on the calling behaviour would help in determining migratory potential of WBC, as well as potential reasons for poorly defined relationship between trap catch data and subsequent crop damage. The objectives of this thesis are:

1) To determine the age at which females mature sexually under different temperature conditions, as it will indicate if the WBC exhibits calling characteristics typical of migratory species

2) To describe the pattern of calling behaviour under different biotic and abiotic conditions to see if there is a greater potential for feral females to compete with pheromone traps under certain environmental conditions.

3) To determine if the pattern of pheromone emission changes with female age.

4) To determine if WBC females are polyandrous.
5) To investigate the length of refractory period of WBC females as a function of their own, and their mate’s, mating status.

This thesis is divided into following Chapters:

Chapter 1 provides background information about communication in mate choice, including communication through chemical signals (pheromones) in Lepidoptera.

In Chapter 2, I determine the age of sexual maturation of WBC females under different cycling temperatures, as well as describe the pattern of calling behaviour as a function of different biotic (age) and abiotic (temperature and relative humidity) conditions.

In Chapter 3, I determine if the pattern of pheromone emission by WBC virgin females changes with age.

In Chapter 4, the incidence of polyandry is determined, followed by investigation of the influence of the mating status (of both males and females) on the refractory period of females.

Chapter 5 summarizes and discusses the results from this thesis, and poses questions about WBC reproductive biology, along with suggestions for future studies.

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CHAPTER 2

Calling behaviour pattern of Western bean cutworm (*Straicosta albicosta*) (*Lepidoptera: Noctuidae*) virgin females is age-dependent, but is not affected by temperature or relative humidity

A version of this chapter will be submitted for publication to *Proceedings of the Royal Society B - Biological Sciences*.

2.1 Introduction

In *Lepidoptera*, female calling behaviour (the extrusion of an ovipositor) is associated with pheromone release and subsequent mate attraction. The age at which females initiate calling, as well as the calling behaviour of virgin females of some nocturnal *Lepidoptera* are affected by both biotic factors such as age and mating status, as well as prevailing abiotic conditions. Once mature, the pattern of female calling can be affected by age, with the mean onset time of calling (the time during scotophase at which calling behaviour is initiated; MOTC) occurring earlier on each successive night (Turgeon and McNeil, 1982; Noldus and Potting, 1990), and the mean time spent calling (MTSC) increasing (Delisle, 1992; Jacas and Pena, 2002).

Furthermore, female calling behaviour is influenced by abiotic factors including temperature, relative humidity (RH), and photoperiod (Webster and Cardé, 1982a; Delisle and McNeil, 1987a; Royer and McNeil, 1991; Delisle and Royer, 1994; Raina, 2003). At lower temperatures (≤ 15°C), females initiate calling earlier (Delisle and McNeil, 1987a; Delisle, 1992), possibly to attract males before their flight ability is reduced (Cardé et al., 1975; Turgeon and McNeil, 1983). Although relative humidity does not affect the age of first calling, fewer females exhibit sustained calling under low humidity conditions to avoid desiccation through the thin membranous layer of extruded ovipositor (Royer and McNeil, 1991; Webster and Cardé, 1982b). Therefore, reproduction of *Lepidoptera* that rely on chemical communication in form of pheromones for mate attraction, can become temporally and spatially limited as the environmental factors change on daily, and seasonal bases.
The Western bean cutworm (WBC) *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), causes substantial damage to corn and dry beans over its geographical range (Michel et al., 2010). Although native to western United States, during the past decade this univoltine insect pest has expanded its range eastward (partly due to changes in climatic conditions and farming practices) and has been found around the Great Lakes region since 2008 (Baute, 2009). While there is evidence that local populations are present and emerge in late July/early August (J. Smith, personal communication), the first males caught in pheromone traps occur several weeks earlier (T. Baute, personal communication), suggesting that each summer the population is comprised of both immigrants and residents.

The migrant and resident WBC females could potentially have different calling behaviour, which could not only result in assortative mating, but also contribute to the competition between the feral females and pheromone traps (set out for population monitoring to estimate subsequent crop loss based on number of catches). Very little is known about the reproductive biology of WBC, therefore this study was initiated to determine how biotic (age) and abiotic (temperature and RH) factors influence the age of sexual maturation and calling behaviour of WBC virgin females. If older virgin females modify their calling behaviour to avoid competition with younger ones, then the MOTC would occur earlier in the scotophase, and the MTSC would increase with virgin female age. Similarly, if environmental conditions are indicative of deteriorating habitat and serve as cues for migratory flight, at lower temperatures females would delay when they become sexually mature, and the MOTC should occur earlier in the scotophase at low temperatures. Furthermore, the calling behaviour should be exhibited less frequently at under low relative humidity conditions.

### 2.2 Methods

*Insect rearing*

WBC adults used were obtained from a laboratory colony established using field material, collected at different times during the flight period at Bothwell, Ontario (42.2509° N, 82.1915° W) in 2011 and restocked in the same manner in 2012. The
colony was maintained at 20 ± 1 °C; 15 ± 1°C (L:D); 70 ± 5% RH; 16L:8D photoperiod. Adults were held in mating cages (approximately 15 females and 25 males per cage) with ad lib 8% sugar water and red bean plants (approximately 4-5 weeks old) as oviposition sites. Eggs were collected daily and the newly emerged larvae were reared on artificial pinto bean diet (modified after Shorey and Hale, 1965) in individual polystyrene cups (~30 mL). This univoltine species has an obligatory diapause in the prepupal stage, so individuals were held at the standard rearing conditions until they pupated (~100 days after hatching). The pupae were washed in 3.7% formaldehyde for 1 min followed by 2 min rinse with distilled water, and then sexed during the pupal stage. Adults were collected daily and the sex confirmed at adult stage based on external anatomy of the reproductive system (Appendix A, panel B). A minimum of 30 newly-emerged virgin females were randomly assigned to the different treatments described below before the onset of their first scotophase and held in the absence of males or host plants. The experimental temperature and humidity conditions tested are biologically relevant as they were based on the 30 year average (1971-2000) weather conditions for London, ON, (http://climate.weatheroffice.gc.ca/climate_normals/index_e.html).

**Calling behaviour**

To observe the calling behaviour, newly emerged WBC adult virgin females were placed in individual clear plastic cylinders (diameter = 4.5 cm; height = 9.5 cm) with cotton wicks soaked in 8% sugar solution as a food source (Appendix A, panel A). Females were observed at 10 min intervals during scotophase (for a total of 8 h), using a flashlight covered with No. 29 Kodak Wratten red filter paper and a layer of tissue paper. In this species, calling and pheromone release are associated with an evident extrusion of the ovipositor. The age of first calling (indicative of sexual maturation), the mean onset time of calling after light out (MOTC), the mean time spent calling (MTSC) and the number of calling bouts, were measured for 3 consecutive days.

To study the effect of temperature on calling, females were held under constant 16L:8D photoperiod and 80% RH, using four different temperature regimes: 25:20; 25:15; 20:15; 20:10 °C L:D. To investigate the effect of RH on the calling behaviour,
females were tested at 20:10 °C L:D, (the regime with the most consistent calling in the previous experiment) under 16L:8D photoperiod, with RH at either 60% or 80%.

