

WINTER MANAGEMENT OPTIONS FOR THE ORCHARD POLLINATOR *OSMIA LIGNARIA* SAY (HYMENOPTERA: MEGACHILIDAE) IN NOVA SCOTIA

C. S. SHEFFIELD¹, S. M. WESTBY², P. G. KEVAN³, R. F. SMITH²

Department of Biology, York University

4700 Keele St., Toronto, Ontario, Canada M3J 1P3

email: corys@yorku.ca

Abstract

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The Blue Orchard Bee, *Osmia lignaria* Say, a commercially available solitary bee, was recently introduced into the Annapolis Valley of Nova Scotia for assessment as a manageable pollinator of apple. An important component of this assessment was to investigate options for wintering this species as few apple growers have storage facilities suitable for the recommended wintering practices. Specifically, winter survival under ambient outside, albeit sheltered conditions was compared to wintering bees in controlled environmental chambers at 4°C. In addition, a comparison of survival was made between bees from two populations; one introduced into Nova Scotia the previous year and reared for a complete generation versus one reared in Utah and imported to Nova Scotia as dormant un-emerged adults.

Populations wintered outside fared slightly but significantly better than those wintered inside, but each location proved suitable for wintering. Bees from both populations had high survival (as measured by emergence from natal cocoons), but rates were significantly greater in bees reared in Nova Scotia for one year. Overall, bees from both populations proved suitably cold hardy for wintering in Nova Scotia, as evidenced by both high rates of survival and by enhanced supercooling capacity. Wintering bees in controlled conditions offers the advantages of predictable climate, and controlled synchrony of emergence with crop flowering. However, access to and/or lack of climate controlled facilities may be a limiting factor in adopting this species for commercial use in the province. This study thus provides evidence that bees wintered outside have natural emergence coinciding favourably with apple flowering, and that placing nests in sheltered outdoor environments in Nova Scotia provides an affordable and safe approach for wintering *O. lignaria* populations for apple pollination.

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¹ Author to whom all correspondence should be addressed.

² Agriculture and Agri-Food Canada, 32 Main St., Kentville, NS, B4N 1J5

³ Department of Environmental Biology, University of Guelph, Guelph, ON, N1G 2W1

Introduction

The last few decades have witnessed many changes in the management of bees (Hymenoptera: Apoidea) for the pollination of crops in North America. One of these changes has involved a small but significant shift from reliance on one species, the ubiquitous honey bee, *Apis mellifera* L., for most of our entomophilous pollination needs. The predictions and warnings of potential problems likely to arise due to almost total reliance on honey bees for crop pollination have been realized as evidenced by the large losses in the number of colonies available for pollination (Kevan 2001; DeGrandi-Hoffman 2003) resulting in recent native pollinator conservation initiatives and literature (Matheson et al. 1996; Stubbs and Drummond 2001; Strickler and Cane 2003; Committee on the Status of Pollinators in North America 2007). In response, interest in the development of non-*Apis* bees as potential crop pollinators has increased, and several potentially useful species have been investigated (Torchio 1990b, 2003; Bosch and Kemp 1999, 2000; Cane 2005).

Some of the earliest research on non-*Apis* bees and their subsequent development as crop pollinators started in the 1940's in the USA (Torchio 1990a). In addition to the need to learn about basic bee biology and diversity, this early research was initiated because of the realization that honey bees are poor pollinators of some crops and that several indigenous species were more efficient crop pollinators (Bohart 1972). More recently, replacement crop pollinators have been sought to fill gaps left by the increased demands on an ever decreasing number of honey bee colonies, and these efforts have resulted in a growing number of non-*Apis* bees being developed for management in North America (Parker et al. 1987; Torchio 1990a; Richards 1993; Strickler and Cane 2003). However, the number of bees currently managed does not approach 1% of the estimated 3000-4000 species in America north of Mexico (Krombein et al. 1979).

