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Migratory movements of the true armyworm (Mythimna unipuncta) (Haworth): An investigation using naturally occurring stable hydrogen isotopes

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Abstract

A number of lepidopteran agricultural pests cannot overwinter in Canada and the annual populations each summer are the result of immigration. In most years densities are low but sporadically there are major outbreaks resulting in serious economic losses. Currently, the detection of potential epidemics is based on pheromone trap catch data in the spring, which leaves a very short window for farmers to intervene if there are high numbers of immigrants. It is accepted that immigrant moth species originate from the USA but we are unsure of whether they originate from one geographic source area every year or if it varies considerably between years. Recent advancements using stable isotopes as endogenous markers offers a new approach to more effectively address certain questions about insect migration. I used the true armyworm, *Mythimna unipuncta* (Haworth), a spring immigrant into Canada to determine the validity of using stable hydrogen isotope measurements from wing chitin (δ^2Hw) to infer the natal origin of adults captured in London. I first examined the isotope profiles of moths captured from April to October in 2016, and the results provide strong evidence that the moths captured in spring are immigrants, while adults captured in summer and fall are generally locally derived populations. Furthermore, data from moths captured in Texas early in the fall of 2012 were of northern origin, confirming the hypothesis of a return fall migration. I then compared the δ^2Hw values of individuals captured in London throughout four years, 2008, 2013, 2015, and 2016. There was considerable intra- and inter-year variability, and while stable isotopes clearly provide insight into patterns of insect migration, significantly more data are required to refine the model and to allow greater precision when assigning the geographic origin of individuals. Furthermore, including information on other stable isotopes, such as carbon or nitrogen, could be used to determine whether immigrants come from natural or agroecosytems.

Keywords

Migration, stable isotope, deuterium ($\delta^{2}\mathrm{H}$), true armyworm, *Mythimna unipuncta*, seasonal variation

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Co-authorship statement:

Intellectual input:

I participated in developing the experimental designs relating to the interpretation. Jeremy McNeil and Keith Hobson also took part in helping to design experiments.

Experiments and data analysis:

I reared all of the insects used in laboratory experiments, monitored all the field traps daily, conducted the calibration experiments, and prepared all material for isotopic analysis. I also prepared all data sets and ran the first analyses.

Keith Hobson, Jeremy McNeil and Kevin Kardynal assisted in data analysis. Kevin Kardynal helped to produce visual representations of assignments and isoscapes.

Writing:

All chapters were written with the help of Jeremy McNeil and Keith Hobson. Chapter 2 was co-authored with Keith Hobson, Kevin Kardynal and Jeremy McNeil.

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Chapter 1

1 General Introduction

1.1 Introduction

Many insect species undertake long-distance migration in response to either predictable (seasonal) or unpredictable (e.g., flooding) changes in habitat quality (Kennedy, 1961; Dingle, 1971; Southwood, 1977; Solbreck, 1978; Dingle, 2014). The onset of migration may be in response to abiotic (e.g., temperature, photoperiod, relative humidity) and/or biotic (such as host plant availability, population density) factors (Dingle, 2014) A number of these highly-mobile species are among the most economically important pests on Earth (Chapman, 2015) as they exploit temporarily available habitats such as agricultural crops (Dingle, 1985; Johnson, 1969, Rabb & Kennedy,1979).

There is clear evidence that the geographic range of many non-migrant insect species is changing as a result of changing climatic conditions. For example, the Southern green stink bug, *Nezera viridula*, is now able to overwinter much further north than previously documented (Yukawa *et al.,* 2007). Similarly, the Mountain pine beetle, *Dendroctonus ponderosae*, is expanding its range into newly exploitable habitats at higher elevations where populations previously did not exist (Carroll *et al.*, 2003). The migratory desert locust, *Schistocerca gregaria*, which historically only affected certain African countries now occurs in southern

Europe (Pedgley, 1989). However, there is less work examining the effects of climate change on the biology of migratory species, such as the monarch butterfly which has distinct summer and winter geographic ranges. In the case of migrant pest species, if conditions in overwintering ranges become more favourable, this may result in increased outbreaks in the summer range. If conditions become less favourable, fewer epidemics may be observed. The same logic holds with respect to changing conditions in the summer range. However, currently we have very little idea of the overwintering sites for many of the migrant pest species that occur in Canada (other than "south of the border") and without such information it is hard to predict to what extent the probability of outbreaks could increase or decrease.

Furthermore, there is the question of changes in wind patterns, for during migration most species fly above their flight boundary layer (FLB; where controlled, oriented flight is possible) and exploit rising thermals and high-altitude winds during long distance movement (Taylor, 1974). While such behaviours may conserve energy and allow individuals to cover greater distances than through active flight some individuals are blown off course by changing weather patterns, potentially into unsuitable areas (Riley *et al.*, 1983; Rabb & Kennedy, 1979). For example, Pose *et al.*, (1975) reported the presence of the corn aphid and the fall armyworm in Ontario during the fall when subsequent survival would not have been possible. Similarly, a peak in fall armyworm, *Spodoptera frugipera,* observed in St. Hyacinthe, Quebec in October 1984, was the result of moths inadvertently blown northward, the result of a low- pressure system from Hudson's Bay that favours northward dispersal at a time when the habitat was clearly unsuitable for this species (Mitchell *et al*., 1991). Will such "wrong direction" movements occur more frequently as the result of climate change and if so, how will they impact the population dynamics of important migratory pest species?

Mark and recapture, radar or the use of light and pheromone trap catch data have been used to provide some insight into the movement of migratory insects (Rabb & Kennedy, 1979; Chapman *et al*., 2002; Chapman *et al*., 2011)., although the data obtained is of limited use. Only 125 of >100,000 Monarch butterfly (*Danaus plexippus*) marked and released from 1975−1998 were recaptured (Hobson *et al.*, 2012,) and while radar can detect insects in flight it cannot easily identify the species. While Global Positioning System (GPS) geolocators can be used to determine the migratory movement of larger species they are of very limited use when studying insects because of their small body size. Furthermore, these approaches require human, ground-based observations making them somewhat impractical for monitoring nocturnal insects travelling at high altitudes (Muller, 1986; Gatehouse, 1997; Chapman *et al*., 2011).

Endogenous markers such as stable isotopes are proving to be a highly useful tool in the study of different migratory species as diverse as birds and insects (Hobson & Wassenaar, 1998; Hobson *et al.*, 2014). In the case of insects, measurements of stable hydrogen isotopes (δ^2H) in metabolically inert tissues, such as wings, have been compared to continent-wide patterns expected from δ^2H of amount-weighted mean growing-season precipitation δ^2H (δ^2H_p; Hobson & Wassenaar, 2008). For example, this approach has been successfully used to trace the origins and movements, as well as other life-history events, of the monarch butterfly in eastern North America (Hobson *et al.*, 1999; Wassenaar & Hobson, 1998; Flockhart *et al.*, 2013; Miller *et al.*, 2012; Yang *et al.*, 2016; Altizer *et al.*, 2015; Hanley *et al.*, 2013; Flockhart *et al.*, 2017). The approach is based on the fact that naturally occurring stable isotopes abundant in primary production can be passed to consumers higher up the food web, where they become locked-in during the formation of metabolically inert tissues. Various biogeochemical processes also result in gradients or patterns of isotope abundance and, once known, these patterns can be used to interpret potential origins of individuals.

Hobson & Wassenaar (1995) showed that there was negligible change in the δ^2H ratio between songbird feathers and diet, which is also the case between food and chitin when insects were reared on known isotope diets (Miller,1984). The wings of holometabolous insects, such as lepidopterans, come from imaginal discs made up of larval-derived nutrients (Oberlander, 1985; Svacha, 1992), so these metabolically inert, chitinous structures are records of larval life-history (Hamback *et al.*, 2016; reviewed by Hyodo, 2015; see also Ponsard *et al.*, 2004; Schallhart *et al.*, 2009) and thus are ideal material to use when inferring natal origin using stable hydrogen isotopes. Stable carbon and nitrogen isotopes can also be of use to provide more information about the biology of an insect. Stable carbon isotopes (C^{12}/C^{13}) provide insight into larval diet as C4 plants are more enriched in C^{13} than C3 diets due to less enzymatic discrimination Marshall *et al.*, 2007). Similarly differences in nitrogen stable isotope ratios in tissues help determine if the larvae originated from natural ecosystems or from agroecosystems receiving nitrogen supplements (Pardo & Naddlehoffer, 2010). Data on both carbon and nitrogen may also help to constrain geographic assignments.

The true armyworm (*Mythimna unipuncta* (Haworth), Lepidoptera: Noctuidae) is a pest of cereals and forage crops throughout Eastern North America, with sporadic outbreaks occurring every 5-20 years (Beirne, 1971). The true armyworm is unable to overwinter in Canada (Breeland, 1958; Beirne, 1971; Fields & McNeil, 1984; Ayre, 1985) and uses environmental cues to assess habitat quality, with cool temperatures and short daylengths causing endocrine changes resulting in delayed reproduction (Turgeon & McNeil, 1982; Delisle & McNeil, 1986). Consequently, it has been postulated that this species is a seasonal migrant, with the 2-3 locally produced generations observed annually as the result of a spring immigration (McNeil, 1987). Therefore, I hypothesized that adults captured in London during the spring, being immigrants from further south, would have significantly different δ^2H profiles than those observed during the summer (local and reproducing) and fall (local and emigrating). Consequently my prediction was that spring individuals would have significantly more positive δ^2H_w values than the locally produced summer and fall populations. I first tested this hypothesis using moths captured in pheromone and light traps throughout the 2016 flight periods in London, Ontario. I was able to confirm that individuals captured in the spring were significantly different than those captured in the summer and fall. I also provided the first direct evidence of a return migration in *M.unipuncta*. as individuals captured during September, 2012 in Texas had more negative δ^2H_w values (reflective of moths produced in more northern locations) than those captured in November, 2012 (locally produced Texas moths). Then using moths captured in London in 2008, 2013, 2015 and 2016, I examined both the intra- and inter-year variability to determine if δ^2H profiles would shed light on whether migrants originate from the same specific region each year, or if in different years they have different origins. The data showed that there was considerable intra- and inter year variability, and further research will be necessary to determine to what extent these differences are due to immigrants originating from different sources and/or differences in annual climate (El Nineo/El Nina years). However, all the data obtained provide strong support for the idea that the use δ^2H measurements could help overcome current barriers limiting our ability to track, small highly-mobile pest insect species. This work also provides a foundation for further research using stable isotopes in migratory insects.

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Chapter 2

2 Inferring origins of migrating insects using isoscapes: A case study using the true armyworm, *Mythimna unipuncta*, in North America

3

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2.1 Abstract

Many important insect pests undertake seasonal migrations at continental scales in response to changes in resource quality and availability. The frequency and timing of these events could be influenced by the impact of climate change on the suitability of the different sites exploited throughout the year yet, in many cases, we know little about the origin of seasonal populations, as tracking insect movements is extremely challenging due to their small body size.

The use of stable isotope measurements in insect tissues combined with the development of tissue specific "isoscapes" of modeled geographic patterns presents a potentially valuable but rarely used approach for obtaining such information on important pest species. In this paper we illustrate how stable hydrogen isotope analyses (δ^2H) in wing chitin of the true armyworm (*Mythimna (Pseudaletia) unipuncta* Haworth), a seasonal migrant, clearly delineated between 2016 spring immigrants and later locally produced moths in southern Ontario, Canada.

We showed that adult moths captured in early fall in Texas were immigrants from farther north, the first direct confirmation of a southward return migration of this species. Stable carbon isotope (δ^{13}C) measurements indicated spring immigrants in Ontario and fall immigrants in Texas were from exclusively C3 biomes. Stable nitrogen isotope (δ^{15}N) measurements also provided information on probability of individuals coming from agriculturally intensive (i.e. higher $\delta^{15}N$) sites. Finally, we make several recommendations for future research that could improve the Bayesian assignment models and thus improve assignment accuracy.

Key words: deuterium, carbon-13, nitrogen-15, dispersal, migration, probabilistic assignment.