Data analysis
Data that did not satisfy the assumptions of normality and equality of variance were analyzed using non-parametric tests. Data relating to the effect of temperature on sexual maturation were log transformed to ensure normality and equality of variance, and analysed using a one-way ANOVA followed by Tukey’s post hoc test ($\alpha = 0.05$). Data relating to the onset and duration of calling by WBC females at different temperatures as a function of age were square root transformed, and a MANOVA (using Pillai’s trace followed by two-way ANOVAs and Tukey’s post hoc tests at $\alpha = 0.05$) was performed. Data on number of calling bouts were analyzed using Kruskal-Wallis test with temperature and age separately.

Data relating to the effect of RH on sexual maturation were analyzed using a two-tailed t-test, while those relating to the onset of calling, and calling duration data were analyzed using a MANOVA, followed by two-way ANOVAs. Data on the onset of calling and calling duration were square root transformed before running MANOVA. Kruskal-Wallis (age as a factor), followed by Dunn’s multiple comparison test, and Mann-Whitney U test (RH as a factor) were used to determine if there are differences in the number of calling bouts. All tests were done using IBM SPPS v. 20 (NY, USA) statistical package. PRISM v 4.0 (Graphpad; CA, USA) was used to run Dunn’s multiple comparison test.

2.3 Results

The age at sexual maturation (as indicated by first calling) of WBC females varied significantly ($F_{(3,143)} = 14.07, P < 0.0001$) between treatments. The difference in sexual maturation was not a direct effect of temperature, but rather the result of the differential temperature change between the photophase and the scotophase. Females experiencing a 10°C difference between day and night temperatures took significantly less time to mature than those experiencing only a 5°C change (Figure 2.1). There was
higher survival over time at the lower temperatures and the most consistent calling was seen at 20:10 °C (Table 2.1).

The MOTC, (Fig. 2.2; $F_{(2, 341)} = 35.8, P < 0.0001$) and the MTSC (Fig. 2.3.; $F_{(2,342)} = 42.9, P < 0.0001$) were significantly different with age ($V = 0.24, F_{682} = 23.1, P < 0.0001$), as females called earlier and spent more time calling on successive nights. However, neither the MOTC ($F_{(3, 341)} = 2.01, P = 0.11$) nor the MTSC ($F_{(3, 342)} = 2.18, P = 0.09$) differed significantly under the different temperature regimes tested ($V = 0.27, F_{682} = 1.55, P = 0.16$). There were no significant interactions between age and treatment for either MOTC ($F_{(3, 341)} = 0.21, P = 0.98$) or MTSC ($F_{(3, 342)} = 0.84, P = 0.54$). The number of calling bouts did not differ with either female age ($H_{2} = 5.89, P = 0.07$) or temperature conditions ($H_{3} = 0.98, P = 0.81$) (Fig. 2.4).

In the experiment looking at the effect of relative humidity on the calling behaviour, there were significant age ($V = 0.24, F_{4, 328} = 11.3, P < 0.0001$) effects observed under the different relative humidities, with older females starting to call earlier ($F_{(2,164)} = 13.09; P < 0.0001$) and maintaining calling for a longer time ($F_{(2,164)} = 22.89; P < 0.0001$). However the relative humidity conditions tested did not affect the age of sexual maturation (on average 4.5 days; $t_{59} = 0.05, P = 0.96$), or the calling behaviour ($V = 0.02, F_{2,163} = 1.22, P = 0.30$): the MOTC ($F_{1,164} = 0.07; P = 0.79$), the MTSC ($F_{1,164} = 2.25; P = 0.14$). As in the previous experiment, the number of calling bouts was not affected by age ($H_{2} = 1.18 ; P = 0.55$), but females held under 60% RH had more calling bouts compared to those at 80% RH ($U = 2984, N_{1}=N_{2}= 30, P < 0.05$) (Fig. 2.7).
Table 2.1 Percentage of Western bean cutworm (*Striacosta albicosta*) virgin females surviving to and calling on second and third consecutive scotophases since the onset of calling at 80% RH and four different cycling temperature settings.

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<th>Temperature setting (°C L:D)</th>
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<td>Survival (%)</td>
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Figure 2.1 Mean (days ± SE) age of first calling for Western bean cutworm (*Striacosta albicosta*) virgin females at 80% RH and four different cycling temperature settings. Bars with the same letters are not significantly different based on Tukey’s post hoc test (P > 0.05).
Figure 2.2 Mean (min ± SE) onset time of calling after the onset of the scotophase for the first three consecutive nights of calling (calling age) of Western bean cutworm (*Striacosta albicosta*) virgin females at 80% RH and four different cycling temperature settings. Bars with the same letters are not significantly different based on Tukey’s post hoc test (P > 0.05). Upper and lower case letters refer to differences among temperatures and ages, respectively.
Figure 2.3 Mean (min ± SE) time spent calling for the first three consecutive nights of calling (calling age) of Western bean cutworm (*Striacosta albicosta*) virgin females at 80% RH and four different cycling temperature settings. Bars with the same letters are not significantly different based on Tukey’s post hoc test (P > 0.05). Upper and lower case letters refer to differences among temperatures and ages, respectively.
Figure 2.4 Mean (± SE) number of calling bouts exhibited by Western bean cutworm (Striacosta albicosta) virgin females for the first three consecutive nights of calling (calling age) at 80% RH and four different cycling temperature settings. Bars with the same letters are not significantly different based on Kruskal-Wallis test (P > 0.05). Upper and lower case letters refer to differences among temperatures and ages, respectively.
Figure 2.5 Mean (min ± SE) onset time of calling after the onset of the scotophase for the first three consecutive nights of calling (calling age) of Western bean cutworm (*Striacosta albicosta*) virgin females under 20:10°C L:D cycling temperature and two RH settings. Bars with the same letters are not significantly different based on Tukey’s post hoc test (P > 0.05). Upper and lower case letters refer to differences between RH and among ages, respectively.
Figure 2.6 Mean (min ± SE) time spent calling during the first three consecutive nights of calling (calling age) of Western bean cutworm (*Striacosta albicosta*) virgin females under 20:10°C L:D cycling temperature and two RH settings. Bars with the same letters are not significantly different based on Tukey’s post hoc test (P > 0.05). Upper and lower case letters refer to differences between RH and among ages, respectively.
Figure 2.7 Mean (± SE) number of calling bouts exhibited by Western bean cutworm (*Striacosta albicosta*) virgin females for the first three consecutive nights of calling (calling age) under 20:10°C L:D cycling temperature and two RH settings. Bars with the same letters are not significantly different based on Kruskal-Wallis (P > 0.05) and Mann-Whitney U (P > 0.05) tests for the RH and calling age respectively. Upper and lower case letters refer to differences between RH and among ages, respectively.
2.4 Discussion

Based on the observation that the age of sexual maturation of the true armyworm (*Pseudaletia unipuncta*) occurred several days post emergence under conditions suitable for reproduction, McNeil (1986) hypothesised that the age at which female moths first express calling behaviour (indicative of sexual maturation) could be used to identify migrant species. It has been proposed that extended pre-calling period (PCP) would provide an extended time window for migratory flight prior to sexual maturation and/or for the accumulation of resources for extended migratory flight (McNeil, 1986; Han and Gatehouse, 1991; McNeil et al., 1995). Similar delays in the PCP have subsequently been reported for the oriental armyworm (*Pseudaletia separata*), the black cutworm (*Agrotis ipsilon*) and the sunflower moth (*Homoeosoma electellum*) (Swier et al., 1976; Hirai, 1984; McNeil and Delisle, 1989; Han and Gatehouse, 1991), all known to be migrant species. Conversely, under the same ecological conditions non-migrant species, such as the oblique banded leafroller, *Choristoneura rosaceana*, females are generally sexually mature and initiate calling within 48h of emergence (Delisle, 1992).