The main differences between managing honey bees and all other bee species currently used for crop pollination in North America stem from differences in social complexity and differing life histories. No other species of bee exhibits levels of social behaviour as complex as that observed in the genus *Apis* (Michener 1974, 2007). In America north of Mexico, all indigenous bee species have a solitary stage in their life history, making it the world's largest land area with no social species wintering as a colony. As such, most temperate zone bee species have dormant periods corresponding to the winter season (Stephen et al. 1969).

Despite the fact that most bee species spend the largest proportion of their life cycle in a non-reproductive/non-feeding stage often overlapping the winter months, little is known about the behavioural and eco-physiological adaptations of bees leading up to and during the winter in temperate zones (Sakagami et al. 1981; Hoshikawa et al. 1992; Rust 1995). For insects in general, properties of the wintering hibernacula such as moisture levels, ground cover, nesting depth, the ground's slope and sun exposure levels are often as important as the physiological and behavioural adaptations of the insect (i.e., cryoprotectant synthesis, supercooling) for enhancing survival (Danks 1978, 1991; Leather et al. 1993). Therefore, such factors are important and must be considered when developing management techniques for solitary pollinators, as evidenced in the past by the works of Krunic and Hinks (1972), Fairey et al. (1987), and more recently by Wilson and Abel (1996), Bosch and Kemp (2000, 2003, 2004), Bosch et al. (2000), Kemp and Bosch (2005),

and Kemp et al. (2004).

Cavity-nesting megachilid bees (Megachilidae) are among the forerunners for management for crop pollination (Sheffield et al. 2008) and for these species, wintering in controlled climate conditions is recommended (Fairey et al. 1987; Richards et al. 1987; Bosch and Kemp 2001, 2003, 2004). However, in Nova Scotia and elsewhere in North America, most growers do not own or have access to controlled cold storage facilities. Therefore, finding alternative methods to safely overwinter *Osmia lignaria* Say for orchard pollination is a priority for managing this species. Considering that *O. lignaria* is indigenous throughout temperate North America (Krombein et al. 1979) though not confirmed in Nova Scotia (Sheffield et al. 2003, 2008), and that its flight period naturally corresponds with flowering of many rosaceous tree fruits, it seems a reasonable assumption that this species can overwinter successfully under ambient conditions within apple producing regions. From 2000-2004, populations of *O. lignaria* were imported into Nova Scotia for evaluation as a replacement pollinator for apple, and to develop region specific management strategies. The objective of this study was to investigate *O. lignaria* winter storage options available to Nova Scotia apple growers or groups interested in rearing this species for commercial tree fruit pollination. Specifically, comparisons in survival and winter physiology were made between *O. lignaria* from two populations wintered outside in a sheltered environment versus in a traditional cold storage facility in the winter of 2001-2002. One population consisted of the offspring of bees imported into Nova Scotia in the fall of 2000 and released for pollination trials in 2001; the second was offspring of a population imported from Utah following the active season of 2001.

Materials and Methods

Nest Preparation

Following the active adult season (late May until early July 2001), nesting blocks containing developing larvae of *O. lignaria* were placed in an unheated screened building (insectary) maintained at the Atlantic Food and Horticulture Research Centre, Kentville, Nova Scotia, to allow development to continue through to the adult stage (i.e., the wintering stage) within the natal cocoons. In early autumn, samples of randomly selected nesting tubes were removed from nesting blocks and split lengthwise to expose cocoons and determine the number and sex of bees; caution was used to ensure the tunnel end plug was left intact. Sex was determined based on cocoon size and position within the nesting tube. Each cocoon was removed in sequence from the nesting tube and brushed lightly to remove faecal particles and other debris. Undamaged cocoons were selected and weighed to 0.001g, and placed into nesting tubes with the same orientation; females positioned in the rear. Nesting tubes were then sealed longitudinally with masking tape. The same procedure was repeated on a portion of dormant bees received from Utah in the autumn of 2001, which were already split upon arrival as part of quality assessment by the supplier (Torchio Enterprises, North Logan, UT). Pre-winter weights of male and female bees from both locations were compared using Analysis of Variance (ANOVA).

