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The Effects of Extrinsic and Intrinsic Factors on the Reproductive Biology of Male Western Bean Cutworm Moths, Striacosta Albicosta (Smith)

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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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THE EFFECTS OF EXTRINSIC AND INTRINSIC FACTORS ON THE
REPRODUCTIVE BIOLOGY OF MALE WESTERN BEAN CUTWORM MOTHS,
STRICOSTA ALBICOSTA (SMITH)

(Thesis format: Integrated article)

by:

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

The School of Graduate and Postdoctoral Studies
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Abstract

Pheromones are important in the mating systems of nocturnal moths as they are relied on to find and/or assess mates. Extrinsic and intrinsic factors have been shown to influence female emission of and male response to pheromones. My thesis focuses on the reproductive biology of males of the western bean cutworm, *Striacosta albicosta*, a pest of beans and corn that has recently begun expanding its range eastwardly. I conducted a field-based experiment to determine the effects of extrinsic factors on pheromone trap catches. I also conducted laboratory based mating experiments to determine the effect of male age on acceptance by females and on female reproductive output. Temperature, but not humidity or wind speed, had a significant but minor effect on trap catches in both summers. The age of a male affected both his acceptance by females and female reproductive output. The two were not directly linked, although 4 day-old males were optimal mates. My results suggest that intrinsic but not extrinsic factors affect the reproductive biology of the WBC.

Keywords

*Striacosta albicosta*, Western bean cutworm, pheromone traps, trap catch, temperature
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Chapter 1

1 General Introduction

1.1 Introduction

The ideal outcome for sexually reproducing organisms is the maximization of reproductive success. However, the driving forces that determine reproductive success differ significantly between the sexes. Darwin (1871) pointed out that two types of selective forces might operate on reproductive behaviour: (1) intrasexual selection, involving competition within one sex for individuals of the opposite sex, and (2) intersexual selection, involving preferential choice by one sex for individuals of the opposite sex. In general, due to their greater parental investment, females are the choosier sex (Trivers 1972), resulting in males competing for females while females are discriminatory in their choice of males. Intrasexual competition and intersexual selection have in some cases led to exaggerated male secondary sexual characteristics such as large size, aggressiveness, fighting ornaments (like antlers and horns) and bright or elaborate plumage.

The attraction and selection of mates may involve an array of signals, where potential mates can assess the quality of the signaler by the quality of the
signal. Signals may be visual, acoustic, olfactory or tactile and might be performed individually or in combination to attract the opposite sex. The use of these signals is found universally in the animal kingdom, from invertebrates to mammals (Semler 1971, Whitney and Krebs 1975, Landolt and Heath 1990, Hill 1991, Drickamer et al. 2000, Kemp 2007, Uetz et al. 2009). The advantages and limitations vary with each type of signal. For example, acoustic and olfactory modes of communication are advantageous over long distances and in darkness, which is not always the case for visual and tactile cues. However, with these means of communication locating the signaler presents certain difficulties, as acoustic and olfactory signals do not direct a message to a particular receiver. The various means of communication may also be extremely important as reproductive isolation mechanisms to avoid mating with closely related sympatric species, as they are species specific and generally do not elicit the same response behaviours from heterospecific species.

Pheromones are olfactory signals used by many species to locate mates and/or assess mate quality, especially in nocturnal moths. Olfactory signals are the most common means of recruiting a sexual partner in nocturnal Lepidoptera, with females generally emitting a species-specific sex pheromone from the pheromone gland located near the ovipositor (Shorey 1973, Bjostad et al. 1987). However, there is a considerable variability in the location of the pheromone source in the Insecta (Tillman et al 1999). This sex pheromone elicits an immediate response of sexual behaviour in mature, conspecific males, who exhibit an upwind response in search of the pheromone source (Kennedy et al.
When a male approaches the female, in response to a high pheromone concentration and potentially other cues, he is stimulated to display his courtship behaviour, which may consist of chemical cues, with the extrusion of scent glands such as hair pencils (Birch et al. 1989), a series of contact moves (Girling and Cardé 2006) and/or acoustic signaling by wing-fanning (Spangler 1987).

Female emission of and male response to pheromones are affected by extrinsic (e.g. temperature, wind speed, relative humidity) and intrinsic (e.g. age) factors. Age of sexual maturity is the age at which females call (extrusion of ovipositor to expose the pheromone gland) for the first time and the age at which males become responsive to pheromones. The length of time between emergence and sexual maturation differs between species and their life history strategies. Generally, non-migratory moths initiate calling and response behaviour within 1-2 days of emergence [Mamestra configurata (Howlader and Gerber 1986)] while in migratory species there is a significantly longer prereproductive period [e.g. Pseudaletia unipuncta (McNeil 1986), Spodoptera exempta (Page 1988)].