2.2 Introduction

The recent technological revolution in miniaturized devices such as geolocators and global positioning system (GPS) tags are now being used to gain a better understanding of movement patterns in small migratory birds and mammals. However, due to the generally small adult body mass, these techniques offer few opportunities for the study of insect migration (Bridge *et al.*, 2013; Dingle, 2014; Hallworth & Marra, 2015; but see Kissling *et al.*, 2013; Wikelski *et al.*, 2006). In contrast, the measurement of endogenous markers such as naturally occurring stable isotopes has been used successfully to infer natal origins and movements, especially at local scales, of several insect taxa. For example, insect tissues, especially chitinous wing material, can be a faithful isotopic recorder of larval diets (Hamback *et al.*, 2016; reviewed by Hyodo, 2015; see also Ponsard *et al.*, 2004; Schallhart *et al.*, 2009), and strong isotopic differences between stable isotopes of carbon (δ^{13}C) have been used to infer local movement among crop types.

Larger-scale movements of insects are now being investigated using stable hydrogen isotopes (δ^{2}H) in metabolically inert tissues such as wings and comparing those to continent-wide patterns expected from δ^{2}H of amount-weighted mean growing season precipitation δ^{2}H ($\delta^{2}H_{p}$; Hobson & Wassenaar, 2008). For example, using continental patterns, or ‘isoscapes’ (West *et al.*, 2010), of both precipitation δ^{2}H and milkweed δ^{13}C, together with the isotopic values in wings of monarch butterflies (*Danaus plexippus* Linnaeus), this approach has been used to (i) trace the origins and movements in eastern North America (Hobson et al., 1999; Wassenaar & Hobson, 1998), as well as infer (ii) patterns of spring recolonization in eastern

North America (Miller *et al.,* 2012; Flockhart *et al.,* 2013), (iii) the origins of wintering individuals in western North America (Yang *et al.,* 2016), (iv) the effects of natal origin on parasite loads (Altizer *et al.,* 2015), (v) the role of wing coloration in flight distance (Hanley *et al.,* 2013), and (vi) general conservation concerns related to areas of high productivity (Flockhart *et al.,* 2017a,b). Similar large-scale tracking studies using naturally occurring stable isotopes have been used to examine the movement of the red admiral (*Vanessa atalanta* Fruhstorfer) and painted lady (*V. cardui* Linnaeus) butterflies in Europe and sub Saharan Africa (Brattström *et al.,* 2010; Stefanescu *et al.,* 2016), as well as the globe skimmer (*Pantala flavescens* Fabricius) dragonfly from India across the Indian Ocean (Hobson *et al.,* 2012).

Many agricultural pests are seasonal migrants moving between habitats in response to changes in both abiotic (e.g., temperature, photoperiod, rainfall) and biotic (e.g., host plant quality/availability, population density) factors (Johnson, 1969; Kieckhefer *et al.,* 1974; Kevan & Kendall, 1997; Capinera, 2001). However, very little is known about the origin of immigrants, which is not only of importance when developing pest management strategies but also for studying the potential impact of climate change on seasonal migratory patterns. The objective of the current study was to determine the effectiveness of using the stable isotope approach to assigning migratory agricultural pest insects to origins at continental scales. I used the true armyworm (*Mythimna unipuncta* Haworth), as it is a polyphagous pest species feeding on both C3 and C4 plants (Beirne) and, with no evident diapausing stage, it cannot overwinter in Canada (Fields & McNeil, 1984; Ayre, 1985). Consequently it has been considered a seasonal migrant that overwinters in the southern United States to avoid the cold but migrates northward in spring to avoid the negative effects of high summer temperatures (McNeil, 1987).

I first constructed a   using data from individuals reared in the laboratory on substrates differing in δ^2H. These were then used to probabilistically assign individuals caught in the field near London, Ontario, Canada at different times in 2016 using Bayesian-based likelihood models to areas of origin. I also consider the potential influence of crop irrigation where the water used may differ in isotopic composition compared to amount-weighted growing-season average or mean-annual precipitation deuterium values. Further, I used wing δ^{13}C and δ^{15}N to assess differences between capture locations and to determine habitats in which these moths developed (Hyodo 2015). For instance, δ^{13}C can be used to determine the host plants exploited by larvae as there is ~ 13‰ mean isotopic difference between C3 and C4 plants (Marshall *et al.,* 2007). Similarly, δ^{15}N measurements provide information if larvae fed on plants in native habitats (low δ^{15}N) or on fertilized agricultural crops (higher δ^{15}N (Pardo & Naddlehoffer, 2010). Finally, I discuss potential means of constraining assignments using prior information such as the use of wind vectors, genetics and other isotope measurements such as δ^{13}C and δ^{15}N.

2.3 Materials and Methods

Lab and field samples

I raised four groups (*n* = 50/group) of armyworm larvae, the progeny of field-collected females, at 25 ± 0.5 °C, 70 ± 5% RH, under a16L:8D photoperiod on barley irrigated with water differing in their δ^2H values. I used local tap water ((δ^2H): -51.9 ± 3‰), as well as two local tap water samples spiked with 99% deuterated water (Sigma Aldrich) to give samples with δ^2H values of

330.9 ± 5‰, and 599.4 ± 6‰. The enriched water samples were produced in single batches and the δ^2H values confirmed throughout the experiment (n = 4) using an LGR cavity ringdown laser spectrometer (Los Gatos Research, San Francisco). In addition, I tested and used local snowmelt (winter of 2016-2017) which had a δ^2H value of -119 ± 3‰. Newly emerged adults from the four treatments were stored frozen until isotope analysis. Dates of capture and samples sizes were recorded (Table. 2-1).

Pheromone traps, baited with a 2-component pheromone blend (Z11-hexadecenyl and Z11-hexadecenol: 450 mg A. I/Kg from Chemtica,) were deployed at sites around London (42.9849° N, 81.2453° W), Ontario, from April through October, 2016. Lures were replaced every six weeks and all traps were checked daily. Over the same period, I also collected adults of both sexes using 18W light traps at several sites in London. I also obtained wings from armyworm moths that had been captured in light traps at Uvalde, Texas (29.3597° N, 99.68242° W,) between September and November, 2012.

Sample preparation and stable isotope analysis

All wing samples were soaked and washed with a 2:1 chloroform:methanol solution and air dried in a fume hood. A sample was taken from a forewing of 12 randomly selected adults in each treatment of the laboratory experiment and, as densities were very low throughout the season, from all field caught adults. Wing subsamples (0.35 ± 0.2 mg) were weighed into individual pressed silver 3.5 x 5 mm capsules. These were placed in a Eurovector Uniprep autosampler (Milan, Italy; Wassenaar et al., 2015) carousel attached to a Eurovector 3000 Elemental Analyzer coupled with a Thermo Delta V Plus isotope ratio mass spectrometer (Bremen, Germany) in continuous flow mode with He carrier gas (Wassenaar et al., 2015). After the samples were loaded, the Uniprep autosampler (heated to 60 °C) was vacuum evacuated and subsequently flushed with dry helium twice to remove adsorbed atmospheric moisture from the samples. Two USGS keratin standards, EC-01 (δ^2H = −197.0‰) and EC-02 (δ^2H = −54.1‰) were included for every 10 wing samples. Samples were combusted at 1350 °C in a ceramic reactor tube containing glassy carbon. Values of δ^2H of non-exchangeable H of wings were derived using the comparative equilibration approach of (Wassenaar & Hobson, 2003) and calibrated to VSMOW using EC-01 and EC-02. Within-run measurement error based on standards (*n* = 5 each) was estimated as ± 2.0 ‰. Water samples were analyzed using a DLT-100 v.1 Liquid Water Isotope Analyzer (Los Gatos Research). Precision of δ^2H in standard waters is ±1‰ and ±5‰ in enriched samples.

For δ^{13}C and δ^{15}N analyses, ~1.0 mg of wing material was combusted online using a Eurovector 3000 (Milan, Italy – www.eurovector.it) elemental analyzer. The resulting CO_2 was separated by gas chromatography (GC) and introduced into a Nu Horizon (Nu Instruments, Wrexham, UK – www.nu-ins.com) triple-collector isotope-ratio mass-spectrometer via an open split and compared to a pure CO_2 or N_2 reference gas. Stable nitrogen (^{15}N/^{14}N) and carbon (^{13}C/^{12}C) isotope ratios were expressed as parts per thousand (‰) deviation from the primary standards, atmospheric AIR and Vienna Pee Dee Belemnite (VPDB). Using previously calibrated internal laboratory standards (powdered keratin [BWB II: δ^{13}C = -20.0‰, δ^{15}N = 14.1‰ and gelatin: δ^{13}C = -13.6‰, δ^{15}N = 4.7‰]) within run (*n* = 5), precision for δ^{15}N and δ^{13}C measurements was ~ ± 0.15‰.

Probabilistic assignments

I depicted the origins of wild armyworms caught in Ontario and Texas using a likelihood-based assignment method (Hobson *et al.*, 2009; Wunder, 2010; Van Wilgenburg *et al.*, 2012), using the wing chitin δ^2H isoscape (δ^2H_w) and an amount-weighted precipitation-to-wing calibration algorithm (see results) derived from our captive rearing experiment (Fig. 2-1). This was used to convert amount-weighted mean annual δ^2H_p isoscapes (IAEA/WMO, 2015; Terzer *et al.*, 2013) into separate δ^2H_w isoscapes. I used the 13‰ residual SD error from this regression in our assignments. I then created a spatial layer representing the known geographic range for the true armyworm and used this as a mask (i.e. clip) to limit our analysis. For completeness, I also investigated the use of a tap water vs. precipitation isoscape (Bowen *et al.*, 2007) because I was interested in identifying potential large-scale effects of assignments involving agricultural-based foodwebs driven by irrigation.

I estimated the likelihood that a cell (pixel) within the δ^2H_w isoscape represented a potential origin for a sample by using a normal probability density function to estimate the likelihood function based on the observed δ^2H_w, and thus depicted the likely origins of each armyworm by assigning individuals to the δ^2H_w isoscape one at a time. A 2:1 odds ratio was arbitrarily chosen to include only those pixels (coded 1) with at least a 67% probability of origin vs. all others (coded 0). This resulted in a binary map per assigned individual of presence and absence. We then summed the results of individual assignments by stacking the surfaces. Geographic assignments to origin were produced using functions within the R statistical computing environment (R Core Team, 2016) using scripts employing the ‘raster’ (Hijmans, 2016) and ‘maptools’ (Bivand & Lewin-Koh, 2016) packages. Thus the final assignment surface depicted the number of individuals co-assigned at each pixel based on the odds criteria.

Sex comparisons

Sexes were compared for all water treatments used in the laboratory calibration experiments independently for laboratory δ^2H calibrations (fig. 2-1) by t-tests run using R statistical computing environment (R Core Team, 2016).

2.4 Results

Stable H isotopes

I found a strong relationship ($r^2 = 0.96$) between armyworm moth wing chitin δ^2H_w and δ^2H of the water used to irrigate the larval host plants ((δ^2H_p); $\delta^2H_w = -84.4 + 0.40 * \delta^2H_p$) (Fig. 2-1) but no effect of sex for any of the water treatments ($t < 1.4$, $p > 0.1$ for all comparisons). Expected wing chitin isoscapes were then created based on the most recent International Atomic Energy Agency (IAEA) Global Network of Isotopes in Precipitation (GNIP) as presented by Terzer et al. (2013) (Fig. 2-2a) and the tap water isoscape from Bowen et al. (2007) (Fig. 2-2b) in order to spatially assign the origin of armyworm moths collected in southern Ontario and Texas.

There was a significant seasonal difference in δ^2H_w of armyworm moth captured in southern Ontario (Fig. 2-2, $r^2 = 0.96$, $p < 0.001$), with those captured during the spring (24 May – 11 June 2016) being significantly different than those captured in early summer (1 – 29 July 2016) and late summer (30 July – 5 September 2016); ANOVA; $F_{2,88} = 18.39$, $p < 0.01$; 107.5 ± 3.2‰, 127.6 ± 1.7‰ and 115.1 ± 4.1‰). As there was no significant difference between early and late summer samples, I only depict those of spring immigrant (Fig. 2-4 A) and early summer resident (Fig.2-4 B) individuals.