Samples from the two locations were divided into 24 groups (twelve each from Nova Scotia and Utah), each group having at least 20 females among the nesting tubes. The

nesting tubes of each group were then placed into a wooden laminate nesting block with 81 tunnels (arranged in a 9 x 9 pattern), each tunnel approximately 0.85 cm in diameter and 15 cm deep (i.e., able to house the nest liners) (Fig. 1a). The nesting tubes were randomly selected and placed into the inner 49 nesting tunnels (i.e., a 7 x 7 pattern) of each nesting block until all were accounted for.

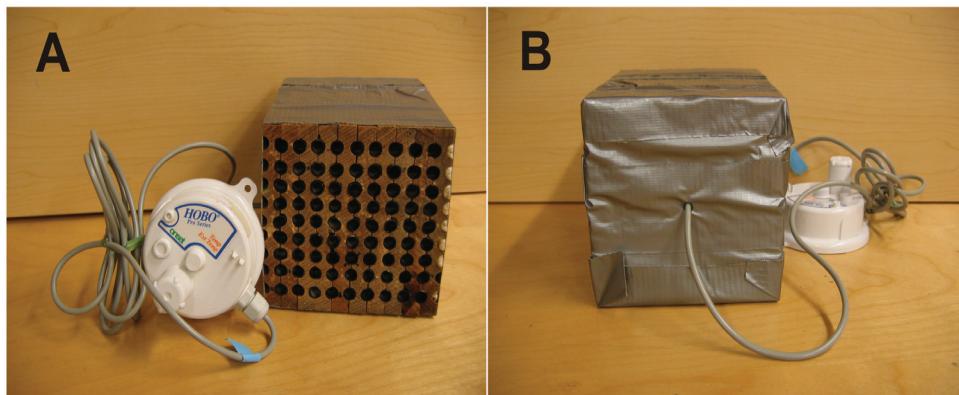


FIGURE 1. A) Nest used for *Osmia lignaria* overwintering studies, showing B) data logger probe position.

Winter Survival

The nesting blocks were randomly assigned to the two wintering conditions, resulting in six blocks from each population in each wintering site. Data loggers (Onset; HOBO7 H8 Pro Series model H08-031-08 and HOBO7 H8 4-Channel model H08-006-04) with external probes were used to record ambient temperature (°C) at each of the wintering sites (six channels per site) and within the nesting tubes (six channels per site, with three allocated to the Nova Scotia and Utah populations) (Fig. 1b).

In the spring of 2002, bees wintered inside were slowly warmed to 25°C, and emerged bees from each block were collected. Following the emergence period, nesting tubes were examined for dead un-emerged bees (i.e., adult bees which were still inside their cocoons and presumably died during wintering). Bees that had chewed out of their cocoons and died due to nesting tube blockage were included in the analysis as “live bees” as it was apparent they survived the winter. The number of cocoons containing dead larvae or pupae were subtracted from the starting total as death would have occurred prior to winter (these made up a very low proportion (<1%) of the population).

Bees wintered outside were allowed to emerge naturally to determine emergence phenology of this species in Nova Scotia. Bees emerging daily from each nesting block were collected with numbers and sex recorded. Following the emergence period, nests were examined for un-emerged adult bees as above.

This experiment was set up and analysed as a multi-factorial design; the three fixed factors of interest were 1) wintering site (inside at 4°C, 50-70% RH versus outside ambient

conditions in an unheated screened building), 2) location of origin (Nova Scotia population versus Utah population), and 3) sex. In addition, interactions between each factor and among all three factors were studied. The proportion of bees surviving for each nesting block was subject to arcsine transformation (Zar 1999) to achieve normality and homoscedacity of variance, which was successful. All analyses were therefore conducted in the transformed form, although the reported population parameters are of non-transformed data.