Once males become mature, they exhibit increasing levels of response to the pheromone source over the first few days [e.g. Grapholita molesta (Baker and Cardé 1979), Mamestra brassicae (Tomescu et al. 1981) and P. unipuncta (Turgeon et al. 1983)] but decline later in life. Furthermore, older males may have reduced mating success compared to younger and intermediate-aged individuals [Choristoneura rosaceana, (Delisle and Bouchard 1995)], suggesting that females may prefer younger males since they are least likely to have previously
mated and hence will have a full supply of secretions and larger spermatophores (Rutowski 1982). This is advantageous as females who receive more supply of accessory gland secretions and larger spermatophores generally have higher fecundity and longevity than females who receive smaller nuptial gifts (Rutowski 1982, Torres-Vila and Jennions 2005). Also, female selection against older males may be due to a decrease in fertilization success with age due to a decline in sperm quality (Kokko 1998). In some insects, these age-related effects may be reflected in the change observed in the duration of mating which increases with male age (Delisle 1995), possibly due to difficulty in the transfer of the ejaculate.

Once initiated, the onset of calling by females often advances earlier in the scotophase (the dark phase of the light and dark cycle) and the time spent calling generally increases with age as seen with *M. configurata, Pseudoplusia includens, Copitarsia consueta, Palpita unionalis, Estigmene acrea, and Condylorrhiza vestigialis* (Howlader and Gerber 1986, Mason and Johnson 1989, Rojas and Cibrian-Trovar 1994, Mazomenos et al. 2002, Del Mazo-Cancino et al. 2004, Ambrogi et al. 2009). Furthermore, the content of the pheromone and effectiveness of pheromone produced may decrease with age (Delisle and Royer 1994). It has been suggested, as in the case of *Platynota stultana*, that older females advanced the onset time of calling possibly to increase the probability of attracting a mate through reduced competition with younger females (Webster and Cardé 1982a).

Male reproduction may be affected by many factors, such as food quality/quantity and extrinsic conditions during larval development (Delisle and
In addition it may vary as a result of previous mating history [eg. *C. rosaceana* (Marcotte et al. 2007) and *Ostrinia nubilalis* (Schlaepfer and McNeil 2000)] due to the costs associated with sperm and spermatophore production. This possible cost of mating on male quality further emphasizes the importance of the first mating event and the age of sexual maturation of males. While the size of virgin male spermatophore increases with age (Delisle and Bouchard 1995), spermatophore volume produced by the generally decreases with consecutive matings [eg. *P. unipuncta* (Marshall and McNeil 1989), *O. nubilalis* (Royer and McNeil 1993), *Utetheisa ornatrix* (LaMunyon and Eisner 1994), *C. rosaceana* (Delisle and Bouchard 1995), *Utetheisa ornatrix* (Smedley and Eisner 1996) and *Agrotis segetum* (Svensson et al. 1998)]. As a consequence both male and female reproductive output may be affected by the reduced spermatophore size, although in most examples studied, spermatophore size and reproductive output are only weakly correlated.

Female calling and male response behaviours can also be affected by temperature, wind speed and relative humidity (McNeil 1991 and refs within). Female calling and male responsiveness advances at low temperatures (Cardé et al. 1973, 1977, Schal and Cardé 1986, Linn et al. 1988), a shift considered to be an adaptation since flight is limited at lower temperatures (Taylor 1963). Females may reduce calling duration at low and high wind speeds and at low relative humidity (Kaae and Shorey 1972, Webster and Cardé 1982b) possibly due to desiccation, for while the large, membranous surface area of the gland is ideal for the release of the sex pheromone, it also allows for considerable water
loss. Low and high wind speeds will also reduce the number of males flying and responding to pheromones possibly because at low wind speeds the pheromone does not get disperse very far while at high wind speeds oriented flight becomes difficult and dangerous (Kaae and Shorey 1972, Sanders 1985). In addition, relative humidity has also been shown to affect male response to sex pheromones, possibly due to low humidity decreasing the efficiency of the pheromone receptors on the antennae (Royer and McNeil 1993).

The aim of this thesis is to investigate the reproductive behaviour of the Western bean cutworm (WBC), Striacosta albicosta, more specifically the responses and behaviour of males. Understanding both the emission of, and response to pheromones could help us better interpret trap catch data when using pheromones in Integrated Pest Management programs to estimate subsequent larval infestations. While pheromone traps effectively catch males of the WBC there is no correlation between male moth densities and subsequent infestations in crops (Mahrt et al. 1987). Thus having basic knowledge of the mating system of the WBC could help explain this anomaly.

The WBC was first described as a pest of edible beans, Phaseolus vulgaris, in 1915, and of corn, Zea mays, in 1954 (Hoerner 1948, Douglass et al. 1957). It is a univoltine noctuid that overwinters in the soil as a prepupa (Hoerner 1948, Douglass et al. 1957). The WBC belongs to a group of climbing cutworms that feed on foliage and fruit, unlike other species of cutworms that cut down young plants and then feed (Douglass et al. 1957). Adults emerge in early July (Hagen 1962) and oviposit preferentially on corn or beans. The young larvae
initially feed on the leaves, pollen and anthers while later instars feed on pods or kernels (Hoerner 1948, Hagen, 1962).