The delay in sexual maturation of WBC females under conditions that are suitable for reproduction is similar to that observed in other migratory moths suggesting this species is also a migrant. Since WBC females become sexually mature 4-6 days post emergence, and may take up to 12 days, this species could undertake extended migratory flights, that would account for the recently observed eastward range expansion (Michel et al., 2010). As the WBC is univoltine, with a flight period occurring in June/July, the cues initiating migration probably relate to the availability of host plants and mates, rather than climatic conditions associated with a deteriorating habitat.

In moths, the onset of calling is related to ovarian development (Gerber and Howlader, 1987; Cusson and McNeil, 1989), so exposure to low constant temperatures during pupal or adult stages result in lower metabolic rates (Colvin and Gatehouse, 1993; Del Socorro and Gregg, 1997) causing a delay in the age of first calling (Turgeon and McNeil, 1983; Delisle and McNeil, 1987b; Han and Gatehouse, 1991; Del Socorro and Gregg, 1997). In the case of the WBC, rather surprisingly, no direct effect of temperature on sexual maturation was observed, but rather the observed changes were due to the difference between day and night temperatures.
In migratory species, the delay in the onset of calling is controlled by juvenile hormone (JH) production (Cusson and McNeil, 1989; Gadenne, 1993; Zhou et al., 2000). Very little research has been carried out examining the diel periodicity of JH production, but in the boll weevil JH biosynthesis was higher during the scotophase than the photophase (Taub-Montemayor et al., 2005), and in the sand field cricket it peaked at the end of photophase-early scotophase (Zhou and Zera, 2004), so the drop in temperature may stimulate change in JH production. It is therefore possible, that a 10°C change in temperature between day and night stimulates higher JH production by WBC females than a 5°C change, resulting in the observed shorter PCP. The idea that temperature shifts may stimulate JH biosynthesis is supported by the observation that 24h after being transferred from fall to summer conditions, JH biosynthesis by P. unipuncta females was higher than in individuals held continuously under summer conditions (Cusson et al., 1990). Furthermore, P. unipuncta females under a cycling thermoperiod became sexually mature at a significantly younger age that those at constant temperature, even though the mean temperature was identical in both experiments (El Ouartassi, 1991). Interestingly in the non-migratory moth, M. brassicae, there was no difference in the PCP between the two temperature regimes (El Ouartassi, 1991), suggesting that migratory species respond differently than non-migrants to cycling temperatures, an idea that merits further examination.

The effect of age on female calling behaviour has been well documented in Lepidoptera, with MOTC usually advancing, and MTSC increasing with age (Turgeon and McNeil, 1982; Noldus and Potting, 1990; Delisle, 1992; Hou and Sheng, 2000; Xiang et al., 2010). A similar effect of age was observed in WBC. It has been postulated that these age related changes in calling behaviour are an adaptation allowing older females to avoid competition with younger ones (Swier et al., 1977; Turgeon and McNeil, 1982; Webster and Cardé, 1982a), as female attractiveness declines with age (Delisle 1992), probably related to lower pheromone production (Webster and Cardé, 1982a; Delisle and Royer, 1994). A drop in temperature at the onset of the scotophase may also result in an advance in the MOTC (Delisle and McNeil, 1987a; Delisle, 1992; Zhang and Paiva, 1998), an adaptation to ensure mating before low temperatures inhibit flight. In P. unipuncta, changes in both the MOTC and the MTSC were a function of
temperature differential that the females experience (Delisle and McNeil, 1987a). Furthermore, there may be marked differences in the patterns of calling behaviour under constant and cycling temperature regimes, as demonstrated in *Phyllonorycter junoniella* and *P. unipuncta* (El Ouartassi, 1991; Mozuraitis and Buda, 2006). However, while there was an age effect on the calling patterns of WBC females, there were no differences in either the MOTC or the MTSC under the different temperature cycling regimes tested. Because WBC is a univoltine species, they are not exposed to a wide range of temperatures and RH during their flight between late June and early August, which could explain why no effect of relative humidity was found on the calling behaviour of WBC females over the range of conditions tested, and the rather limited effects of temperature.

The delay in sexual maturation in WBC confirms migratory strategy of this insect. Since larger drops in temperature caused shorter PCP while calling behaviour was more consistent under lower temperatures, low temperature may actually be favourable for the pheromone mediated sexual behaviour of WBC. Insensitivity of WBC females (in terms of their calling behaviour) to temperature and RH is probably linked to the univoltine life cycle of this Lepidoptera, and their exposure to narrow range of environmental conditions. Environmental variation that WBC experiences under natural conditions is a lot more variable than the one determined by the cycling temperatures and RH in this study, therefore field collected data would confirm the current results.

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CHAPTER 3

Sex pheromone titer at the onset of calling of Western bean cutworm, *Striacosta albicosta* (Lepidoptera: Noctuidae) virgin females is not age-dependent

A version of this chapter will be submitted for publication to *Journal of Chemical Ecology*.

3.1 Introduction

Pheromones are species specific chemical signals, that can elicit a physiological (primer pheromone) or behavioural (releaser pheromone) response in a receiver (Karlson and Luscher, 1959). In many insects, including Lepidoptera, reproduction involves the deployment of sex pheromones, where the sex that signals and the time of signalling are determined by the costs of producing and responding to pheromones (Bergstrom and Lachmann, 1997; Johansson and Jones, 2007). Generally females (the choosy sex) produce the long distance pheromone to attract potential mates, while males may produce short distance pheromones that females use when assessing male quality (Greenfield, 1981; Fitzpatrick and McNeil, 1988; Birch et al., 1990; Svensson, 1996).

The geographic distribution of the Western bean cutworm (WBC) *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae), an important agricultural pest of corn and dry beans, has increased dramatically in the last decade (Michel et al., 2010). The WBC female sex pheromone was characterized (Klun et al., 1983), and while pheromone traps baited with commercially available lures have proved useful for monitoring regional distribution and inter-year population fluctuations, trap catch data are not reliable indicators of subsequent defoliation in either corn or bean crops (T. Baute, personal communication). There may be many reasons for this lack of relationship, including the possibility that the lures do not contain the correct blend/concentration and are thus less attractive to males than calling feral females.

However, without a thorough understanding of the reproductive biology of WBC it is difficult to investigate possible causes for the disconnect between trap catch and population density. Therefore, this study was undertaken to examine different aspects of
pheromone mediated mating in the WBC. The calling behaviour of female moths may be affected by a number of abiotic and biotic factors (McNeil, 1991). For example, as female age, older females initiate calling earlier and, increase the time spent calling (Turgeon and McNeil, 1982; Delisle, 1992; Jacas and Pena, 2002). It was suggested that these changes are adaptations to reduce competition with younger females early in the scotophase (Swier et al., 1977; Turgeon and McNeil, 1982; Webster and Cardé, 1982), as older females may be less attractive males than younger ones (AliNiazee and Stafford, 1971; Delisle, 1992) due to reduced pheromone production (Webster and Cardé, 1982; Delisle, 1992; Delisle and Royer, 1994).