Supercooling Point Determination and Weight Loss

At two intervals throughout the winter (January and March), sub-samples of cocoons were removed from the nesting blocks. These were re-weighed to determine percent weight loss within the interval of wintering. Bees were then removed from their cocoons, placed into a gelatin capsule, and were attached with petroleum jelly to a thermocouple within a Nalgene™ 2.0 ml cryovial. The cryovial was then submerged in coolant in an FTS-Systems ultra-low temperature bath (Stone Ridge, NY) interfaced with a computer using virtual instrumentation (VI) Lab View 5 (National Instruments, Austin, TX) software and hardware (Nubuss port and A/D boards). From an initial bath temperature between 10°C and 15°C, the temperature was dropped at a rate of 1°C /min (after Salt 1966), and supercooling points were measured to the nearest 0.1°C; the supercooling point is the lowest body temperature recorded prior to the increase in temperature due to the latent heat released during crystallization (Lee 1991).

Nonparametric analysis (based on ranked data) was used on both supercooling point and weight loss data sets as they were not normally distributed and variances were unequal. For non-parametric analysis of supercooling points, each datum was transformed to a positive value, T, using the following formula $T = (a^\circ\text{C})^{-1}$, where a represents the supercooling point datum (which is always a negative number). This transformation was done as more negative supercooling points are indicative of enhanced cold-hardiness, and it allowed the most negative values to have the highest rank instead of the lowest.

Results

Overwintering Survival

Populations overwintered outside had significantly higher mean survival rates than those kept inside ($F = 16.59$; $df = 1$; $p < 0.001$), and bees reared in Nova Scotia had significantly higher mean survival rates than populations imported from Utah ($F = 29.78$; $df = 1$; $p < 0.001$) (Fig. 2). Significant interactions were observed between origin x wintering site ($F = 5.28$; $df = 1$; $p = 0.027$), and the main effects plot (Fig. 2) indicated that origin had a larger main effect than the wintering site (indicated by the slightly steeper slope). Sex did not significantly influence wintering survival ($F = 0.04$; $df = 1$; $p = 0.851$) or the two-way interactions of 'origin x sex' and 'wintering site x sex', although these did approach significance ($F = 3.15$; $df = 1$; $p = 0.083$ and $F = 3.22$; $df = 1$; $p = 0.080$, respectively). The three-way interaction of 'wintering site x origin x sex' was not significant ($F = 0.28$; $df = 1$; $p = 0.60$).

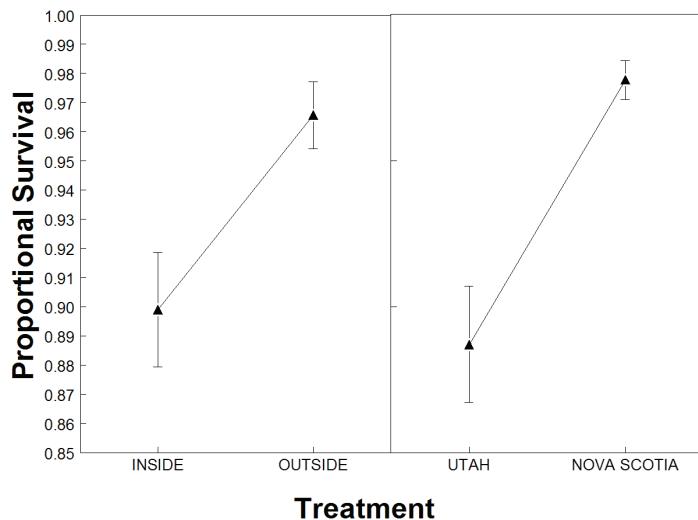


FIGURE 2. Main effects plot of mean proportional survival (\pm s.e.) for *Osmia lignaria* wintered inside versus outside (left graph), and from Utah versus Nova Scotia (right graph).