Before the 1950s, the WBC was only an occasional pest of edible beans and largely confined to the Great Plains region (Hoerner, 1948) but since the 1950s it has become a more destructive pest of beans and corns. The species distribution remained stable for the remainder of the 20th century. However, over the past decade, the WBC has begun a rapid eastward expansion, making its way through most of the Midwestern United States and into southern Ontario and Quebec (O’Rourke and Hutchinson 2000, Dorhout and Rice 2004, Catangui and Berg 2006, Pope 2007, Rice and Pilcher 2007, Cullen and Jyoutika 2008, DiFonzo and Hammond 2008, Baute 2009). Three hypotheses that are not mutually exclusive have been proposed to explain the recent range expansion: (i) Niche opening through planting of maize containing the Cry1Ab protein, which affects other species of Lepidoptera significantly more than the WBC (ii) farm practices such as conservation tillage and reduction in pesticide use and (iii) warmer winters due to climate change. However the relative importance of any of these possible causes remains to be determined, and it is probably a combination of these factors. Regardless of the cause of the range expansion, the WBC clearly has the potential to become a major agricultural pest in Ontario. A 14-year study on the density of the WBC showed a great deal of variability from one year to the next, although peaks were observed every 6 to 8 years (Hagen 1976).

The female sex pheromone of the WBC has been identified (Klun et al.
and is now used for monitoring programs (Cullen and Jyoutika 2008, Baute 2009). Dorhout and Rice (2008) have shown that the type of trap used did not affect trap catch, but the height of the trap did, with traps placed at 1.2 and 1.8 m catching more moths than traps placed at 0.6 m. Furthermore, Mahrt et al. (1987) showed that weekly catches from pheromone traps correlated well with those of light traps. However average daily catch per trap showed a high amount of variability and moth counts were not a good indicator of subsequent larval damage. Consequently a study on the basic reproductive biology of this pest has been funded by Grain Growers of Ontario, of which this thesis is an integral part.

I conducted a field-based experiment to determine the potential effects of temperature, wind speed and humidity on variation in trap catch during the flight period. My hypothesis was that the time of peak trap catch and the number of moths caught should change with decreasing temperatures, expecting there to be fewer males caught and the peak catch occurring sooner after dusk on cooler nights compared to warmer ones. The number of males found in traps should change with increasing wind speed, where at undetectable and at high wind speeds the number of males caught will be low. Finally, a change should also be seen with increasing humidity, with fewer males being caught in pheromone traps at high humidity as the high concentrations of pheromone in commercial lures result in sensory overload of the antennal receptors and avoidance behaviour by males.

I also determined the effect of male age on their acceptance by females. Since the recent range expansion of the WBC, individuals are captured in
pheromone traps prior to local emergence, which suggests that some individuals are immigrants while others are residents. However, as local populations have been present for only a few years, I hypothesized that sexual maturation would occur several days after emergence, as the WBC is a migrant species. I then determined the effect of male age on female reproductive output. I hypothesized that male age would affect female reproductive success, with very young and old males providing inferior ejaculates. Together, these data will help determine the influence of extrinsic and intrinsic factors on the reproductive biology of the WBC.
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Chapter 2

2 The effects of temperature, wind speed and relative humidity on the number of male western bean cutworms, *Striacosta albicosta*, caught in pheromone traps in the field.

2.1 Abstract

A field based study to determine the effects of temperature, humidity and wind speed on the number of the western bean cutworm males caught in pheromone traps was conducted in 2011 and 2012. The peak trap catch/hour was earlier on cool (<15°C) than warm (>17°C) nights, as reported in other species. There was a marked difference in population densities between the two years so the two data sets were analyzed separately. The hourly trap catches were significantly affected by temperature in 2011 and relative humidity in 2012. However, these factors explained <10% of the overall variability.
2.2 Introduction

Foraging is the means by which insects seek essential resources such as food, refugia, oviposition sites and mates. Thus, efficient searching mechanisms will increase an individual’s chances of survival and reproduction. Environmental factors determine both resource availability and the risks associated with acquiring these resources. For example, in the bumble bee, *Bombus terricola*, the energetic costs of foraging increase as temperature drops, as the bee must maintain a high thoracic temperature in order to continue flying, (Heinrich 1979), so because of the interspecific variability in nectar production, flowers yielding low energy rewards are only exploited at relatively high air temperatures (Bell 1990). The location of potential mates by nocturnal moths is often mediated by specific pheromones, but prevailing extrinsic conditions can markedly affect both the emission of, and response to, these chemical cues.

Females, who are usually the emitters of the species-specific pheromones, shift their calling window and become active earlier under cooler temperatures as seen with *Holomelina lamae* (Schal and Cardé 1986), and in some cases, such as *Grapholita molesta*, may also increase the time spent calling (Baker and Cardé, 1979). The time spent calling may also vary with wind speed, being less when winds are low or high (0 and >4 m/s). Similarly, calling is reduced when relative humidity is low (<40%) (*Trichoplusia ni* and *Platynota stultana*) probably associated with desiccation (Kaae and Shorey 1972, Webster and Cardé 1982), as during calling, water is lost from the large, membranous surface of the pheromone gland.
The male activity window, although broader than that of females, also occurs earlier on cooler than warmer nights in a diversity of moth species (Cardé et al. 1973, 1977, Landolt and Curtis 1982, Linn et al. 1988). This may just reflect a response to the shift in female calling window or an adaptation to locate a mate before the temperatures drops below the threshold that actually inhibits flight. The majority of studies showing an advance under cooler conditions use synthetic pheromone lures, which would not be exhibiting periodicity in release and thus supports the second hypothesis.