The δ^2H_w distribution for moths captured in Texas throughout the late summer and fall was bimodal (Fig. 2-5), and using a δ^2H_w threshold of -120‰, it is clear that those individuals captured in September originated from much farther north (Fig. 6a), while those captured later were likely of local origin (Fig. 6b; early mean: -129 ± 11.4‰, late: -91.9 ±3.7‰; $F_{1,13} = 3.1$, $p = 0.004$). Assignment exercises were repeated using a tapwater δ^2H surface from the United States (Bowen et al., 2007) instead of the GNIP-based precipitation isoscape (Supplementary Material).

Stable carbon and nitrogen isotopes

All 2016 spring immigrants in southern Ontario fed exclusively on C3 plants during larval development (mean $\delta^{13}C$ = -27.7 ± 2.8‰, *n* = 58) (Fig. 2-7a). On the other hand, the Texas sample was clearly bimodal. The early immigrants that originated from a broad range of latitudes had all fed on C3 plants as larvae (early mean $\delta^{13}C$ = -24.6 ±1.6‰), while the later local populations included individuals where larvae had fed on C3 and C4 host plants (Fig. 2-7a; late mean -17.0 ±1.5‰; $F_{1,20} = 3.5$, $p = 0.001$). In the case of spring immigrant moths captured in Ontario, there was a tight distribution of $\delta^{15}N$ values ($\bar{x}$ = 5.0 ± 2.4‰) while the values from Texas moths had a broad range (0.3 to 22.2‰; early mean: 7.3 ±1.7‰, late mean 8.3 ±1.3‰; $F_{1,20} = 0.5$, $p = 0.3$). The highest $\delta^{15}N$ values were associated with those moths of C4 origins captured in late fall (Fig. 2-7b) suggesting they originated from fertilized agroecosystems.

2.5 Discussion

Fields & McNeil (1984) reported that the true armyworm cannot overwinter in Canada (a finding confirmed in 2015 field trials: Appendix A) and postulated that the populations observed in Canada each year are derived from immigrants. The results of this study provide the first direct evidence that the early season adult armyworms captured are immigrants from farther south, while those caught in mid- and late summer were consistent with locally produced individuals. McNeil (1987) also postulated that the true armyworm undertakes a southward fall migration and the early fall isotope data I report for individuals captured in Texas in September 2012 provided the first concrete evidence that this is the case.

While the general assignments provide strong support that the true armyworm undertakes a two-way seasonal migration, there is still considerable variation in the wing deuterium values observed within any given flight period. For example, while the majority of individuals captured in the summer and fall were assigned to local populations, there were some that would be classified as immigrants from farther south. In all cases this could be accidental movement, the result of prevailing wind conditions. However, in the summer it can be an active response to unfavourable local habitat conditions, such as low food quality and the high density of conspecifics, as proposed for the northward summer migratory movement observed in *M. separata* Walker in China (Zhao *et al.,* 2009). In contrast fall immigrants arriving in Ontario enter an unsuitable habitat and clearly would not survive, as previously reported for the fall armyworm (Rose *et al.,* 1975; Mitchell *et al.,* 1991).

A summary of stable isotope calibrations used to date for insects, while few, underline the fact that there appears to be considerable variation in wing chitin to environmental water δ^2H calibrations and the underlying causes must be taken into account in future studies. For example, it is not clear to what degree derivations observed from laboratory studies (e.g., 2-1) are comparable to those from field collected individuals that would have incorporated other sources of environmental variability (Hobson *et al.,* 1999). Furthermore, laboratory calibrations assume there is a linear relationship over a large range of water δ^2H values. However, this has to be confirmed using water δ^2H values sampled from more northern latitudes and/or high altitude sources (Clark & Fritz, 1997).

The δ^2H values for species feeding on wild host plants (e.g., monarch butterflies on milkweed, *Asclepias* sp.) in natural habitats are driven exclusively by natural precipitation. However, insect pests derived from agroecosystems present an additional challenge as the irrigation water may be derived from reservoirs subject to evaporation, ground waters that may differ from long-term mean annual/growing season precipitation values or river systems with geographically distant origins (Clark & Fritz, 1997; Bowen *et al.,* 2011). Our assignment of the armyworm origin using a tap water isoscape available for the contiguous United States (Supplementary Material) did not qualitatively differ from those generated using amount-weighted mean precipitation isoscapes. However, this may be an important regional issue and comparing wing chitin values from known locations with values predicted from precipitation, surface and tap water models (Bowen *et al.,* 2007) would likely improve assignments. Although the derived true armyworm wing δ^2H isoscapes provide useful information on latitudinal origins, there is much ambiguity with respect to longitudinal resolution (Hobson *et al.*, 2009; Van Wilgenburg & Hobson, 2011). Resolving this issue would certainly help in the assignment for species that undertake more east west oriented migrations, such as noctuids that migrate to higher altitudes at similar latitudes during the summer (e.g., Kevin & Kendall, 1997). The use of additional isotopes may also help refine assignment (Hobson & Wassenaar 2008). For example, if δ^{13}C and δ^{15}N values of immigrants are consistent with values expected from the landuse patterns previously described and if they are similar to those I measured in 2016, then one could limit investigations to unfertilized C3 ecosystems and exclude C4 agroecosystems as potential sources of immigrants. Isotopic data could also be combined with other techniques that could refine origin assignment, such as pollen analysis (Hendrix & Showers, 1992) or the use of molecular makers through the creation of genescapes (Rundel *et al.*, 2013; Ruegg *et al.*, 2014), as with previous studies on the fall armyworm (Nagoshi *et al.*, 2007; Westbrook *et al.*, 2016). The appropriate wind pattern data would also be taken into consideration when assigning origins in general or at the level of individual fallout events (Stefanescu *et al.*, 2007; Chapman *et al.*, 2008; Chapman *et al.*, 2015; Westbrook *et al.*, 2016).

In conclusion, I believe that probabilistic assignments using stable isotopes, with the refinements discussed above, offer considerable promise when addressing basic questions about insect migration, including how climate change may affect long-distance, seasonal movement patterns, as well for the development of more effective management strategies for migrant pests.

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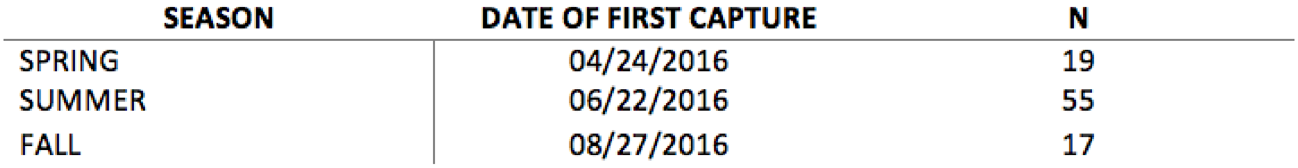
Table. 2-1. Season, date of first capture and sample size of *M. unipuncta* caught in London, Ontario in 2016.



SEASON	DATE OF FIRST CAPTURE	N
SPRING	04/24/2016	19
SUMMER	06/22/2016	55
FALL	08/27/2016	17

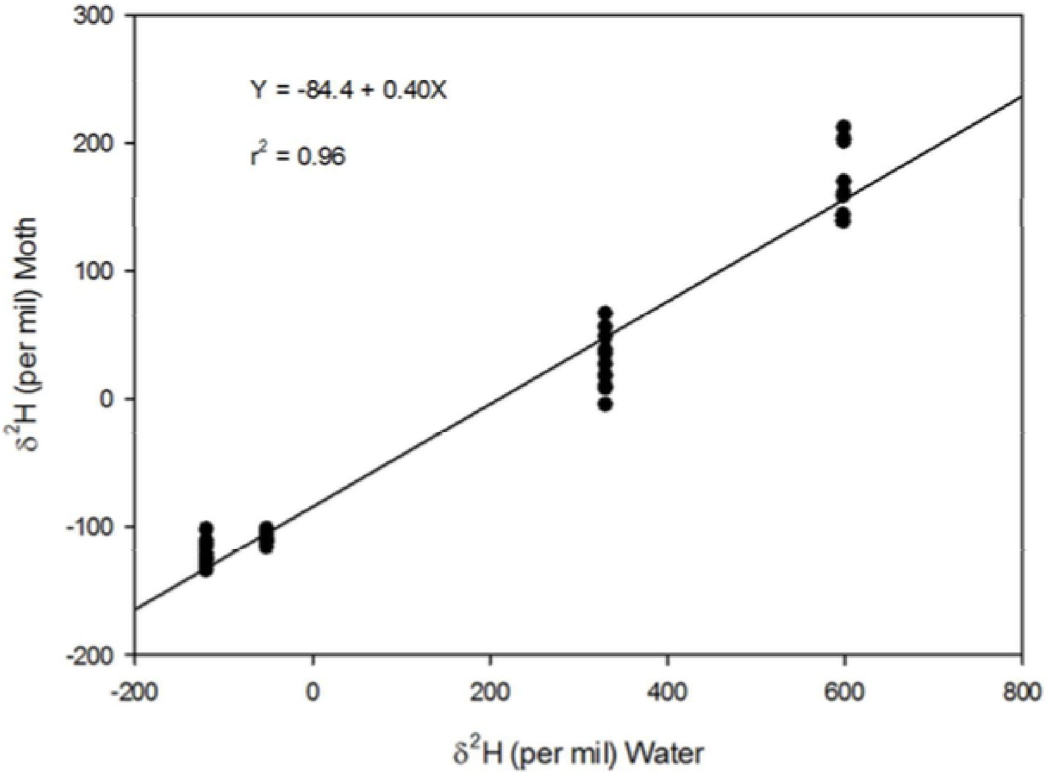


Fig. 2-1. Relationship of wing chitin δ^2H (δ^2H_w) and environmental waters from true armyworm, *Mythimna unipuncta*, adults raised on barley irrigated with various waters.

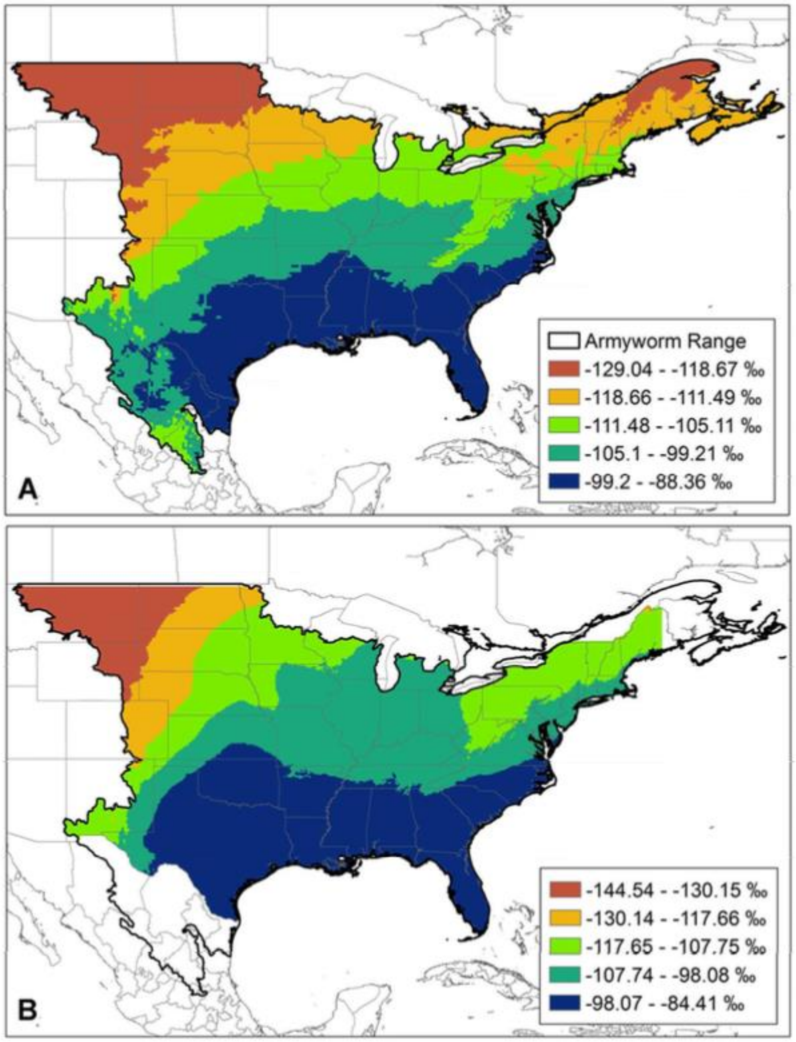


Fig.2-2. True armyworm, *Mythimna unipuncta*, wing chitin isoscape using A) mean annual deuterium in precipitation (Terzer 2013; IAEA/WMO 2015) and B) tapwater (Bowen et al., 2007) isoscapes (see Methods).