Limited information is available on the reproductive biology of WBC, but recently (see Chapter 2) the calling behaviour of WBC virgin females was shown to change with age, with older females shifting their calling activity to earlier in the scotophase suggesting age-dependent changes in sex pheromone content. As noted above, this age related shift has been proposed as an adaptation to avoid competition with younger females who produce more pheromone. Therefore this study examined the pheromone gland content of virgin WBC females on the first three nights of calling. If earlier calling is an adaptation by which older females reduce competition with younger, more attractive ones, a decline in pheromone production with age is expected.

3.2 Methods

*Insect rearing*

Adult WBC were obtained from laboratory maintained colony (restocked annually with field collected individuals from Bothwell, Ontario; 42.2509° N, 82.1915° W) held at 20 ± 1°C: 15 ± 1°C (L:D); 70 ± 5% RH; 16L:8D photoperiod. Colony cages contained red bean plants as oviposition sites and 8% sugar water supply as a food source. Egg batches were collected daily and the newly hatched larvae reared individually on pinto bean diet (modified after Shorey and Hale, 1965) in polyester cups (~30 mL). Pupae were sexed (Breeland, 1958), washed in 3.7 % formaldehyde (1 min) followed by a rinse with distilled water (2 min), then kept at 20 ± 1°C: 10 ± 1°C (L:D); 80 ± 5% RH; 16L:8D photoperiod until adults emerged. Only females were kept for the experiments.
**Pheromone gland collection and pheromone extraction**

Newly emerged virgin WBC females were transferred to individual clear plastic cylinders (diameter = 4.5 cm; height = 9.5 cm) with sugar (8%) soaked cotton wicks (Appendix A, panel A). Females were held at 20 ± 1°C: 10 ± 1°C (L:D); 80 ± 5% RH; 16L:8D photoperiod conditions, under which WBC females become sexually mature about four days after emergence (Chapter 2). Females were observed every 10 minutes throughout the scotophase (using a flashlight covered with No. 29 Kodak Wratten red filter paper and a layer of tissue paper) until they initiated calling for the first time. Since pheromone titer was shown to be independent of female body weight in several lepidopteran species (Miller and Roelofs, 1980; Charlton and Cardé, 1982; Schal et al., 1987), I did not correct for female body mass. Pheromone glands were extracted at the onset of calling (time of maximum amount of pheromone produced in most Lepidoptera; Schal et al., 1987; Delisle and Royer, 1994) on the first, second, or third night of calling (n = 23 females for each age). Females were killed, the abdomen was gently pressed to expose the pheromone gland (Appendix A, panel C), which was excised with a pair of fine scissors and submerged in 15 µL solution of heptane in gas chromatography (GC) vials with glass inserts (Agilent Technologies, Mississauga, Canada) for 25 sec (chosen for optimal extraction time as longer extraction times result in non-pheromone components being extracted, which complicates subsequent peak identification and quantification; J. McNeil, personal communication). The solvent solution contained 0.5 ng/ µL of Z-9-teradecenyl acetate (Z9-14Ac) as an internal standard. The total time of extraction from the onset of calling took approximately two min. Samples were kept at -20°C until they were analyzed.

**Gas chromatography (GC) analysis**

One microliter of each individual gland extract was analyzed with an Agilent 7890A gas chromatograph equipped with splitless injector (225°C), flame ionizing detector (FID) (300°C), and a CP-Sil 5 MS LB column (27 m x 320 µm x 0.25 µm). Nitrogen (N₂), at a flow rate of 2.5 mL/min, was used as a carrier gas. The column temperature program was: 100°C for 1 min, increased to 130°C at 40°C/min, increased to 145°C at 1°C/min,
increased to 200°C at 10°C/min, held for 3 min. Retention times of the four WBC female sex pheromones, Z-5-dodecenyl acetate (Z5-12:Ac), Z-7-dodecenyl acetate (Z7-12:Ac), Z-11-dodecenyl acetate (Z11-12:Ac), dodecyl acetate (12:Ac), and the internal standard, Z9-14Ac, were 11.89, 12.15, 12.37, 12.70 and 19.85 min, respectively.

**Statistical analysis**

MANOVAs (with Pillai’s trace) using SPSS v. 20.00 (IBM, New York, USA) statistical software were performed to determine if the concentration and the ratio of pheromone components differed across the ages. Samples with no detectable pheromone were excluded from the analysis (a total of 54 and 24 samples were used in the titer and ratio analyses). Ratios were calculated based on Z7-12:Ac with a reference value of 1 (since A7-12:Ac had the lowest concentration), therefore samples that did not contain this acetate could not be included in the calculation. Since there were no differences in pheromone titer among ages, the data were pooled and a one-way ANOVA was used to determine if there are differences in relative proportions of individual acetates in the sex pheromone.

**3.3 Results**

The sex pheromone of WBC virgin females had been reported as a four component blend of Z-5-dodecenyl acetate (Z5-12:Ac), Z-7-dodecenyl acetate (Z7-12:Ac), Z-11-dodecenyl acetate (Z11-12:Ac), and dodecyl acetate (12:Ac) at a 5:1:5:5 ratio (Klun et al., 1983). We observed the same four acetates reported previously (Fig. 3.1), but there was considerable inter-individual and between day variability with respect to the number of different acetates detected in the gland of any given female (Table 3.1). There was a significant difference in the relative proportions of the four components ($F_{(3, 92)} = 19.6$, $p < 0.001$) (Fig. 3.2), but there was no difference in either the mean concentration of individual sex pheromone components ($V = 0.46$, $F_{(8, 38)} = 1.42$, $p = 0.22$) (Table 4.1) or ratios ($V = 0.25$, $F_{(3, 19)} = 0.96$, $p = 0.47$) (Figure 3.2) as a function of calling age of virgin females. Based on the mean values obtained, the ratio of Z5-12:Ac, Z7-12:Ac, Z11-12:Ac and 12:Ac, was 4:1:4:4.
**Figure 3.1** A gas chromatogram of a sex pheromone extract of a single Western bean cutworm (*Striacosta albicosta*) virgin female containing four acetates: 1 = Z-5-dodecenyl acetate (Z5-12:Ac); 2 = Z-7-dodecenyl acetate (Z7-12:Ac); 3 = Z-11-dodecenyl acetate (Z11-12:Ac); 4 = dodecyl acetate (12:Ac). IS = internal standard, Z-9-teradecenyl acetate (Z9-14Ac) at 0.5 ng/µL.
Table 3.1 Mean concentration (ng/µL ± SE) and mean relative abundance of sex pheromone components of Western bean cutworm (*Spisicosta albicosta*) virgin females at the onset of calling over the first three days of calling based on gas chromatography analysis of sex pheromone gland extracts.