When flying to a pheromone source, males maintain a constant upwind ground speed by adjusting their flight speed to compensate for changes in wind velocity (Sanders 1985). However, in the absence of wind, there is no dispersion of the pheromone plume, so males have a difficult time locating the source, while on windy nights (>4 m/s) males are unable to sustain oriented flight (Kaae and Shorey 1972). Thus, wind velocity is important in the ability of males to locate potential mates. In addition, relative humidity has also been shown to affect male upwind flight response to sex pheromones (Royer and McNeil 1993). The authors proposed that the pheromone receptors function maximally under high humidity and that efficiency declines due to receptor desiccation under conditions of low humidity. In contrast, at high humidity, the system becomes very sensitive and if pheromone concentrations are too high (most commercial lures) compared with levels emitted by calling females, males will leave the plume (Royer and McNeil 1993).

The western bean cutworm, Striacosta albicosta, (WBC) has recently
expanded its geographic range and is now a potential agriculture pest in the Great Lakes region. While the female sex pheromone has been identified and is being used in monitoring the change in the geographic distribution of this univoltine (one generation per year) species, the correlation between trap catch and subsequent larval damage is low (Mahrt et al 1987). An understanding of how extrinsic factors influence and modify trap catch will provide basic information that could improve our ability to use pheromone trap catch data in Integrated Pest Management programs. Therefore a field-based study was undertaken in 2011 and 2012 to determine the impact of temperature, relative humidity and wind speed on hourly trap catch of the WBC males over the flight period.

2.3 Methods

Twelve green Universal Moth Traps (Great Lake IPM, Michigan USA) and two light traps were operated during the flight period of the WBC in Bothwell, Ontario, in 2011 and 2012. The trials started once WBC adults were consistently captured in light traps operated from early May. The pheromone traps (on 2.1 m tall wooden posts) were evenly spaced over 300 m (with 20 m between traps) on the outer edge of a cornfield. A commercial lure (rubber septum) loaded with female WBC pheromone blend composed of (Z)-5-dodecenyl acetate, (Z)-7-dodecenyl acetate, 11-dodecenyl acetate, and dodecenyl acetate in a 5:1:5:5 ratio (Great Lake IPM, Michigan USA) was used. The actual concentration was unknown, as the company did not divulge this information.
Traps were emptied every hour from 9 pm to 6 am EDT on 10 (2011) and 13 (2012) randomly selected nights throughout the 3-4 week flight period. The moths were counted, placed in individual Eppendorf tubes and frozen in dry ice. Temperature, wind speed and relative humidity were also recorded each hour from a portable weather station (Deluxe Weather Station – La Crosse Tech, Wisconsin USA), placed at the same height as the pheromone traps.

Data were analyzed using an ANOVA to test if extrinsic factors and trap catch data differed between years. A multivariate regression analysis was conducted to determine the relationships between extrinsic factors and trap catch data for each year. To determine if the hour of peak trap catch shifted with temperature, I plotted average nightly trap catch as a function on the mean nighttime temperature (Fig. 2-1). Base on these data I compared the pattern of trap catch data on cool (<15°C) and warm (>17°C) nights using an ANOVA.

2.4 Results

There was a significant difference in both mean nightly temperature ($P < 0.001$, $F = 20.6$, $df = 1$) and number of males caught in pheromone traps ($P < 0.001$, $F = 24.0$, $df = 1$) in the two years, with 2011 having higher temperatures and trap catch numbers than 2012 (Fig. 2-2). Given the significant difference in moth density and temperature, the data from each year were analyzed independently.

Multiple regression analysis found that in 2011 there was a significant, positive relationship between trap catch and temperature ($P < 0.007$, $R^2 = 0.10$, $F = 20.6$, $df = 1$).
df = 1; Fig. 2-3), however humidity ($P= 0.301, F= 1.08, df= 1$) and wind speed ($P= 0.932, F= 0.01, df= 1$) had no effect. In contrast, in 2012, there saw a significant negative relationship between trap catch and relative humidity ($P < 0.05, R^2= 0.05, df= 1$; Fig. 2-4), but trap catch was no affected by temperature ($P= 0.836, F=0.04, df= 1$) or wind speed ($P= 0.246, F=2.03, df= 1$). However, in both years the observed significant relationship between trap catch and extrinsic factors explained < 10% of the observed variability.

Peak hour of trap catch was around midnight on cool nights (9.5-14.5°C) and 03:00 on warm (17.5-26°C) nights (Fig. 2-5).

2.5 Discussion

Significantly fewer males were caught in 2012 than 2011, in agreement with the marked inter-year variability reported by Hagen (1976) in a 14-year study. There are a number of possible explanations for this difference, which are not mutually exclusive. The immigrant moth population may have been smaller in 2012 and/or local populations may have been reduced due to the widespread use of insecticides on corn at Bothwell in 2011. The average temperatures during the flight period were cooler in 2012 (Fig. 1) but were well within the range for normal flight for noctuids; therefore, it is unlikely that flight activity was limited by ambient temperatures. This is supported by the fact that the relationship between trap catch data and temperature was only seen in 2011 and only explained less than 10% of the variation observed.