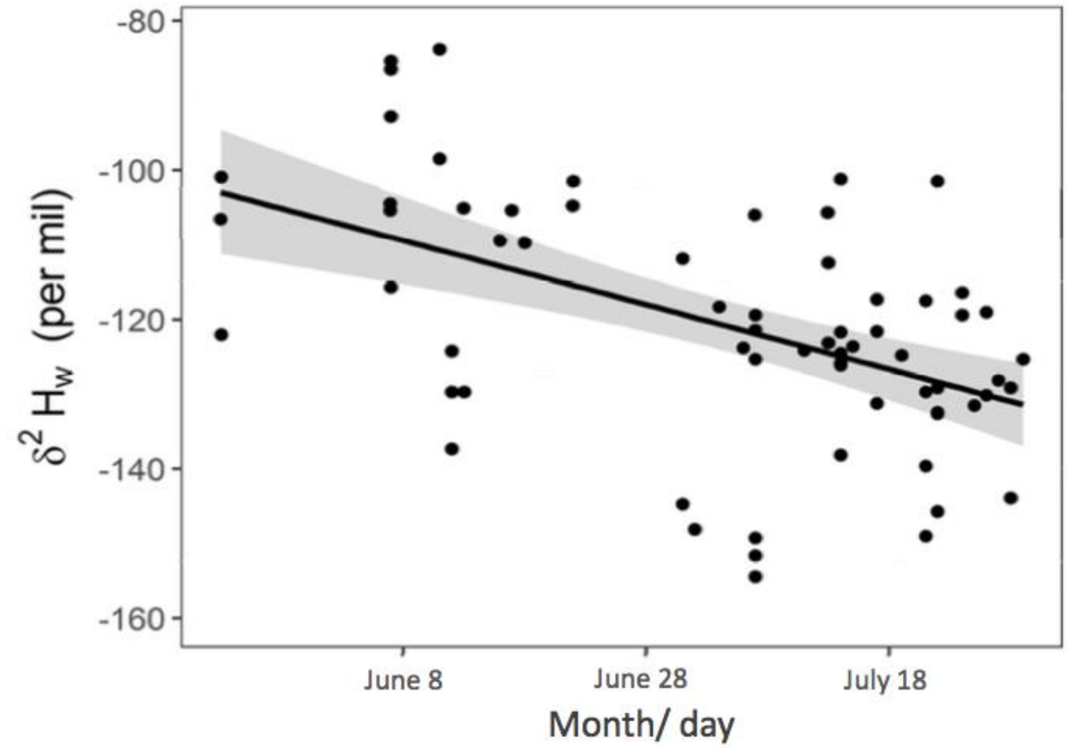


Fig. 2-3. Relationship between wing chitin δ^2H (δ^2H_w) and sampling date for true armyworm, *Mythimna unipuncta* adults captured in southern Ontario, 2016. The first sample was collected on May, 24. The black line represents the regression for δ^2H_w vs. time, and the shaded area represent the 95% confidence interval of the regression.

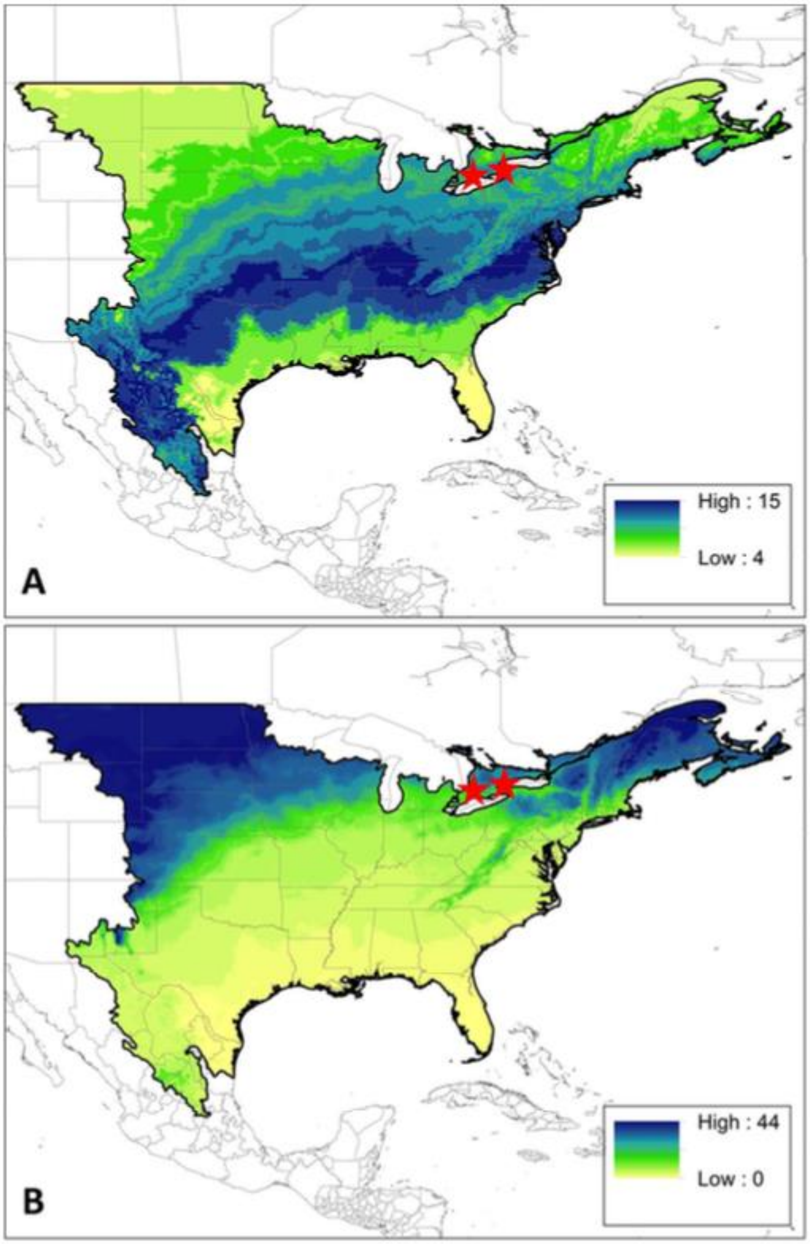


Fig. 2-4. Depiction of probable origins of true armyworms, *Mythimna unipuncta*, collected in southern Ontario in May and June 2016 (early, A) and 1 – 29 July 2016 (late, B) assigned using mean annual precipitation isoscapes (Terzer *et al.*, 2013; IAEA/WMO 2015). The number of individuals assigned to each cell a cell in the isoscapes is indicated by the legend values. Stars indicate sampling sites.

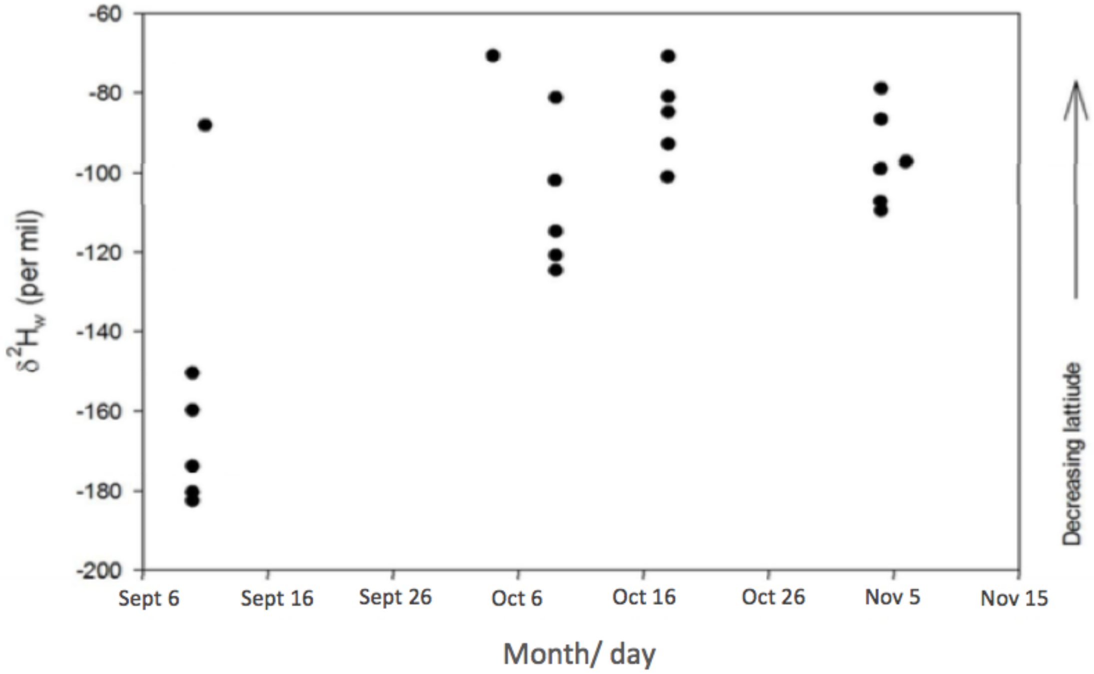


Fig. 2-5. Pattern of wing δ^2H (δ^2H_w) values of true armyworms, *Mythimna unipuncta*, captured between September and November, 2012 in Uvalde, Texas. The first sample was collected on 11-September.

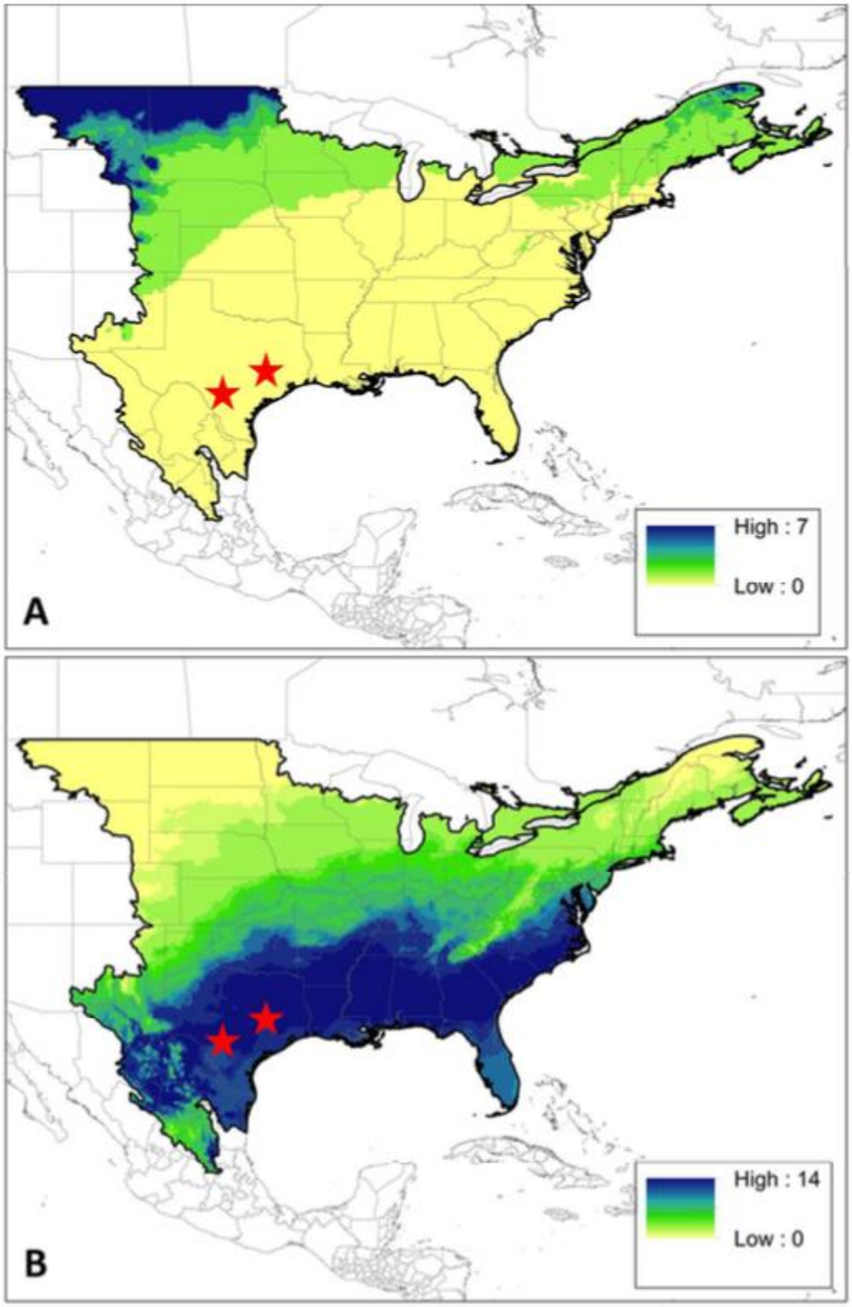


Fig. 2-6. Depiction of probable origins of true armyworm, *Mythimna unipuncta*, moths captured between September (A) and November (B), 2012 in Uvalde, Texas (A: $\delta^{2}H_{w} < -120‰$, B: $\delta^{2}H_{w} > -120‰$) assigned using mean annual deuterium in precipitation isoscapes (Terzer *et al.*, 2013; IAEA/WMO 2015). The number of individuals assigned to each cell in the isoscapes is indicated by the legend values. Stars indicate sampling sites.