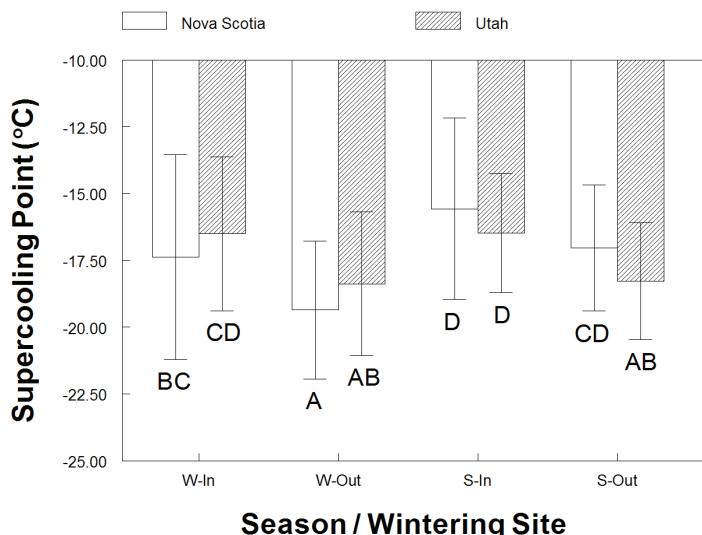


FIGURE 3. Mean supercooling points ($^{\circ}$ C \pm s.e.) of Nova Scotia and Utah populations of *Osmia lignaria* wintered in environmental chambers (IN) and outside in a screened building (OUT) in the winter (W) and late winter/early spring (S). Bars sharing letters are not significantly different (ANOVA of ranked data with Tukey's HSD test; $p = 0.05$).

Supercooling

No significant differences in supercooling points were observed between sexes within any of the treatment conditions, so sex-data within treatments were pooled. Significant differences were found among the treatments ($F = 15.62$; $df = 7$; $p < 0.001$), so Tukey's HSD test was used to separate means of the ranked data (Fig. 3). Early in the winter (i.e., January), bees wintered outside (W-Out) from both locations of origin had significantly lower supercooling points than their respective counter parts wintered inside (W-In) (Fig. 3). At this time, bees from Nova Scotia-Outside had slightly lower supercooling points (mean = -19.4°C; $n = 71$; range = -10.1°C – -23.1°C) than those from Utah-Outside (mean = -18.4°C; $n = 45$; range = -9.5°C – -22.7°C), but the differences were not significant. The same trend was observed for bees wintered inside in the early winter: Nova Scotia (mean = -17.4°C; $n = 52$; range = -6.8°C – -22.3°C); Utah (mean = -16.5°C; $n = 47$; range = -7.4°C – -20.9°C).

In the spring (i.e., March) outside (S-Out) treatment, bees from Utah (mean = -18.3°C; $n = 43$; range = -8.3°C – -21.2°C) still had significantly lower supercooling points than those inside (S-In) (mean = -16.5°C; $n = 52$; range = -10.7°C – -20.2°C), but no differences were observed between the supercooling points of winter and spring bees in the respective wintering site (Fig. 3). In the spring, the supercooling points of Nova Scotia bees kept outside (mean = -17.0°C; $n = 73$; range = -9.6°C – -21.2°C) were lower but did not differ significantly from those inside (mean = -15.6°C; $n = 54$; range = -5.3°C – -19.3°C). Unlike the Utah population, bees from Nova Scotia (inside and outside) had significantly reduced cold-hardiness in spring versus the winter (Fig. 3). Only within the S-Out treatment did bees from both populations differ significantly.

Weight Loss

Significant differences were observed between the weights of bees from both populations ($F = 1011.4$; $df = 3$; $p < 0.001$), so means were separated using Tukey's HSD test ($p = 0.05$). The pre-winter weights of bees from Utah were significantly greater than bees reared in Nova Scotia (Fig. 4). Males from both populations were significantly lighter than their respective females, but Nova Scotia females were significantly heavier than Utah males (Fig. 4).

Although significant differences were observed among all treatments ($F = 4.55$; $df = 15$; $p < 0.001$), no significant differences in weight loss were observed among females and males, or between wintering sites for bees reared in Nova Scotia (Figs. 5a and b). Weight loss ranged between 1.2% - 2.1% total body wt in early winter, 1.8% - 2.6% by the spring. Similar trends were observed in bees from Utah. Weight loss in the winter ranged from 0.9% - 1.7%, overlapping the results from Nova Scotia bees. By the spring bees had lost between 2.1% - 3.3% body weight (Figs. 5c and d). Utah females wintered inside lost more weight by the spring than any other group (Tukey's HSD test; $p = 0.05$). In both populations, early winter weight loss was slightly greater (though not significantly so) in bees wintered outside than inside, while the reverse was observed in the spring (Fig. 5).