There was very little effect of temperature and relative humidity on trap
catch in either year. Also, there was no noticeable effect of wind speed. These findings are contrary to most reports in the literature, which show a significant effect of these extrinsic factors on male responses. This is probably due to the fact that the WBC only has one flight period, lasting roughly 3-4 weeks from the middle of July to early August, and during this rather narrow time period climatic conditions are usually favourable, with quite small variability. It is possible that there is little selection on middle of summer univoltine species, such as the WBC, to evolve traits to cope with marked differences to extrinsic factors, as the year-to-year climatic conditions are relatively stable. This is in contrast to univoltine species that fly in spring or fall, and to those that have multiple generations in a year.

For example, studies on the true armyworm, *Pseudaletia unipuncta*, a migratory species with several generations a year, have demonstrated changes in mating behaviour with changes in extrinsic conditions (Turgeon et al. 1983). In Ontario adults immigrate into the region in spring (April-early June) to breed, the second generation (late June, July) is a resident one and breeds locally, while the adults of the third generation emigrate south in the fall (August-October). Consequently, the three generations experience significantly different local environmental conditions, and will also encounter markedly different ones following migration. Thus, to survive and reproduce this species has evolved plasticity in its reproductive behaviour to overcome variation in the extrinsic conditions experienced over its multiple generations in both time and space.

Generally, there is a positive relationship between temperature and male
response to pheromone within a given night (Turgeon et al. 1983). However, the upwind flight response of males may be affected by pheromone concentration, as reported for Grapholita molesta and Pectinophora gossypiella (Linn et al. 1988). At 20°C with lower pheromone dosage, responses were reduced compared to responses at 26°C. However, at a high pheromone concentration, male response was similar (nearly 100%) at both 20 and 26°C, while at a lower dose males were significantly less responsive at 20°C. This is probably due to the increase in the pheromone release rate at higher temperatures. In an effort to extend field life, commercial lures usually contain much higher concentrations of pheromone than feral females, so it is possible that concentration of the lures used in this study (unknown as the company would not divulge this information) may have largely negated any possible overall temperature effects.

Royer and McNeil (1993) showed that male European corn borer, Ostrinia nubilalis, responses to synthetic pheromone lures decreased with increasing relative humidity, with the highest response reported when relative humidity was around 40-60%, but the responses decreased as humidity levels rose. It was postulated that the sensitivity of the antennal sensillae that detect the pheromone increases with increasing humidity and that the concentrations released from the lures resulted in sensory overload and the males left the plume. However, the WBC males did not experience a large variation during my field trails, as the average humidity was 90% with little variation.

Similarly, studies on the effect of wind speed on male oriented flight (Kaae and Shorey 1972, McClure and McNeil 2009, Rousse et al. 2009) demonstrated
an effect when wind speed was either very low (0m/s) or very high (3-4m/s). Low and high wind speeds were rarely experienced in the field during the flight period of the WBC and so it is not really surprising that no effect of wind speed on trap catch was detected.

Oriented flight in response to pheromone cues is a major component of mate location in Lepidopteran males and there is evidence that males of many Lepidoptera modify mate location behaviours in response to changes in extrinsic factors, this is not the case for the WBC, other than the observed shift in diel periodicity of flight. Clearly this could just be that in the two years of the study there were few extreme changes in the extrinsic conditions measured. Alternatively, the WBC may not have evolved the plasticity in reproductive behavior observed in other species that are subject to wider ranges of climatic conditions. It would be of interest to study other insects that only have one flight period in the middle of summer to determine if they also show limited responses to changes in extrinsic factors.
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Physiol. Entomol. 11, p. 75-87.


Fig. 2-1. The average number of males caught in pheromone traps in Bothwell Ontario each night as a function of mean nighttime temperature in 2011 and 2012.
Fig. 2-2. Average (±SE) nightly trap catch of males in pheromone traps (columns) and mean nightly temperature (squares) (°C±SE) during the Western bean cutworm flight period at Bothwell Ontario in 2011 (N=10) and 2012 (N=13). Columns with the same letter are not significantly different (α<0.05). Boxes with the same letter are not significantly different (α<0.05).
Fig. 2-3. Relationship between temperature (°C) and number of male Western bean cutworm moths caught in pheromone traps in Bothwell, Ontario in 2011 ($P < 0.007$, $R^2 = 0.09$). $N=72$. 

$y = 1.82x - 19.88$

$R^2 = 0.09913$
Fig. 2-4. Relationship between relative humidity (%) and number of male Western bean cutworm moths caught in pheromone traps in Bothwell, Ontario in 2012 ($P < 0.05$, $R^2 = 0.04$). $N=90$. 

$y = -0.2659x + 29.249$

$R^2 = 0.04836$
**Fig. 2-5.** Proportion of male Western bean cutworms caught in pheromone traps in Bothwell Ontario at different hours during the scotoperiod under cool (<15°C) and warmer (>17°C) nights. Arrows point to the 50% mark for trap catches for each temperature range.
Chapter 3

3 Observations on the effects of male age on the incidence of mating, mating time and female reproductive success in the Western bean cutworm, *Striacosta albicosta*.