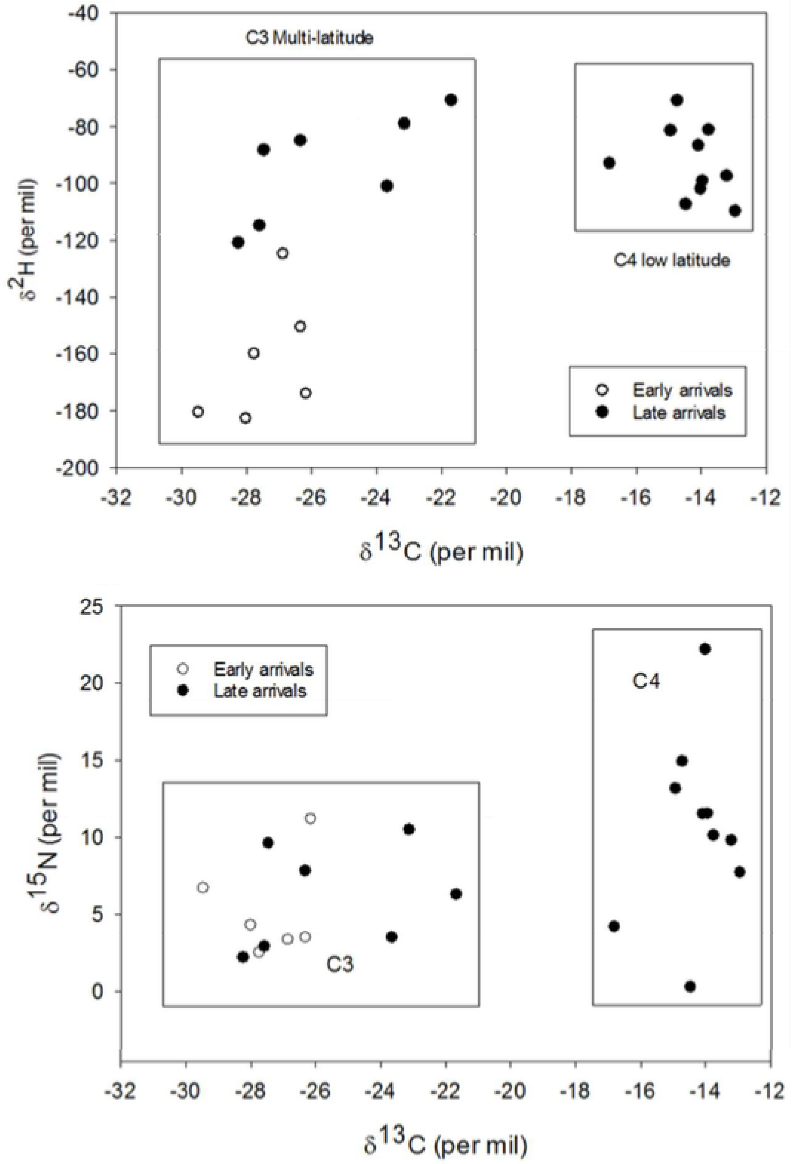


Fig. 2-7. Isotopic ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$) structure of true armyworm, *Mythimna unipuncta*, moths captured between September and November, 2012 in Uvalde, Texas. a) Origins from locally produced (i.e. Texas) armyworms from both C3 and C4 habitats and a range of latitudinal origins from C3 habitats. b) Individuals from C3 and C4 habitats showing a range of $\delta^{15}N$ values likely reflecting differential agricultural N inputs. Open symbols were individuals captured earlier than Julian day 255 (11-September) and dark symbols indicate individuals captured after that (see Fig. 5).

Chapter 3

3 Intra- and inter-year variation of wing δ^2H measurements in the migratory true armyworm moth (*Mythimna unipuncta)*

3.1 Abstract

A number of lepidopteran pests of agriculture, such as the true armyworm, *Mythimna unipuncta* (Haworth), cannot overwinter in Canada. Sporadic epidemics are the result of immigration and are currently predicted based on high numbers of moths caught in pheromone traps at the beginning of the season. This provides farmers very little time to intervene. To develop more appropriate management strategies we need better information on seasonal migratory patterns, yet we currently have no idea from where immigrants originate. To address this question, I analyzed the wing δ^2H profiles (δ^2H_w) of moths captured in London, ON during the flight periods of 2008, 2013, 2015, and 2016 to examine both the intra-year and inter-year variability. My objective was to determine if stable isotope profiles could help determine if immigrants always originated from one general area or whether it varied between years. There was considerable variability both within and between years but no consistent seasonal trends, so more work is required to determine the influence of the impact on soil hydrology, as well as weather and climatic patterns on the inter or intra-year variability in δ^2H_w of this species.

Keywords

Migration, stable isotope, deuterium (δ^2H), true armyworm, *Mythimna unipuncta*, seasonal variation

3.2 Introduction

Many insect species are seasonal migrants and most travel long distances using high-altitude winds (Muller, 1986, McNeil, 1987), which are well above their flight boundary layer (FBL), where they can actually control the direction of movement. However, the wind patterns can vary considerably from day to day (Chapman *et al.*, 2011) and consequently individuals that successfully reach a given location may arise from different sites of origins (Rabb & Kennedy, 1979; Riley *et al.* 1983). Due to the small body-sizes of insects, it is difficult to track individuals (Chapman, 2015) and thus, in many cases, we have little data about actual patterns of movement. This lack of information has applied implications as many of our agricultural lepidopteran pests are migratory, and without a good understanding about the origin of infestations one cannot develop really efficient management programmes. For example, does an immigrant pest species in Ontario originate from the same general location every year or do spring populations originate from different sites between years?

The true armyworm (*Mythimna unipuncta*), is an important agricultural pest that cannot overwinter in Canada (Breeland, 1958; Beirne, 1971; Fields & McNeil, 1984; Ayre, 1985; Doward & McNeil, appendix), and given the changes in its reproductive biology in response to abiotic cues such as temperature and daylength (Turgeon & McNeil, 1982; Delisle & McNeil, 1986) it has been classified as a seasonal migrant (McNeil, 1987). The profiles of the stable isotope deuterium, provided the first concrete support for the hypothesis that individuals observed in the spring are immigrants from a southern location (Chapter 2) and in the present study I used the same approach to determine if the intra- and inter-year in the wing δ^2H (δ^2H_w) of field captured true armyworm moths captured in different years in Ontario (2008, 2013, 2015, 2016) would provide insight into seasonal patterns of movement. I predicted that the immigrant moths would have more positive δ^2H values from those captured in summer and fall, and will show very little inter-year variability than if they originate from the same general area each year.

3.3 Materials and Methods

The wings used for the analyses were obtained from *M. unipuncta* adults that had been collected in pheromone and light traps at the Environmental Sciences Western field station (42.9849° N, 81.2453° W) in 2008, 2013, 2015 and 2016. The dates of first capture varied between years (Table 3-2) but as the life cycle of the true armyworm requires approximately 6 weeks (Breeland, 1958) for any given year we classified all individuals captured in the four weeks following the first capture as spring immigrants. All individuals captured after the first four weeks but before August 13th were considered as the being part of the resident summer population, while those captured on or after August 13th were considered emigrants.

Stable Isotope analysis of wings

I first soaked and washed all wing samples in a 2:1 chloroform:methanol solution and then air dried them in a fume hood. A 0.35 ± 0.2 mg sample was taken from a forewing of all adults collected each year and placed into individual, pressed silver 3.5 x 5 mm capsules. These were subsequently placed in a Eurovector Uniprep autosampler carousel (Milan, Italy), attached to a Eurovector 3000 Elemental Analyzer coupled with a Thermo Delta V Plus isotope ratio mass spectrometer (Bremen, Germany) in continuous flow mode using He carrier gas (Wassenaar et al., 2015) for analysis.. The Uniprep autosampler (heated to 60 °C) was vacuum evacuated and subsequently flushed with dry helium twice to remove adsorbed atmospheric moisture from the samples. The samples were combusted at 1350°C in a ceramic reactor tube containing glassy carbon, with two USGS keratin standards, EC-01 (δ^2H = —197.0‰) and EC-02 (δ^2H = —54.1‰) being included for every 10 wings sampled. Wing values of δ^2H of non-exchangeable H were derived using the comparative equilibration approach of Wassenaar & Hobson (2003) and calibrated to VSMOW using EC-01 and EC-02. Based on the values obtained for the standards I estimated the within-run measurement error to be ± 2.0 ‰.

Statistical analyses

Inter-year and intra-year comparisons of the δ^2H*w* values for the spring, summer and fall flight periods were analyzed with a two-way ANOVA followed by post hoc comparisons Tukey HSD test using R statistical computing software (R Core Team, 2016).

3.4 Results

Variation in wing δ^2H

There were significant inter-year differences in mean δ^2H*w* of true armyworm moths captured in Ontario (ANOVA; F_3= 31.76, p<0.01), as well as a significant interaction between year and seasonal flight period (ANOVA; F_6= 9.95, p<0.01). The values for moths captured in the spring of 2008 (-99.4 ± 11.9 ‰) and 2016 (-108 ± 15.4‰) were markedly more positive than those captured in 2013 (-131.5 ± 8.6 ‰) and 2015 (-130.6 ± 8.9 ‰) (Figure 3-1). Significant inter-year differences in mean δ^2H*w* were also observed during the summer and fall seasons as well as moths captured during the summer of 2008 (-123.5 ± 14.8 ‰) differed significantly from those captured during the summer of 2013 (-131.7 ± 11 ‰) and 2015 (-135.6 ± 6.6 ‰), but not

2016 (-126.9 ± 13.4 ‰) (Fig. 3-1). Mean δ^2H_w of moths captured during the fall of 2015 (-134 ± 7.8 ‰) differed significantly from those captured in 2016 (-119.79 ± 15.5 ‰) (Fig. 3-1)

There were intra-year significant differences in mean δ^2H_w for 2008 and 2016, but not for 2013 or 2015 (ANOVA; F_2= 22.97, p<0.01) (fig. 3-2). In 2008 the mean spring δ^2H_w values were significantly more positive than both summer and fall values (Fig. 3-2), while in 2016, the mean spring δ^2H_w values differed significantly from summer, but not from fall (fig. 3-2).