<table>
<thead>
<tr>
<th>Pheromone component</th>
<th>Concentration (ng/µL)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>n</td>
<td>Day 2</td>
<td>n</td>
<td>Day 3</td>
</tr>
<tr>
<td>Z5-12:Ac</td>
<td>0.232 ± 0.03 a 19</td>
<td></td>
<td>0.315 ± 0.06 a 13</td>
<td></td>
<td>0.270 ± 0.03 a 19</td>
</tr>
<tr>
<td>Z7-12:Ac</td>
<td>0.097 ± 0.03 a 8</td>
<td></td>
<td>0.097 ± 0.02 a 4</td>
<td></td>
<td>0.076 ± 0.01 a 12</td>
</tr>
<tr>
<td>Z11-12:Ac</td>
<td>0.204 ± 0.04 a 19</td>
<td></td>
<td>0.200 ± 0.04 a 13</td>
<td></td>
<td>0.212 ± 0.02 a 20</td>
</tr>
<tr>
<td>12:Ac</td>
<td>0.230 ± 0.04 a 20</td>
<td></td>
<td>0.239 ± 0.03 a 18</td>
<td></td>
<td>0.224 ± 0.02 a 23</td>
</tr>
</tbody>
</table>

*Note: Means within a row followed by the same letter are not significantly different based on MANOVA. Sample sizes refer to number of samples that contained a particular component, not number of samples analysed.*
Figure 3.2 Relative proportions of sex pheromone components in the pheromone glands of Western bean cutworm (*Striacosta albicosta*) virgin females at the onset of calling on the first three days of calling. Sex pheromone components: Z-5-dodecenyl acetate (Z5-12:Ac), Z-7-dodecenyl acetate (Z7-12:Ac), Z-11-dodecenyl acetate (Z11-12:Ac), and dodecyl acetate (12:Ac). Bars with the same letters are not significantly different based on Tukey’s post hoc test (P > 0.05).
3.4 Discussion

The amount of individual sex pheromone components of WBC ranged from 1.2 ng/gland (Z7-12:Ac) to 4.8 ng/gland (Z5-12:Ac), which is small compared to other Noctuids that contain between 20 and 600 ng of their respective pheromone components in gland extracts (Delisle and McNeil, 1987; Park et al., 1996; Gemeeno and Haynes, 2001; Raina, 2003). The pheromone titer of WBC females did not differ with age which is quite different from previous studies that reported a decline in pheromone gland content with female age (Webster and Cardé, 1982; Delisle and Royer, 1994). Assuming that gland content reflects amount of pheromone released, lack of age-dependency in the WBC sex pheromone titer suggests that at the onset of calling, older virgin females are as attractive to receptive males as younger ones. Since the onset of calling in older WBC females is shifted to earlier time of the scotophase (see Chapter 2), by extending their calling window, older virgin females are able to attract potential mates before the calling activity of the younger females starts. The absence of an age effect on pheromone production does not lend support the hypothesis, that older females call earlier to avoid competition with younger conspecifics, that I postulated in chapter 2. In contrast the results suggest that older females actually could have competitive advantage in selecting good quality mates.

There was a lot of variability in the presence and absence of the different components observed in the pheromone glands of WBC females both within and between days. Klun et al. (1983) reported highest trap catches with the three component blend of Z5-12:Ac, Z7-12:Ac, and Z11-12:Ac, with the presence or absence of 12:Ac having no effect. Traps baited with a binary blend of Z5-12:Ac and Z11-12:Ac (the two most consistently detected compounds in WBC pheromone glands in the current study), also captured males at a level similar to traps baited with females. Thus, it is possible that the relative attractiveness of females could vary based on the concentration of Z7-12:Ac present. This aspect needs further investigation, by looking at gland content at different times through the calling period.

The ratio of the four pheromone components, calculated only using the results from individual females containing Z7-12:Ac, was 4:1:4:4, which differs from the 5:1:5:5 ratio reported by Klun et al. (1983). However, it should be noted that the results of Klun...
et al. (1983) were based on pool sample of 4 females of unknown age and mating status. However, given the pronounced inter-female differences in both the number and concentration of the compounds found in individual glands, it is clear that the ratios for individual females would be much more variable. The ratio of pheromone components is not always the same in different geographic populations of the same lepidopteran species (Toth et al., 1992; Park et al., 1996, El-Sayed, 2003). Considering the migratory capacity of WBC (see Chapter 2) and its range expansion, the observed ratios of WBC female sex pheromone reported previously and presently might differ because of different insect collection sites (Idaho USA and Ontario Canada, respectively).

Furthermore, pheromone gland extracts may not reflect the ratios of the volatiles emitted during calling (Pope et al., 1982; Heath et al., 1991). Pheromone traps have proven effective for monitoring the presence of WBC (Mahrt et al., 1987; Dourhout and Rice, 2008) but male trap catch data do not provide a good measure of subsequent damage in crops (T. Baute, personal communication). The commercially available lures are formulated using the results from Klun et al. (1983) that were obtained from a pooled pheromone gland extract from 4 females. The results obtained from current analysis of individual pheromone glands suggest that additional research looking at the pheromone blends actually emitted by calling females may lead to a more effective pheromone lure.

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CHAPTER 4

Previous mating status regulates post-mating refractory period in
Western bean cutworm, *Striacosta albicosta* (Lepidoptera: Noctuidae)
females

A version of this chapter will be submitted for publication to *Animal Behaviour*.

4.1 Introduction

The optimal reproductive strategies of males and females differ: males benefit from acquiring as many mates as possible (Parker and Birkhead, 2013; see Dewsbury, 2005 for critique), while females typically select fewer but high quality mates. Since Bateman (1948) showed that males of *Drosophila melanogaster* benefit more from multiple mating than females, monoandry became the assumed norm. However, polyandry (multiple mating by female) is common among insects and a meta analysis of insect studies found that in most cases, females gain direct fitness benefits from multiple matings (Arnqvist and Nilsson, 2000). The benefits may include avoiding sperm shortage (Baker et al., 2001, Evans and Marshall, 2005), increasing sperm competition and ensuring genetic diversity of the offspring (Parker, 1970; Drummond, 1984), and avoiding genetic defects resulting from long term storage of the sperm (Halliday and Arnold, 1987). Although there are many direct benefits of multiple mating such as obtaining additional nutrients (Drummond, 1984; Gowaty et al., 2010), polyandry may just be a convenient means by which females avoid energy and time expenditure required to avoid harassment by coercive males (Andersson, 1994). Certain models also predict that polyandry should be a common strategy to minimize the risk of virgin death, even if polyandry is not optimal, or carries an increased risk of mortality (Kokko and Mappes, 2013).

Additional nutritional resources either as a prey item or directly from the male ejaculate (Thornhill, 1976; Gwynne, 1984) may be used directly for female maintenance and/or invested into offspring (Smedley and Eisner, 1996), thereby increasing female fitness. In Lepidoptera, males transfer a spermatophore that contains sperm and other
secretions. Although certain substances from male accessory ejaculate stimulate egg production and egg laying (Webster and Cardé, 1984, Proshold, 1995), thus increasing female fitness, there may be a reduction in female lifespan (Fowler and Partridge, 1989; Chapman et al., 1998), and influencing female mating behaviour (Chen, 1984; Eberhard, 1996; Klowden, 1999).