3.1 Abstract

Virgin males, 2, 4, 6, 8 or 10-days old, were paired with sexually mature females to determine the effect of male *Striacosta albicosta* age on female acceptance, duration of copulation and female reproductive output. Female acceptance did not differ with 2, 4 and 6 day old males but declined at older ages. Copulation time and the size of the spermatophore transferred to the female increased with male age. However, very different patterns were observed for female lifetime fecundity (eggs laid) and fertility (% hatching) as females mated with 2-day old males had a significantly lower reproductive output than those mated with 4 and 6-day old individuals.
3.2 Introduction

In most insects the female is the choosy sex and the best male reproductive strategy is to mate as often as possible (Thornhill 1976). However, there is an associated cost, as the quantity and quality of both sperm and accessory gland secretions decline with repeated mating. This is reflected in a significantly reduced reproductive output of once mated females when paired with a previously mated male relative to a virgin male (Delisle and Bouchard 1995, Karlsson 1998, Svensson et al. 1998, Savalli and Fox 1999, Hughes et al. 2000, Marcotte et al. 2005, Torres-Vila and Jennions 2005, Marcotte et al. 2007). Furthermore, in a few cases female longevity may be inversely related to the number of previous matings her partner has had (Hughes et al. 2000). Thus, there should be strong pressure on females to detect male quality and choose those of highest quality (Delisle and Bouchard 1995, Kokko and Lindstrom 1996, Kokko 1997).

The age at which males reach sexual maturity may be affected by the life history of any given species. Generally, non-migratory insects become sexually active shortly after emerging as adult forms, while migratory insects delay sexual maturation as they initiate migration to more suitable habitats as sexually immature individuals (Rankin and Burchsted 1992, McNeil et al. 1996, Zhao 2009).

During copulation, male moths transfer a spermatophore, which not only contains sperm, but also accessory gland secretions and other compounds that serve as a form of a nuptial gift (Thornhill 1976). The timing of sexual maturation
has been linked to the developmental time of both the testes and the accessory gland (Baker et al. 2003), thereby ensuring that males transfer a high quality ejaculate when mating. Although the male’s reproductive strategy is to remate often, one of the associated costs is that spermatophore size decreases with increased remating events (Delisle and Bouchard 1995, Svensson et al 1998, Hughes et al 2000, Torres-Vila and Jennions 2005, Marcotte et al. 2007) and may result in a lower female reproductive output (Royer and McNeil 1993). However, in polyandrous species, females mated with previously mated males often have a shorter refractory period (Oberhauser 1989, 1997), allowing them to remate more quickly, thus increasing the probability of sperm competition, which may have a significant effect on male reproductive success (Svärd and McNeil 1994, Cook and Gage 1995).

Data from the laboratory and the field show that the Western bean cutworm, *Striacosta albicosta* (WBC) females are polyandrous (personal observations), and this could result in strong intrasexual competition. However, little is known about male reproduction in this species. Therefore, I undertook a study to test the hypothesis that male age would not only affect his acceptance by a receptive female, but that it would also affect female age specific and lifetime fecundity (eggs laid) and fertility (percent hatching).

### 3.3 Methods

A colony of the WBC, established using field collected egg masses from London and Bothwell Ontario, Canada in the fall of 2011 and restocked in 2012,
was maintained under a 16L (20°C):8 (15°C) D photoperiodic cycle and 70±5 % R.H.. Larvae were reared individually on an artificial pinto bean diet (Shorey and Hale 1965), and upon pupation individuals were sexed based on morphological characteristics of the last abdominal segments. Newly emerged adults were held in individual containers with an 8% sucrose solution. All mating experiments were conducted at the same rearing conditions, with pairs being held in 20X30 cm cages with 8% sugar water.

**Mating experiment**

To determine the effect of male age on female acceptance, 20 pairs were established using either 2, 4, 6, 8 or 10 days old virgin males and a sexually mature female (initiated calling behaviour for the first time during the previous scotophase). The weight of both male and female was recorded prior to the mating assay. The couples were monitored every 15 minutes throughout the scotophase to document if and when mating occurred. At the end of the scotophase, couples were separated, males were weighed and the mated females were dissected to obtain the spermatophore. The duration of mating and spermatophore mass for each male as a function of age was determined.

The effects of male age on female reproductive output were determined using the same protocol, described above. However, in this case, following mating, females were placed in an individual cage with an oviposition site (fanned wax paper) and kept under standard laboratory conditions. Eggs were collected, counted and then observed daily to record the number of eggs that
hatched. Females that did not lay fertile eggs but had been observed copulating were dissected after death to determine if a spermatophore had been transferred. A spermatophore was present in all females that were judged to have copulated but never laid any fertile eggs.

Female acceptance of males at each age was analyzed using chi-squared tests. Copulation duration, spermatophore mass, female lifetime and daily fecundity (eggs laid) and fertility (percent hatched), and time to egg laying were analyzed using ANOVA with male and female weight as co-variables followed by Tukey’s HSD test.