Wintering Temperatures

Temperatures inside the environmental chamber fluctuated slightly between 4°C and 6°C throughout the experiment (Dec 2001 - May 2002) with a few notable exceptions

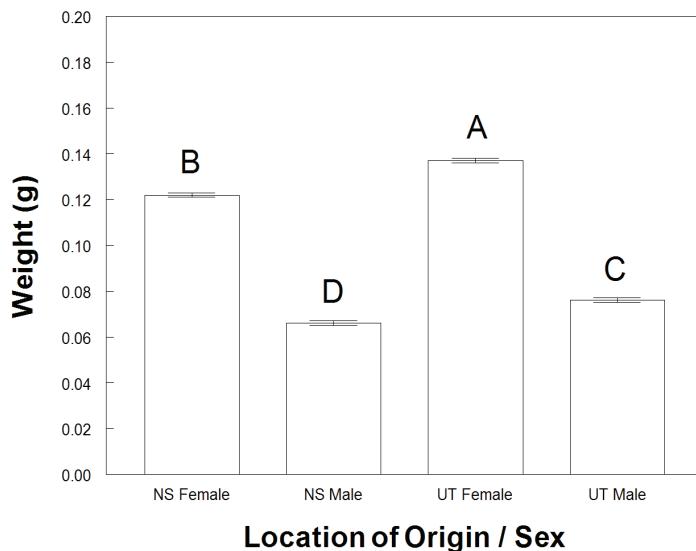


FIGURE 4. Mean pre-winter weights ($\text{g} \pm \text{s.e.}$) of female and male *Osmia lignaria* from Nova Scotia and Utah populations. Bars sharing letters are not significantly different (Tukey's HSD test; $p = 0.05$; $n = 250$).

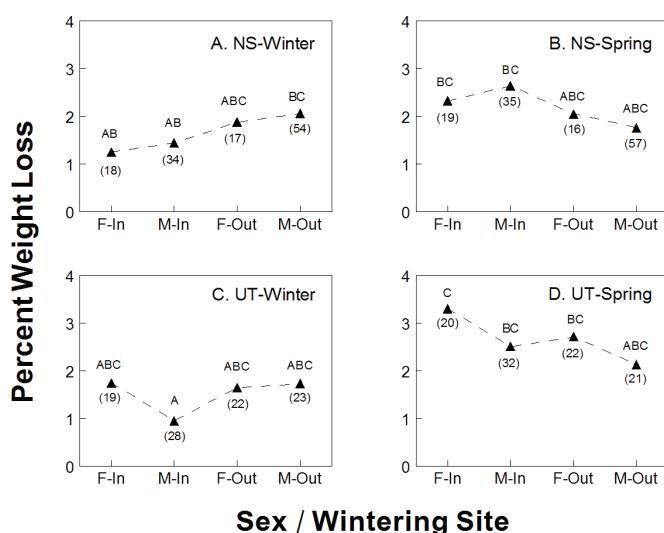


FIGURE 5. Percent weight loss during two wintering periods (A and C; Winter: Oct – early Jan; B and D; Spring: mid Jan – late Mar) in *Osmia lignaria* populations from Nova Scotia (NS) and Utah (UT) wintered in environmental chambers (In) and outside in a screened building (Out). Data markers sharing letters are not significantly different (Tukey's HSD test; $p = 0.05$); sample size in () below each data marker.

(Fig. 6): on 12 Dec 2001 the temperature ranged between -2°C and 10°C; 07 Feb 2002 the temperature ranged between 0°C and 11°C; 16 Mar 2002 the temperature ranged between -3°C and 8°C. The corresponding temperatures recorded inside the nesting tubes had a range falling within those observed within the chamber, but followed the same trend.