In insects, there may be a transient or permanent loss of sexual receptivity following mating (Raina et al., 1986; Giebultowicz et al., 1991). In species that rely on pheromones for mating, the refractory period (loss of sexual receptivity following mating; RP) is accompanied by a decline in pheromone production (pheromonostasis). This process is frequently modulated by sex peptides and other accessory gland secretions transferred by the male during copulation, which reduce the probability of sperm competition, and thus could increase male reproductive success. Species that mate multiply resume sexual activity after a RP, which varies with male quality and the amount of viable sperm provided (Taylor, 1967; Marcotte et al., 2005). The length of the RP may also be affected by substances that females produce, post mating, to counteract the effects of male derived compounds (Kingan et al., 1993, Kingan et al., 1995; Fan et al., 1999).

A preliminary analysis of field collected Western bean cutworm (WBC) Striacosta albicosta (Smith) (Lepidoptera: Noctuidae) females suggested that this species is polyandrous. Therefore, this study was undertaken to determine the incidence of polyandry in WBC, and the effect of mating status of both sexes on the RP of WBC females. If female RP varies with male quality, then virgin females mated with previously mated males would have shorter refractory periods than virgin females mated with virgin males.

4.2 Methods

Insect rearing

WBC adult were obtained from an laboratory colony established using field collected material from Bothwell, Ontario in 2011 and 2012, and maintained at 20 ± 1 °C/ 15 ± 1 °C (L:D); 70 ± 5% RH; 16L:8D photoperiod. Adults were kept in mating cages with an 8% sugar water solution and 5-6 week old red bean plants as oviposition
sites. Eggs were collected daily, and newly emerged larvae were reared individually on artificial pinto bean diet (modified after Shorey and Hale, 1965) in polystyrene cups (~ 30 mL). Pupae were sexed (Breeland, 1958) and washed in 3.7 % formaldehyde for 1 min followed by a 2 min rinse with distilled water. Male and female pupae were kept separately at 20 ± 1 °C/ 15 ± 1 °C (L:D); 80 ± 5% RH; 16L:8D photoperiod in mesh cages (22 cm x 15 cm x 15 cm) in vermiculite sprayed with distilled water daily to maintain moisture. Newly emerged adults were transferred into individual clear plastic cylinders (diameter = 4.5 cm; height = 9.5 cm), with cotton wicks soaked in 8% sugar water for feeding. Females were held in one incubator at 20 ± 1 °C/ 10 ± 1 °C (L:D); 80 ± 5% RH; 16L:8D while males were held in another at 20 ± 1 °C/ 15 ± 1 °C (L:D); 80 ± 5% RH; 16L:8D, until needed.

**Spermatophore dissection**

WBC females were collected over the flight period (July 11- August 3) in a light trap at Bothwell, Ontario, (42.2509° N, 82.1915°) in 2012. In addition dead females were collected from the bottom of the colony cage in the laboratory. Females were dissected and the number of spermatophores in the bursa copulatrix (indicative of the number of matings) was counted. A total of 1682 and 556 field and laboratory collected females were dissected.

**Mating**

Virgin females (4 days old) were set in individual mesh cages (22 x 15 x 15 cm) at the start of scotophase with a supply of 8% sugar water, and either a virgin male (4-6 days old) or one that had previously mated once (7-9 days old) or twice (11-13 days old) before. Previously mated males were allowed a 48 hour rest period between matings. Pairs were checked every 20 minutes using a red light (flashlight covered with No. 29 Kodak Wratten red and a layer of tissue paper). The total duration of each mating was recorded and at the end of the scotophase females that successful mated were held in individual plastic cylinders and their calling behaviour observed every 10 minutes during subsequent scotophases to determine the duration of the RP and the MOTC when calling.
resumed. The same experiment was repeated but in this case virgin males were paired with either virgin females, or ones that had mated once or twice before. In all combinations there was \( n \geq 30 \) except virgin male x twice mated female where \( n = 15 \).

**Data analysis**

A two-way ANOVA (\( \alpha = 0.05 \)), followed by a Tukey’s post hoc test was used for the data on the duration of mating. Data on the length of the refractory period and the time spent calling were analysed by one-way ANOVAs (\( \alpha = 0.05 \)) followed by a Tukey’s post hoc tests or Kruskal-Wallis tests (\( \alpha = 0.05 \)) followed by Dunn’s multiple comparison tests for the parametric and nonparametric data sets, respectively. All analyses were performed using SPSS v. 20.00 (IBM, New York, USA) and PRISM v 4.0 (Graphpad; CA, USA) statistical softwares.

**4.3 Results**

**Incidence of polyandry**

Seventy eight percent of WBC females from the laboratory colony had mated, similar to the 75% observed in field collected individuals. Eighty eight percent of mated individuals from the laboratory and 93% from the field had mated more than once, with an average in both cases around three times (Fig 4.1).

**Mating duration and female refractory period**

The duration of mating was affected by male (\( F_{(4, 141)} = 6.94, P < 0.001 \)), but not female (\( F_{(4, 141)} = 1.08, P = 0.34 \)) previous mating history (Fig. 4.2). Matings involving virgin males took about ~ 215 min, regardless of female mating status, while mating involving previously mated males took ~ 240 min to complete (production and transfer of a spermatophore).

While male previous mating history did not affect the length of female RP (\( H = 4.17, df = 2, P = 0.13 \)) female mating status did (\( H = 9.13, df = 2, P < 0.01 \)) as once mated females resumed calling significantly sooner than virgins.(Fig. 4.3) Similarly, while previous male mating history did not affect refractory onset time of calling.
(ROTC) \( F_{(2, 101)} = 0.10, P = 0.91 \), female mating status did \( H = 25.4, df = 2, P < 0.001 \), with previously mated females calling much earlier (by \(~100\) min) during the scotophase than virgins (Fig. 4.4).
Figure 4.1 The frequency of repeated mating in Western bean cutworm (*Straicosta albicosta*) females captured in light traps in Bothwell, ON during the peak flight (July 11-August 3, 2012) (field), and those collected from laboratory colony (lab).
**Figure 4.2** Mean (min ± SE) duration of Western bean cutworm (*S. albicosta*) mating where males and females of different mating status were paired with virgin conspecifics. Bars with the same letter are not significantly different based on Tukey’s post hoc test (P > 0.05). Upper and lower case letters refer to differences for the male and female mating status, respectively.
Figure 4.3 Mean (days ± SE) refractory period of Western bean cutworm (*Straicosta albicosta*) females following mating where males and females of different mating status were paired with virgin conspecifics. Bars with the same letter are not significantly different based on Dunn’s multiple comparison test (P > 0.05). Upper and lower case letters refer to differences for the male and female mating status, respectively.
Figure 4.4 Mean (min ± SE) time of female calling after the onset of scotophase, indicating refractory onset time of calling (ROTC) of Western bean cutworm (*Straicosta albicosta*) females following mating where males and females of different mating status were paired with virgin conspecifics. Bars with the same letter are not significantly different based on Tukey’s post hoc test (P > 0.05) and Dunn’s multiple comparison test (P > 0.05). Upper and lower case letters refer to differences for the male and female mating status, respectively.
4.4 Discussion