3.5 Discussion

There was considerable year-to-year variation in the δ^2H_w measurements of field-captured *M. unipuncta* in London, Ontario as the spring immigrants, captured in 2008 and 2016, had more positive δ^2H_w values than those in 2013 and 2015. This suggests that moths captured in 2008 and 2016 originated from more southerly habitats than those in 2013 and 2015 (Figure 3.1), and would mean that the immigrant populations do not always originate from the same general geographic region. There were very marked differences in the date of first capture: in 2008 and 2013 the first adults were captured in the last week of April, while in the other two years it was not before late May. Interestingly, in 2008 and 2013 moths arrived at the same time but had significantly different δ^2H_w profiles (Figure 3-1). This underlines the necessity of integrating atmospheric weather pattern data (that would influence the movement patterns of immigrants) when studying inter-year patterns, as the origin of immigrants may be purely the result of prevailing wind patterns, or may be the result of differential overwintering survival in different geographic areas.

The armyworm does not overwinter in Canada, yet the data indicate that some spring immigrants originate from a geographic area sufficiently close to London that cannot be delineated by δ^2H_w values alone. However, the fact that spring δ^2H_w values were generally significantly more positive than in summer and fall still supports the idea that all spring individuals would be immigrants. Interestingly, the only year where the spring and summer values did not differ significantly was 2013, even though the first captures (April 18th) were the earliest for any year of the study. The expected mean δ^2H_w value for local moths is -110.7 ‰ and the mean SD of δ^2Hw for all summer (i.e. presumed of local origin) individuals included in my analyses (2008, 2013, 2015, 2016) was 12‰. Thus an expected range of δ^2H_w values for a local moth, based on a 2:1 odds ratio, would be -122.7 to -98.7 ‰. Thus, moths with a δ^2H_w more positive than -98.7 ‰ would be assigned to breeding ground considerably further south. Based on this simple threshold criterion 52.2% (n=22) of 2008 spring moths had clearly immigrated considerable distances, while in 2016 it was 23.8% (n=23). Using the same values, moths captured in the summers of 2008, 2013 and 2016 included 7.0 (n=56), 3.4 (n=87) and 1.5 (n=63) % immigrants, respectively. These individuals probably emigrated from their natal site, not in response to environmental cues such as daylength and temperature, but rather in response to resource availability or population density, as proposed for the summer migration of *M. separata* (Zhao et *al.*, 2009). Interestingly, in the fall of 2013 and 2016 , 18.5 (n=16) and 5.8 (n=17) % of moths would be classified as immigrants, probably the result of upper air masses carrying moths in an inappropriate direction, as reported on two separate occasions for the fall armyworm (Mitchell *et al.,* 1991, Rose *et al.,* 1975). However, unlike the spring and summer immigrants, it would be a dead end as conditions would not be suitable for reproduction. However, the existence of significant differences in inter-year δ^2H_w values between locally produced moths in both summer and fall (Fig. 3-1) suggest that other factors also contribute to variation in the stable isotope values. The causes for such variation merit further research as they need to be taken into account when interpreting field data. The inter-year variation in δ^2H_w values of local moths could be related to the water sources used by the larvae during development. Water derived from snowmelt typically has significantly more negative δ^2H values than rain at a given location, with actual values driven by ambient temperature (Clark and Fritz, 1997). Thus, after a cold winter with a heavy and late thawing snowpack, the water δ^2H_w values in spring could differ from those following a warm winter with little snow. Furthermore, differences in precipitation δ^2H_w values, due to El Niño and La Niña events, may exist (Welker, 2012). In addition, if moths originate from agroecosystems, one also must consider the origin of water used for irrigation as it could be local ground water or taken from a river system with its origin a considerable distance away. These factors could be incorporated if spatially explicit Bayesian-based models are used for assignment of individuals to origin, , but these causes of variability would be more troubling when using a simple 2:1 odds ratio threshold model based on a range of 2 SD.

As armyworm populations in Ontario do not originate from the same geographic location each year considerably more information will be required if one hopes to develop more effective management practices. A north-south trapping network to obtain samples from different geographic locations throughout the year, together with specimens reared on unirrigated vegetation to allow for ground-truthing at each site, would be ideal to clarify the causes for the observed differences. Furthermore, any effort to develop predictive models will have to include data on (i) overwintering conditions in potential source areas, and (ii) large scale atmospheric conditions (Muller, 1986) to allow for atmospheric backtracking (McNeil, 1987; Westbrook, 2016) during periods of migration. In addition, using measurements for stable nitrogen and carbon isotope ratios would also provide useful information regarding both the habitat (natural or agroecosystems) and host plants utilised.

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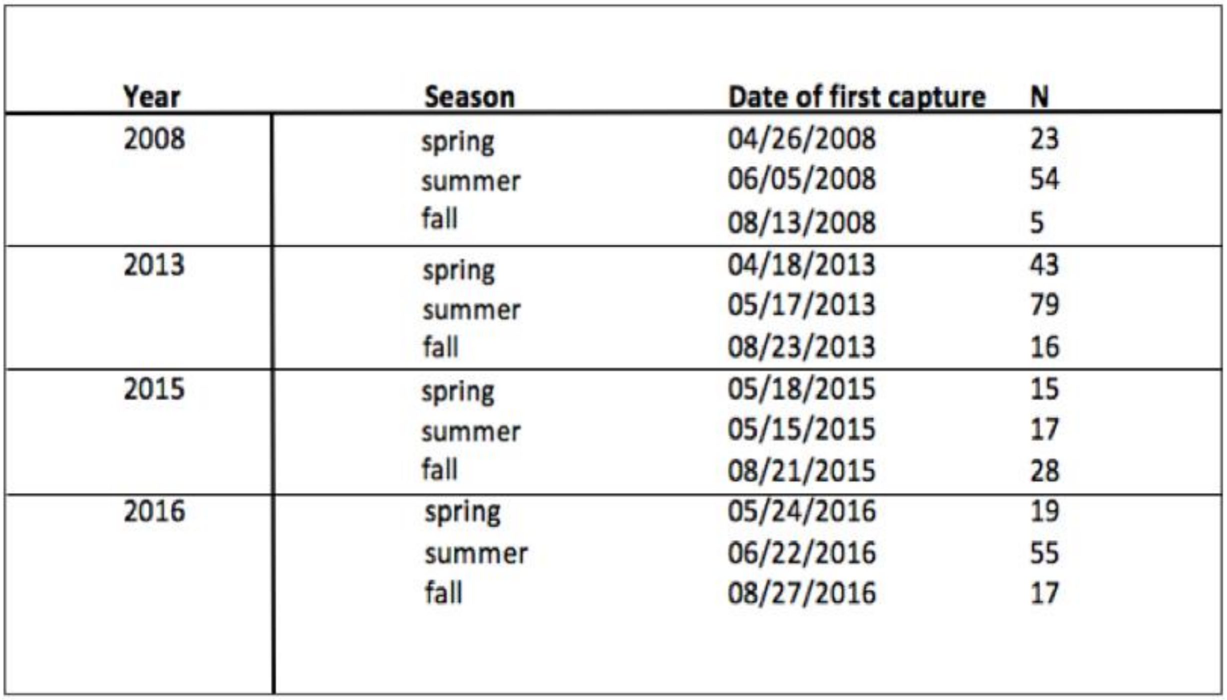
Table. 3-1. Analysis of variance in the δ^2Hw of adult *M. unipuncta* for year, season, and year x season interaction.

Source of variation	Degrees of freedom	F-value	P
Year	3	31.76	<0.01
Season	2	22.97	<0.01
Year x Season	6	9.95	<0.01

Table. 3-2. Date of first capture and sample size of *M. unipuncta* captured during all seasons of 2008, 2013, 2015, and 2016 in London, Ontario.



Year	Season	Date of first capture	N
2008	spring	04/26/2008	23
	summer	06/05/2008	54
	fall	08/13/2008	5
2013	spring	04/18/2013	43
	summer	05/17/2013	79
	fall	08/23/2013	16
2015	spring	05/18/2015	15
	summer	05/15/2015	17
	fall	08/21/2015	28
2016	spring	05/24/2016	19
	summer	06/22/2016	55
	fall	08/27/2016	17

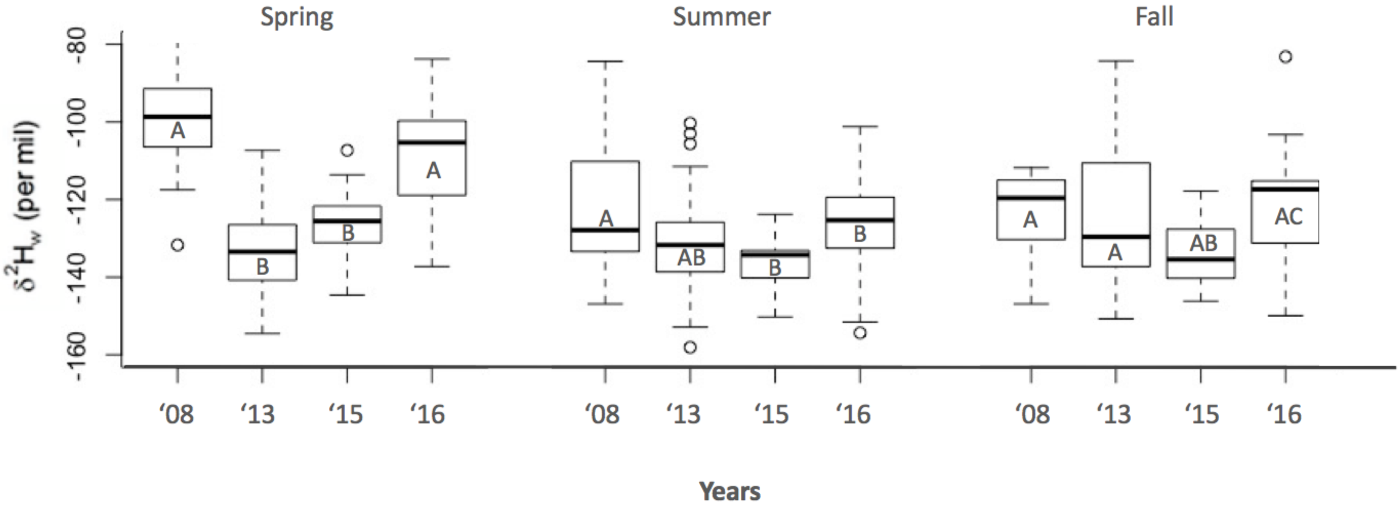


Fig. 3-1. Boxplot of seasonal wing δ^2H (δ^2H_w) of adult *M. unipuncta* captured in 2008, 20013, 2015, and 2016. Black bars represent the median δ^2H_w. Different letters within one season indicate a statistical difference in mean δ^2H_w ($p<0.05$).