### 3.4 Results

Male acceptance by females was significantly affected by male age, as fewer 8 and 10 day-old individuals were successful when compared to younger individuals (Fig. 3-1). The duration of mating was also affected by male age, with the 8 day-old males taking significantly longer (Fig. 3-2) and producing significantly larger spermatophores, which represented a significantly higher proportion of their body mass (Fig. 3-3), than younger males.

**Fecundity experiment:**

The effect of male age on the mean lifetime fecundity of females was marginally significant ($P=0.054$, $F=2.81$, $df=3$, Fig. 3-4) and highly significant for fertility ($P<0.001$, $F=16.2$, $df=3$, Fig. 3-4). Females mated with either 2 or 8 day-old males were significantly less successful than those mated with 4 or 6 day-old
individuals. Furthermore, females mated with young or old males took significantly longer after mating to start ovipositing \((P<0.001, F=19.0, df=3, \text{Fig. 3-5})\). In the case of age-specific fecundity the effect of male age was only observed on the first day \((\text{Day 1: } P < 0.029, F = 3.2, df = 3), (\text{Day 2: } P = 0.145, F = 1.9, df = 3), (\text{Day 3: } P = 0.171, F = 1.8, df = 3), (\text{Day 4: } P = 0.079, F = 2.6, df = 3), (\text{Day 5: } P = 0.326, F = 1.2, df = 3), (\text{Day 6: } P = 0.697, F = 0.4, df = 2), (\text{Day 7: } P = 0.823, F = 0.201, df = 2), (\text{Day >8: } P = 0.693, F = 0.2, df = 1; \text{Fig. 3-6})\), while the impact on fertility was seen throughout the oviposition period \((\text{Day 1: } P < 0.001, F = 8.9, df = 3), (\text{Day 2: } P < 0.001, F = 14.5, df = 3), (\text{Day 3: } P < 0.001, F = 7.8, df = 3), (\text{Day 4: } P < 0.016, F = 4.3, df = 3), (\text{Day 5: } P = 0.014, F = 4.8, df = 3), (\text{Day 6: } P < 0.001, F = 30.9, df = 2), (\text{Day 7: } P < 0.001, F = 36.6, df = 2), (\text{Day >8: } P < 0.006, F = 47.4, df = 1; \text{Fig. 3-7})\).

### 3.5 Discussion

Males of the WBC take several days to reach sexual maturity. This suggests that this insect is a migrant species, which fits with the current pattern of geographic expansion. Data from traps across the province suggest that the populations observed are made up of immigrants, who arrive before the emergence of the local populations.

The low fertility of eggs from females mated with 2 day-old males suggests that these males are still not sexually mature. Their spermatophores are smaller and thus may not contain a full complement of sperm/accessory gland secretions, even though these young males have a high level of acceptance by
females. This may well be an artifact of the laboratory assay, as females were confined to a cage with the male. Female of many moth species assess male quality by the short distance pheromone released during courtship (Fitzpatrick and McNeil 1988, Birch et al 1990). In nature a female will fly away from an unacceptable male but in a small cage the female may not have been able to escape and thus may accept persistent males to reduce the possibility of injury (Ringo 1996). This idea is supported by the delay in oviposition after mating seen with females mated to younger males, which was significantly longer than those mated with 4 day-old males. The latter group of females began ovipositing the night after mating and had both higher fecundity and fertility.

The prolonged copulation time and the production of larger spermatophores by older males may be due to more accessory fluid being transferred in the ejaculate, which could be the result of diel periodicity of sperm release from the testes to the duplex, and/or the inability of older males to effectively transfer sperm rapidly (Giebultowicz et al 1988; Jones and Elgar 2004, Liu et al. 2011). The first two possibilities would suggest higher quality ejaculates, although there may not be a direct relationship between spermatophore size and female fecundity (Royer and McNeil 1993, Wedell 1993, Wedell and cook 1999, Jones and Elgar 2004, Liu et al. 2011). When considering female reproductive output, the idea of a superior ejaculate is not supported, but rather that older males produce low quantities and/or quality ejaculates.

Given the results obtained I believe that under natural conditions WBC females would discriminate between different aged males, preferring and
selecting 4-6 day-old individuals. However, as females of this species are polyandrous, one has to ask to what extent does this repeated mating overcome "mistakes", especially if females become receptive more quickly when mated with poor quality males as reported in other species (Lum and Brady 1973, Hagan and Eugene 1981, Delisle and Hardy 1997, Marcotte et al. 2007).
References


Giebultowicz, J. M., Bell, R. A. and Imberski, R. B. (1988). Circadian rhythm of sperm movement in the male reproductive tract of the gypsy moth,