After mating, female moths enter a period of reduced sexual receptivity and lowered sex pheromone production, which can be permanent in monoandrous (e.g. *Lymantria dispar*) (Giebultowicz et al., 1991), and transient in polyandrous (e.g. *Helicoverpa zea*) (Raina et al., 1986) species. Since paternity is not guaranteed in polyandrous species, theory predicts that males would try to induce and maximize the refractory period of their mating partner (Eberhard, 1996). The inhibition of female sex pheromone production by the males may be related to humoral factors originating from male accessory gland secretions (Raina et al., 1994), testicular factors such as ecdysteroids (Ramaswamy et al., 1996), or signals transmitted via the ventral nerve cord (Jurenka et al., 1993; Ando, 1996). The latter may be influenced by spermatophore size that decreases with successive matings (Outram, 1971; Pivnick and McNeil, 1987; Royer and McNeil, 1993; Marcotte et al., 2005), and the transfer of larger spermatophores induces longer refractory period in females (Svard and Wiklund, 1991; Wedell and Cook, 1999). Furthermore, the refractory period is also associated with sperm dynamics and viability within the female reproductive tract (Giebultowicz et al., 1991). Thus, previously mated males may be less effective at inducing prolonged pheromonostasis, as reported in a number of lepidopterans, where females mated with previously mated males have a shorter refractory period than those mated with virgins (Torres-Vila et al., 1997; Wedell and Cook, 1999; Marcotte et al., 2005, Marcotte et al., 2006). Conversely, in the WBC, previous mating history of males did not have a significant effect on the length of the refractory period. No effect of male mating status on the female refractory period suggests that there are few changes in male ejaculate quality (e.g. eupyrene and apyrene sperm densities and the concentrations of accessory gland secretions) with successive matings, which may be associated with longer time taken by previously mated males to transfer the spermatophore. There is also a possibility that females are able to metabolise and/or neutralise male derived compounds that would extent the RP (Boggs, 1990; Kingan et al., 1993, Kingan et al., 1995; Eberhard, 1996; Vahed, 1998; Fan et al., 1999). To resolve this question additional studies are needed to determine the extent to which age and previous mating history affects the size and content of spermatophores transferred by WBC males.
The intensity of potential sperm competition may result in males adjusting the quality and/or quantity of sperm produced (Wedell and Cook, 1999; Hunter and Birkhead, 2002, Ingleby et al., 2010; Garbaczewska et al., 2013), to maximize their chance of fertilizing eggs. Given the high level of multiple mating in WBC, it is possible that there was strong selection on WBC males to maintain high ejaculate quality, an aspect than merits further attention. Considered from the potential for increased reproductive output, if previous female mating history was important mated females would be expected to have a longer RP than virgins. However once mated, WBC females had a shorter refractory periods than virgins, suggesting that if the females possess counter adaptations to neutralize male derived compounds that prolong pheromostasis (Arnqvist and Nilsson, 2000), these are more active in mated than virgin WBC females. In polyandrous species, sperm precedence (use of the sperm from the latest mate) is seldom complete, where the second male may provide nutrients that increase female reproductive output without any gain in paternity (Knowlton and Greenwell, 1984; Svard and McNeil, 1994). Therefore, females mated with high quality males may be re-mating just to obtain the male derived nutrients that increase female reproductive success (Boggs and Gilbert, 1979; Pivnick and McNeil, 1987; Markow et al., 1990; Chapman et al., 1994; Svard and McNeil, 1994; Pitnick et al., 1997; Rooney and Lewis, 1999).

The earlier MOTC by multiply mated WBC females indicates a shift in calling activity. As virgin females age, their MOTC occurs earlier and it has been postulated to be an adaptation for older individuals to avoid competition with more attractive younger females, with higher pheromone titers (Swier et al., 1977; Turgeon and McNeil, 1982; Webster and Cardé, 1982; Delisle, 1992; Delisle and Royer, 1994). This age related shift in the calling window has also been observed in the WBC (Chapter 2) but previously mated females initiate calling even earlier after the onset of the scotohase than older virgins, which could also be an adaptation to avoid competition with more attractive virgin females. The possible difference in relative attractiveness of virgin and mated females could be investigated by quantifying pheromone production in mated and virgin females.

Interestingly, a proportion of WBC females (both from the field and laboratory maintained colony) were unmated. In the field, females were captured over the entire
flight period, thus it is possible some individuals were captured before they reached sexual maturation as WBC females take 4-6 days after eclosion to mature (reported in Chapter 2). Captured females may have reached sexual maturity before they mated. Gowaty and Hubbell (2013) suggested that females might die as virgins, depending on variation in lifespan, mate encounter, and choosiness. Consequently, some WBC females may never mate, which would be most likely at the beginning and end of the flight period, when females might not encounter any males of good enough quality to accept (Rhainds, 2010).

In conclusion, the previous mating history of WBC females influenced the refractory period, and thus the likelihood that those individuals will re-mate. Opposing interests in terms of reproductive success of sexes were evident, with males attempting to prolong, and females to shorten the RP. As in all cases the refractory period was quite short, possibly reflecting the univoltine life history and migratory strategy of this insect.

4.5 References


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5.1 General discussion

Sexual reproduction allows for gene recombination and production of diverse offspring with variable responses to changing environmental conditions. Sexual reproduction is costly, and as females usually invest more resources in their offspring, they tend to be choosier than males when selecting a mate. The location, attraction, and selection of best available mate(s) require communication between potential partners, and may be based on different modalities, involving visual, auditory, tactile or chemical cues. The use of chemical signals (sex pheromones) is widespread among insects, including nocturnal Lepidoptera. Effective production and reception of sex pheromones in Lepidoptera are affected by both biotic and abiotic factors (Webster and Cardé, 1982a; Turgeon and McNeil, 1982; Delisle and McNeil, 1987) and each species is adapted to the prevailing conditions over its geographical range.

Over the past decade, the Western bean cutworm (WBC), *Striacosta ablicosta* (Smith) (Lepidoptera: Noctuidae) has expanded its range into Canada, and now threatens Ontario corn and bean crops. Since WBC was not considered to be a primary pest until it recently replaced other corn pests following a niche opening (due to changes in farming practices such as use of Bt crop varieties), there was little interest in studying its reproductive biology. Because of the recent threat to economically important Ontario crops, a laboratory colony (re-stocked annually with field collected material to maintain high diversity in the colony) of WBC was successfully established and used to investigate some aspects of WBC reproductive behaviour. The general objective of this thesis has been to improve our understanding of the reproductive biology of WBC females by determining pre and post mating behavioural aspects, in terms of sexual maturity, courtship (calling behaviour and sex pheromone synthesis), and sexual receptivity.