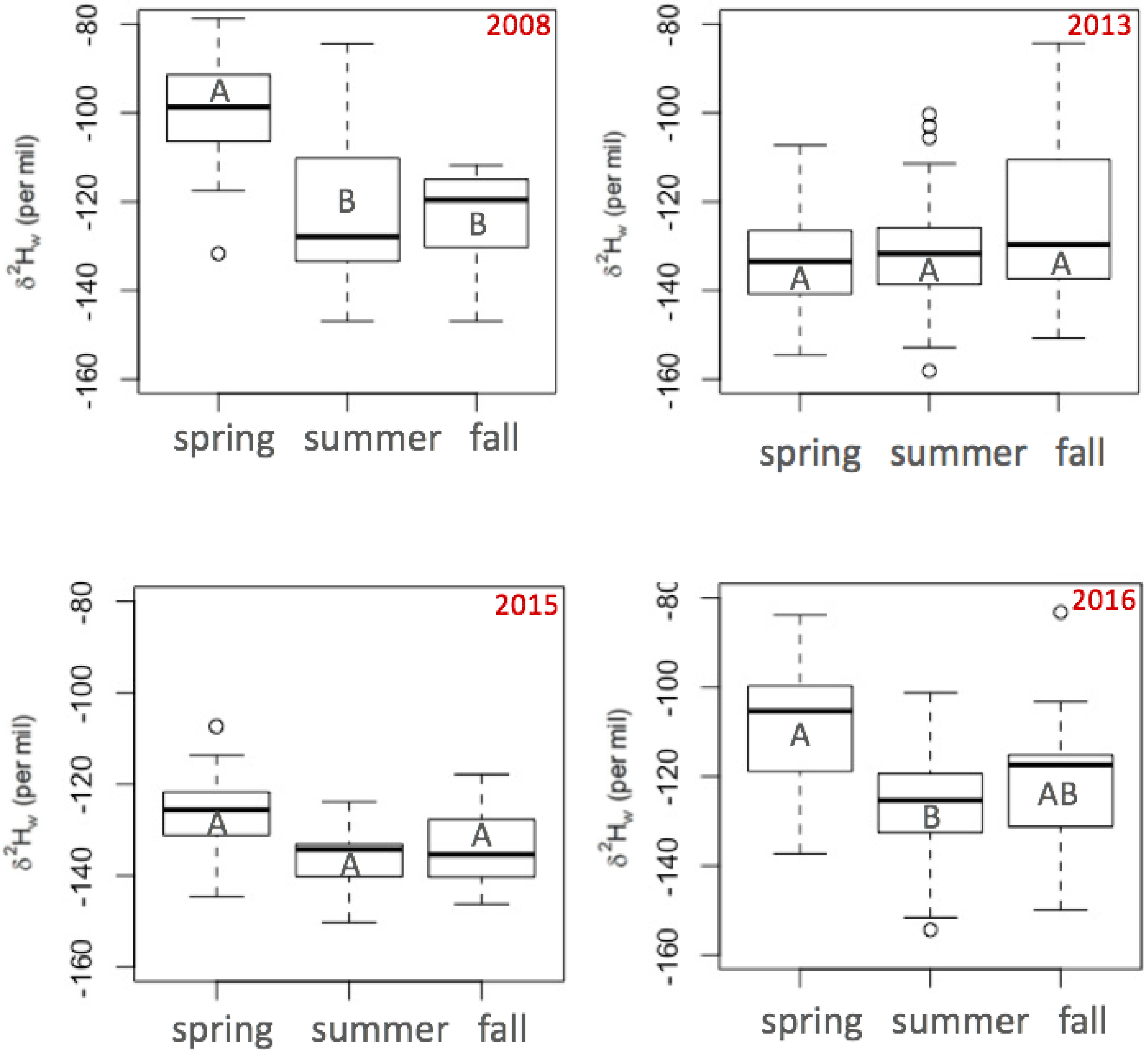


Fig. 3-2. Boxplots depicting Wing δ^2H (δ^2H_w) of moths captured in spring, summer, and fall within years 2008, 2013, 2015, and 2016. Black bars represent the median δ^2Hw. Different letters within a year indicate a statistical difference in mean δ^2H_w ($p<0.05$).

Chapter 4

4 General Conclusion

Results comparing the differences in the δ^2H_w profiles of moths captured in the spring flight and those during summer and fall of 2016 (Chapter 2), together with data of the overwintering study showing 100% mortality by December (Appendix 1) lend support to the hypothesis that the spring populations of armyworm observed in Canada are founded by immigrants. Furthermore, the δ^2H_w profiles of the moths captured in Texas in early fall provide the first concrete support that the armyworm is a two-way seasonal migrant, as originally postulated by McNeil (1987). In addition, the data from the inter-year comparison also suggest that the source of the immigrants into the area may vary from year to year. Thus, as seen for other insects (see Hobson & Wassenaar, 1998; Hobson *et al*., 2012; Stefanescu *et al*., 2016) wing δ^2H values can be used, at least to some extent, to infer the natal origin of important pest species.

However, the intra and inter-year variability underlines the need to better understand how other factors might impact the δ^2H values obtained and how this knowledge could be integrated to better use stable isotopes to study seasonal migration. Obviously, any variability in the δ^2H values resulting from precipitation (be it rain or snow) and/or local irrigation practices at natal sites will be passed to the insects though the host plants they consume. Thus, datasets establishing the variability of these parameters at a number of sites along a north-south axis could help when assigning origins to migrants using isotopic techniques. On a broader scale, further detail must be given to potential variation between El Niño and La Niña events (Welker, 2012). Another component relates to the prevailing climatic conditions, for while they will not affect the actual isotopic values obtained they will determine the probability of moths emigrating from point A actually arriving at point B. The first step will be to use trap catch data from previous years (preferably single days with high catches) and carry out atmospheric (i.e. wind vector) backtracking using recently available modelling systems, such as HYSPLIT, to more precisely estimate trajectories (see McNeil, 1987; Westbrook, 2008). Future research should also include data on both carbon and nitrogen isotopes, as done with some non-pest migrants (Hobson & Kardynal, 2016; Still & Powell, 2010; Werner et al., 2016). As species like the armyworm are oligophagous, data on δ^{13}C and δ^{15}N in wing chitin would provide insight into both larval diet (C_3 versus C_4 plants) and whether they came from both natural and /or agroecosystems (associated with the application of artificial fertilizers): as done with the small sample of 2012 Texas individuals in Chapter 2. A large, long-running north-south network of trapping would be ideal to obtain the appropriate data on *M. unipuncta* to develop a truly integrated, large-scale model of seasonal lepidopteran migration. With increased knowledge surrounding the migration of invasive and economically important insect pest species such as *M. unipuncta*, early warning systems, and more temporally and spatially focused management procedures can be developed.

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Appendix A

A-1. Investigating the overwintering capacity of the true army worm (*Mythimna unipuncta*) in Ontario

Fields & McNeil (1984) clearly demonstrated that the armyworm did not survive the winter conditions in Quebec, leading McNeil (1987) to classify this species as a seasonal migrant, overwintering in the southern United States but migrating northward in spring to avoid hot summers. However, given that recent winters have been less severe, it was possible that as a result of climate change the armyworm could now overwinter here. Therefore, I repeated the Fields and McNeil (1984) field experiment to reevaluate *M. unipuncta's* overwintering capacity in Ontario. Furthermore, in the laboratory I determined the supercooling points (SCP) of different developmental stages when insects were reared under insectary conditions on both artificial and natural diet, again using the same techniques as Fields and McNeil (1984). In addition, I determined the SCP of insects from the non-migratory population found in the Azores. The complete data will be written up and submitted to an international journal in the next months.

It is clear from both field and laboratory experiments that, (i) the armyworm cannot overwinter in Ontario (Table. A-1) and the SCPs obtained for the 2015 Ontario population (Figure A-1) were not significantly different than those previously reported by Fields & McNeil (1984). Furthermore, neither overwintering survival or SCPs were affected by diet or rearing conditions (Fig. A-1), and SCPs do not vary between migrant and non-migrant populations (Table. A-2). These data support the idea that the true armyworm is an insect with no real capacity to survive cold conditions and migrates in response to seasonal habitat deterioration.

Methods

Field trials to determine overwintering survival

To evaluate the overwintering capacity of *P. unipuncta*, I used the same protocol as Fields & McNeil (1984). I placed 40 cylindrical cages (25 cm diameter, 60 cm height) at randomly selected locations in a timothy grass/ alfalfa plot at Environmental Sciences Western. Each cage was sunk 20 cm into the ground, the sod replaced and left for several months to allow the earth to settle and the sod to reestablish as the timothy grass served as food for armyworm larvae. I reared four different cohorts of larvae on field collected corn/ timothy grass under natural conditions in an insectary, the first starting August 10th and the last starting on September 20th. Each week, from September 9th until October 5th, I placed 5-20 3rd, 4th, 5th and 6th instar larvae, as well as prepupae, in different cages. The number per cage varied and was based on larval size (estimated by head-capsule width; Breeland (1958), to ensure that the food source available was sufficient to support the population. Twelve cages were excavated at 3 time intervals through the winter to determine the number of living and dead individuals, their developmental state and the depth at which they were recovered from in the soil.

Laboratory studies of super cooling points

I measured supercooling points (SCP) of 15 3rd, 4th, 5th and 6th instars, prepupae and pupae from a single cohort. For feeding larval instars, I used newly moulted individuals to ensure that gut contents were voided as this prevented ice-nucleators from affecting the results (Fields & McNeil, 1984). Larvae were placed into individual 1.5mL Eppendorf tubes, restrained at the bottom by cotton, in contact with a (36 AWG) type- T thermocouple (copper‑constantan, Omega, Laval, QC, Canada) connected to a computer with a TC-08 thermocouple interface (Pico Technology, Cambridge, UK). I recorded the temperature of each sample every 0.5s using Picolog software (v 5.24.2 Picotech) (Anthony *et al.*, 2015). The cooling bath was a 24-well Lauda Proline RP855 circulator (Lauda, Wurzburg, Germany). Individual samples were cooled to -22 °C at a rate of 0.1 °C/min (Lee, 2010). Super cooling points (SCP), the lowest internal body temperature before internal ice formation (Lee et al, 1987), were recorded as the temperature at the exotherm resulting from the heat of crystallization; these temperatures were determined using output plots from Picolog software.

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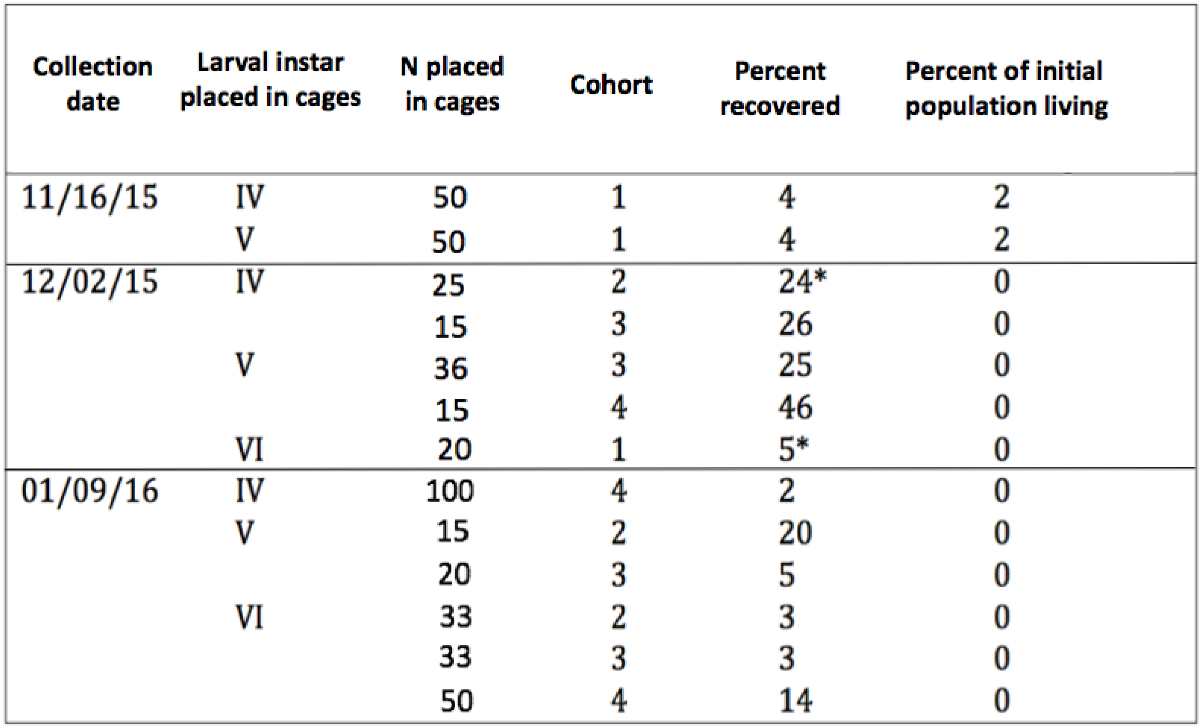
Table. A-1. Results of 2015 field experiment on the overwintering of *M. unipuncta* in Ontario. An asterisk indicates that an adult moth was found.