Fig. 3-1. The effect of male age on female acceptance in 2, 4, 6, 8 or 10 day-old Western bean cutworm, Striacosta albicosta, males. Bars with same letter were not significantly different ($N = 20$, $\chi^2 = 20.44$, $df = 4$, $P < 0.0004$).
Fig. 3-2. Copulation duration (min ±SD) as a function of male Western bean cutworm age (2, 4, 6 and 8 days after emergence). Sample size is indicated in the bars. Bars with the same letter were not significantly different ($P < 0.001$, $F = 15.7$, $df = 3$).
Fig. 3-3. Spermatophore mass (white bars; mg ±SE) and spermatophore mass as a proportion of male mass (hatched lines; % ±SE) as a function of male Western bean cutworm age (2, 4, 6 and 8 days after emergence). Sample sizes are indicated above the bars. Bars with the same letter were not significantly different ($P < 0.001$, $F = 24.7$, $df = 3$).
Fig. 3-4. Mean lifetime fecundity (white bars; ±SE) and fertility (hatched bars; % ±SE) by females mated to male Western bean cutworm at different ages (2, 4, 6 and 8 after emergence). Sample size is 20 females per age. White bars with no letters are not significantly different ($P = 0.054$, $F = 2.81$, $df = 3$). Percent hatched bars with the same letter were not significantly different ($P < 0.001$, $F = 16.2$, $df = 3$).
Fig. 3-5. The effect of male age on the time (day ±SE) following mating for the Western bean cutworm females to oviposit for the first time. Sample size is 20 females per male age. Bars with the same letter were not significantly different ($P < 0.001$, $F = 19.0$, $df = 3$).
Fig. 3-6. The effect of male age on the age specific fecundity of Western bean cutworm females. Same letters within day 1 were not significantly different (Day 1: $P < 0.029, F = 3.2, df = 3$), (Day 2: $P = 0.145, F = 1.9, df = 3$), (Day 3: $P = 0.171, F = 1.8, df = 3$), (Day 4: $P = 0.079, F = 2.6, df = 3$), (Day 5: $P = 0.326, F = 1.2, df = 3$), (Day 6: $P = 0.697, F = 0.4, df = 2$), (Day 7: $P = 0.823, F = 0.201, df = 2$), (Day >8: $P = 0.693, F = 0.2, df = 1$).
**Fig. 3-7.** The effect of male age on the daily fertility (%) of Western bean cutworm females. Same letters within each day were not significantly different (Day 1: $P < 0.001$, $F = 8.9$, $df = 3$), (Day 2: $P < 0.001$, $F = 14.5$, $df = 3$), (Day 3: $P < 0.001$, $F = 7.8$, $df = 3$), (Day 4: $P < 0.016$, $F = 4.3$, $df = 3$), (Day 5: $P = 0.014$, $F = 4.8$, $df = 3$), (Day 6: $P < 0.001$, $F = 30.9$, $df = 2$), (Day 7: $P < 0.001$, $F = 36.6$, $df = 2$), (Day >8: $P < 0.006$, $F = 47.4$, $df = 1$).
Chapter 4

4 General Conclusion

4.1 Conclusion

The results of my study show that in the field, during peak flight, changes in extrinsic factors generally did not significantly affect the numbers of WBC males captured in pheromone traps baited with commercial lures. This may be due to the narrow flight period for the WBC, which usually occurs in peak summer, when extrinsic conditions generally do not vary considerably. These findings are contrary to the results of many studies looking at trap catch density, but in other studies the moths experienced much larger variations in temperature, humidity and wind speed than observed in my study. Furthermore, many of the other species studies are multivoltine, having flights at very different times within the same year. Potentially these species would have evolved greater flexibility in their reproductive biology to cope with wide variations in extrinsic factors than a univoltine species flying in mid summer.

I also showed that male sexual maturity in the WBC took several days following emergence, suggesting that this is a migratory species, which is consistent with the recently observed range expansion. I observed that WBC females had the highest reproductive output when mating with 4-6 day-old males,
and thus, under field conditions, should avoid mating with very young or old males. However, under small cage conditions females accepted younger males but given the very poor reproductive output I suggest this is probably a laboratory artifact. Future research should examine both the content of spermatophores from different aged males, as well as the sperm dynamics post mating. In addition, it would be informative to examine to what extent mate choice actually occurs in the WBC, as well to test how multiple mating in this polyandrous species affects female fertility and fecundity.

My results suggest that intrinsic factors affect the reproductive biology of the WBC significantly more than extrinsic factors. Based on the date looking at reproductive output as a function of male age it is clear that young and old males could be considered poor quality mates. If there is strong female choice in the WBC then it is possible that the males caught in pheromone traps are predominantly those rejected by females. If true, then this could explain the weak link between trap catches and subsequent larval damage, as these males may not represent the breeding population.

In 2011 a number of the pheromone-baited traps contained females as well as males. Obviously if this is a common occurrence but people using the traps assume all individuals are males, they would be overestimating the population. However, it is also possible that males produce a long distance sex pheromone, as reported for the cabbage looper, *T. ni.* (Grant 1970). If true, then the number of individuals captures would be the result of those males responding to the pheromone lure, and females responding to pheromones emitted by the
captured males. In turn the females captured might call and potentially increase the attractiveness for males. If the attractiveness of the trap increased over the night as result of both male and pheromone emission then it would be very difficult of find a direct relationship with trap capture and subsequent larval densities. Interestingly, in 2012, when the WBC densities were low, no females were found in the pheromone traps. Clearly, additional research is needed to determine if there is a long distance male pheromone, and if there so when it is emitted as a function of male age and prevailing climatic conditions.
Reference

Curriculum Vitae

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