To this end, I first investigated the sexual maturation (the initiation of calling) of WBC females under different temperature conditions to determine if WBC has calling characteristics of migratory species. McNeil (1986) proposed that migratory species could be identified by the time taken to express calling behaviour following emergence,
based on the observations that even under conditions suitable for reproduction, migratory moths delayed sexual maturation for several days (Turgeon and McNeil, 1982), while non-migrant ones become sexually mature soon after emergence (Delisle, 1992). I confirmed that WBC has characteristics of a migratory species, and their long pre-calling period suggests the ability of this insect to travel over long distances before finding a suitable habitat for reproduction. Interestingly, there was no direct effect of temperature on the pre-calling period (PCP) as reported in other moth species (Turgeon and McNeil, 1983; Delisle and McNeil, 1987; Han and Gatehouse, 1991a; Del Socorro and Gregg, 1997). Instead, there was an effect of the differential temperature change between the photophase and the scotophase, a finding not previously reported in the pheromone literature. It should be noted that most studies on sexual maturation have been performed by comparing results from different constant temperature regimes, rather than cycling ones. The only study comparing constant and cycling temperatures looked at two moth species and found the PCP of one species (migratory; *Pyralis unipuncta*) was significantly shortened under cycling than constant conditions but was unaffected in the other species (non-migratory; *Mamestra configurata*) (El Ouartassi, 1991). Thus, one avenue for future research would be determining to what extent fluctuating abiotic conditions, which are the norm experienced in nature, affect maturation in both migrant and non-migrant species.

My second objective was to describe the pattern of calling behaviour under different biotic (age) and abiotic (temperature and RH) conditions. Contrary to my hypothesis, abiotic factors did not influence the patterns of calling behaviour, which may be related to the fact that WBC is a univoltine species, so adults experience considerably less variable environmental conditions than multivoltine species that must adapt to a much wider range of conditions. For example, in the same geographic region adults of the true armyworm, *P. unipuncta*, are in flight in May, July, and September (Breeland, 1958; M.A. Marinas, personal communication), compared with the WBC that has one flight period between late June and early August.

Although the abiotic conditions tested did not affect the calling behaviour of WBC, I observed that, similar to other Lepidoptera (Turgeon and McNeil, 1982; Xiang et al., 2010), as WBC females aged, there was an advance in the mean onset time of calling
(MOTC), and the mean time spent calling (MTSC). It was postulated that these changes increase the chances that older females, which have lower pheromone titers (Webster and Cardé, 1982b; Delisle and Royer, 1994), would attract potential mates (Swier et al., 1977; Webster and Cardé, 1982b). However, I found that older WBC females produced comparable amounts of sex pheromone at the onset of calling activity as younger individuals, which suggests that the attractiveness of WBC females is independent of age. As older females call earlier and spend more time calling, they may be at an advantage when it comes to attracting mates.

I also determined that the WBC is polyandrous (multiple mating by female with different males) with an average of three matings per female. In insects, polyandry often leads to direct benefits for the female, especially in species with nuptial gifts (e.g. spermatophores transferred by the males of Lepidoptera which contain nutrients in addition to sperm) (Arnqvist and Nilsson, 2000). The high incidence of polyandry suggests that with high lifetime egg production (Blickenstaff and Jolley, 1982), females need to mate multiply to realize their entire egg complement. Increased number of matings would result in high population densities with a high degree of the genetic variability, increasing the likelihood of survival under unfavorable conditions.

After mating, females of polyandrous species undergo a transient loss of sexual receptivity (Raina et al., 1986), and this period of pheromonostasis varies with male quality and the amount of viable sperm provided by previous mates (Taylor, 1967; Marcotte et al., 2005; Marcotte et al., 2006). Contrary to other species studied, multiply mated WBC females resumed calling activity sooner after mating, regardless of the male's mating status, suggesting than male spermatophore content does not vary with successive ejaculates, or that females rapidly metabolize or neutralize male derived substances transferred during mating (that might prolong pheromonostasis and thus reduce sperm competition) (Boogs and Gilbert, 1979; Rooney and Lewis, 1999; Wedell and Cook, 1999). In addition, multiply mated WBC females resume calling earlier in the scotophase than virgins and thereby potentially reducing competition with virgin females. Additional research is needed to determine the reasons why the mating status of males has no influence on pheromonostasis, by investigating spermatophore size and content (Outram, 1971; Pivnick and McNeil, 1987; Marshall and McNeil, 1989; Royer and
Like other Lepidoptera (Marcotte et al., 2005), the duration of the mating in WBC was under male control, with previously mated males needing more time for production and transfer of spermatophore (Dewsbury, 1982; Svard and Wiklund, 1986). Moreover, an examination of sperm movement in the female reproductive tract following mating could also provide insight into the duration of pheromonostasis (Marcotte et al., 2005; Marcotte et al., 2006).

I also found that the mean content of the WBC female pheromone gland does not change with age in virgin females, but inter-female variability was considerable, both within and between days. Again, this variability between individuals is different than most other moth species studied to date, and certainly merits further attention as it would appear that older females would outcompete younger conspecifics. Furthermore, given the very short refractory period of mated females and the fact that they initiate calling sooner than virgins, it would be interesting to measure their pheromone content, for if there was no reduction in pheromone, mated females would outcompete virgins. Describing the calling behaviour and pheromone production of different aged virgin and mated females, as well as comparing their relative attractiveness in traps, under field conditions would provide insight into the question of competition.

My results also suggest that additional research examining the actual pheromone blend used in pest management programmes for the WBC could be important. As noted previously, while the commercial pheromone lures are effective to monitor the presence or absence of this species, they have proved ineffective as a predictor of infestation levels. The current lure formulation is based on the work of Klun et al (1983), who reported a 5:1:5:5 ratio of the four component blend based on the analysis of four pooled pheromone glands. However, it is clear from my data that the pheromone concentration observed in the gland extracts was much lower than reported in many noctuids, and there is a substantial inter-female variability, both in the number of compounds found in the gland, and their relative ratios. Therefore, as the concentration and ratio of the pheromone blend may change throughout the calling period (Raina et al., 1986; Xiang et al., 2010), investigation of WBC sex pheromone titer throughout the calling window, together with field trials testing different lures could help determine if a different pheromone blend
would be more effective for predicting crop losses. The interpretation of trap catch data would have to take into consideration information from the female-female competition experiments mentioned above, as this would help understand the possible competitive interactions between feral females and pheromone traps.

While this thesis has increased our understanding of WBC female reproductive behaviour, there are several other aspects of WBC reproductive biology, in addition to those mentioned above arising directly from my results, which merit attention. For example, the PCP is a heritable trait (Han and Gatehouse, 1991b; Hill and Gatehouse 1992), and as there are now resident populations of WBC in the Great Lakes region, it will be interesting to see if, over time, there is a reduction in the PCP, as seen in the non-migrant populations of the true armyworm found in the Azores (McNeil et al., 1996). It would also be interesting to study juvenile hormone (JH) biosynthesis in this species, not only because it is involved in sexual maturation of migrant species (Cusson and McNeil, 1989; Zhou et al., 2000), but also to test my hypothesis that the effect of temperature differential between the photophase and the scotophase on the rate of sexual maturation is due to increased JH titers.

In summary, the findings presented in this thesis have characterized previously unknown aspects of the reproductive behaviour of WBC. This includes establishing that the WBC is polyandrous and has characteristics of a migrant species. Furthermore, the information on the patterns of calling behaviour and pheromone synthesis as a function both age and mating status will provide important baseline data for the pheromone trap component of the management program developed for the WBC.

5.2 References


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APPENDICES

Appendix A. Western bean cutworm (*Spadicosta albicosta*). A. Experimental setup for female calling behaviour observations. B. Adult WBC male (with claspers) and female (with ovipositor). C. Close-up of the inverted female ovipositor with the pheromone gland.
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