Collection date	Larval instar placed in cages	N placed in cages	Cohort	Percent recovered	Percent of initial population living
11/16/15	IV	50	1	4	2
	V	50	1	4	2
12/02/15	IV	25	2	24*	0
		15	3	26	0
	V	36	3	25	0
		15	4	46	0
	VI	20	1	5*	0
01/09/16	IV	100	4	2	0
	V	15	2	20	0
		20	3	5	0
	VI	33	2	3	0
		33	3	3	0
		50	4	14	0

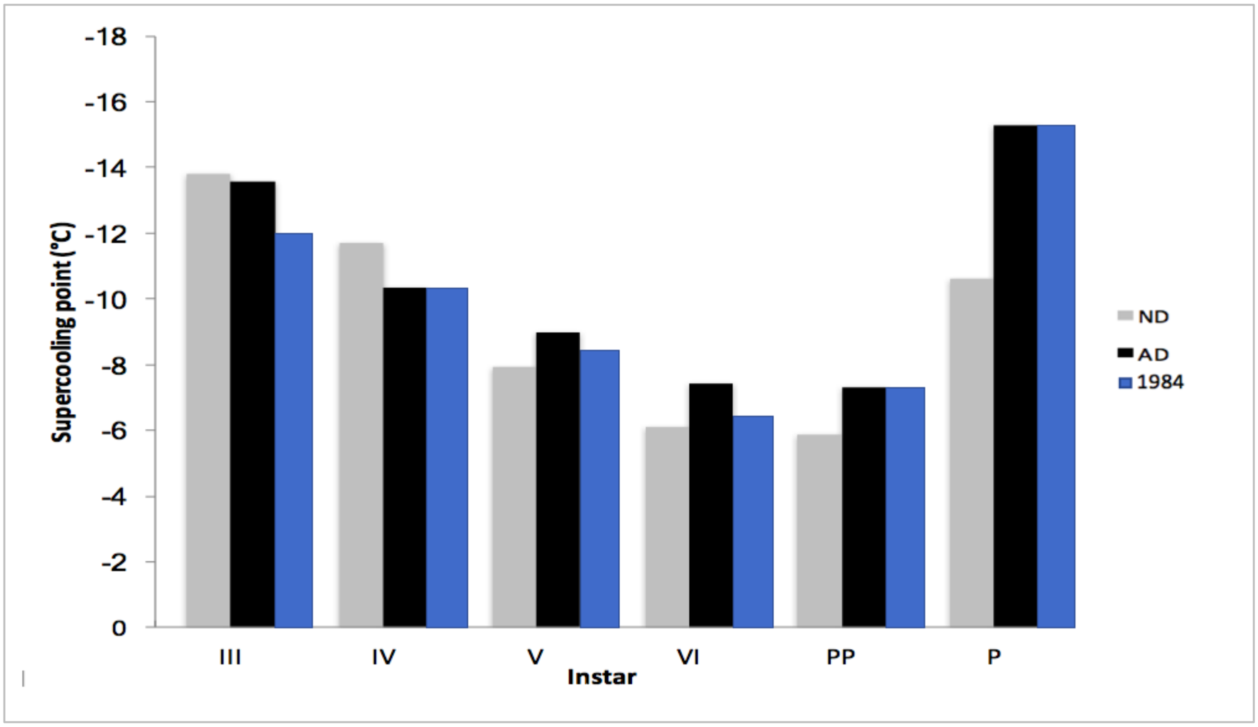


Fig. A-1. Supercooling points of *M. unipuncta,* for all larval stages including pupae fed natural diet (ND) or artificial diet (AD) compared to data collected by Fields and McNeil (1984).

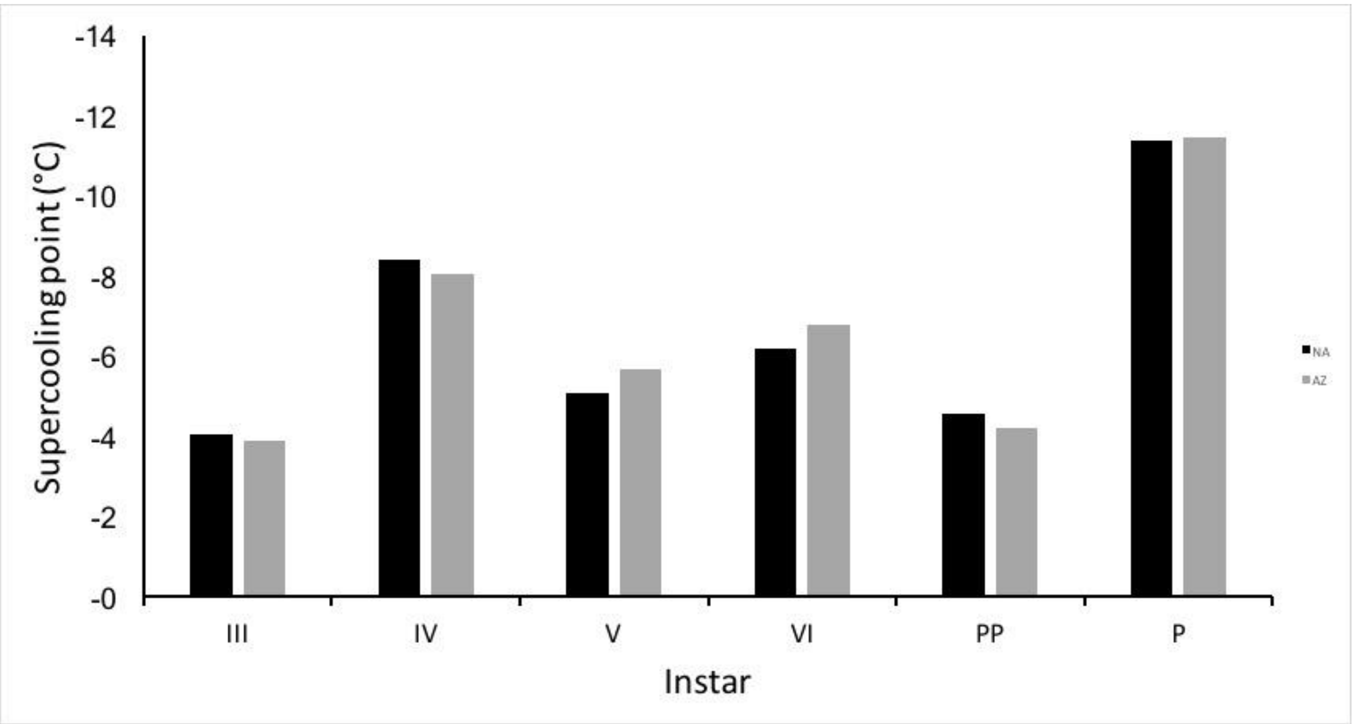


Fig. A-2. Supercooling points of *M. unipuncta* for all larval stages from third to pupae of North American (black) and Azores (grey) populations.

Appendix B

Publisher permission: Chapter 2- Inferring origins of migrating insects using isoscapes: A case study using the true armyworm, *Mythimna unipuncta*, in North America

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Profile

Driven and enthusiastic about learning, and applying my skills to real-world problems. Very independent and able to complete tasks with minimal supervision. Able to apply learned techniques and to ask questions to expand my understanding of concepts. Highly dependable and eager to work with plants and to apply my experience in entomology to my work.

Education

Bachelor of science (B.Sc.), Specialization Biology (2015), Western University, London, ON.

Current M.Sc. candidate under the supervision of Dr. Jeremy N. McNeil, and Dr. Keith A. Hobson (Completion date set for May, 2018)

Experience/skills

- comfortable with plant-maintenance practices within a greenhouse, crop, or natural setting
- comfortable with corresponding with agriculturalists for research
- competent in a field, laboratory or classroom setting under little or no supervision
- proficient in rearing/ maintenance (problem solving) of study species of plants, insects and arachnids
- able to develop and manage projects, and to collect appropriate data

Current work and Research experience

- Horticultural Technician, Western Greenhouses
    - responsible for plant care (watering, fertilizing, pruning, resituating to appropriate temperature/ light conditions), identification, and routine greenhouse maintenance, as well as greenhouse equipment maintenance when required (often requiring heavy lifting/ carrying)
    - responsible for insect pest identification and control by various means (trapping, chemical control, physical removal, or biocontrol).
    - involved in planting, maintaining (according to specified conditions) and delivering plant specimens for use by more than 7 courses in the department of Biology

- Responsible for maintenance of plants at Environmental Sciences Western (sweet corn, field corn, pinto beans, timothy grass) to be used for ongoing experiments.

- I conducted a combined field and laboratory study to reexamine the overwintering capacity of the true armyworm in Ontario. This study confirmed that even in light of recent climate change the true armyworm still is unable to overwinter in Canada, as reported previously by Fields and McNeil (1984). This work is an appendix in my thesis and will be submitted for publication this fall.

- For my thesis, I have been using the stable isotope, deuterium, to examine the seasonal migration of the ecology of the true armyworm (*Psuedaletia unipuncta*). The first part (Chapter 2) is the "proof of principle" paper using the insects captured during 2016, supplemented by controlled laboratory measurements on insects reared on vegetation receiving different levels of deuterium. This paper has been accepted for publication in *Ecological Entomology*

- The third chapter of my thesis looks at both the intra and inter year variability in the deuterium levels of armyworm in Ontario, using specimens from 2008, 2012, 2015 and 2016. There are clear inter year differences in the values for both immigrants (spring) and residents/emigrants (summer/fall): there are several possible explanations that will need to be investigated further.

- I am working in collaboration with my co-supervisor, Prof. Hobson, using isotopes to conduct laboratory and field experiments to study the migration of the Monarch butterfly, *Danaus plexippus*. We are interested to see if the isotope profiles in adult fat body (especially carbon obtains as nectar for host plants) could provide further insight into the migratory behavior of this species.
    - planted several native/ non-native late-season flowering species of plants within a large aviary to provide butterflies with nectar sources under natural fall conditions

- I observed that females of the Bold jumping spider (*Phidippus audax*) frequently set up "tents" in the pheromone traps we are using to monitor the Brown Marmorated Stink Bug, but only in the fall when the bug would normally be establishing overwintering aggregations. I conducted Y tube studies and clearly showed that females respond to the aggregation pheromone, and postulate that females will exploit the stink bugs as a food source during the aggregation period from September to May. I am conducting additional field trials this summer and will conduct a number of additional Y tube assays this fall, before submitting the paper for publication

Previous Work Experience

- Maintenance of plants (corn, beans and mixed grains) in Western greenhouses as well as at Environmental Sciences Western as part of a summer job with Dr. J. McNeil

- Responsible for care of spiders, (2014-2015), Sinclair Lab, Western University

- Conducted a study to test the "Beneficial Acclimation Hypothesis" in *Latrodectus hesperus* (Western black widow). Sinclair Lab, Western University

- Worked in the field with Dr. Leland Humble collecting sawfly larvae and identifying possible new species. (2011), Pacific Forestry Centre, Victoria B.C.

Teaching Experience

- 2nd year Research methods laboratory (fall/ winter & summer sessions)
  - Responsible for instruction and supervision of student laboratory experiments, as well as the evaluation of student laboratory performance and notebook submissions

- 1st year biology laboratory (fall/ winter & summer sessions)
  - Instructed and supervised, and marked laboratory experiments

- 1st year biology skill development sessions (fall/winter session)
  - Independently instructed and evaluated 200 students
    - Focus was on building academic skills, as well as presentation of supplementary course material

- 1st year biology summer course
  - Responsible for facilitating the presentation of in-class lectures to an online audience by live video streaming/ recording, clarifying material during lecture and addressing questions or concerns students may have.

Presentations of my research at Scientific meetings

- Poster presentations*:*
  - 2016 Ontario Society of Entomologists annual meeting in Sault St. Marie, ON
  - 2016 Fallona Family Interdisciplinary Showcase at Western University
  - 2016 Annual meeting for the Entomological Society of Toronto at U of T.
  - 2017 Annual meeting for the Entomological Society of Canada, Winnipeg

Oral presentations:

- 2017 Insect Neuropeptide Conference. Argentina (presented by J. McNeil)

-2017 Entomological Society of Canada. Winnipeg (presented by J. McNeil)
-2017 Entomological Society of America. Denver (presented by J. McNeil)

Awards

- 2016 Winner of the Ontario Society of Entomologists President’s Poster Award for my research on *P. unipuncta* (Sponsored by Algoma University)

Outreach

- 2015, 2016, 2017 Entomological Society of Ontario London Bug Day
- 2015, 2016, 2017 BOOSEUM:
    - A family-oriented, Halloween-themed event at the Children’s Museum London representing the Entomological Society of Ontario by providing, showcasing and presenting information on a variety on arachnids and insects