Temperatures within the screened insectary (outside) showed large fluctuations between night (daily minimums) and day (daily maximums); on several instances throughout the winter a range of 20°C was observed within a 24 h period (Fig. 6). The lowest temperature recorded was below -20°C, observed on 12 Feb 2002. More than half the days in Jan-Feb 2002 stayed below 0°C; Dec 2001 and Mar 2002 had most daytime temperatures above 0°C and night time temperatures below 0°C. In Apr and May 2002, temperatures seldom fell below freezing.

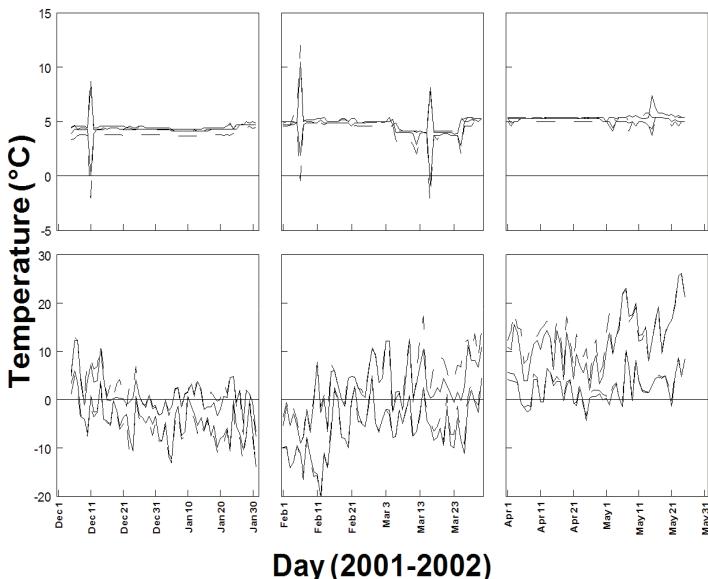


FIGURE 6. Maximum and minimum ambient air (solid lines) and nesting tunnel temperatures (dashed lines) within an environmental cabinet maintained at 4°C (top row) and within an unheated screened insectary (bottom row) from Dec 1, 2001 to May 31, 2002.

Emergence and Bloom Phenology

Bees from both populations had similar emergence patterns, although the sex ratio of bees from Nova Scotia was highly male biased (Fig. 7a). Male emergence began approximately 01 May 2002, with most individuals emerged within one week. Female emergence began approximately one week later than males, its start corresponding to the peak of male emergence. Female emergence peaked approximately ten days prior to commencement of the bloom period of McIntosh apples (Fig. 7).

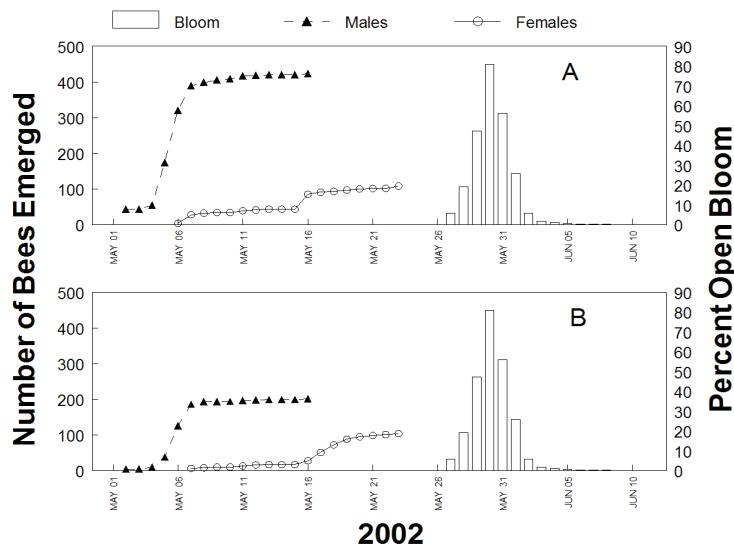


FIGURE 7. Natural emergence of male and female *Osmia lignaria* from A) Nova Scotia and B) Utah wintered in an unheated screened insectary, and the flowering phenology (percent open bloom) of McIntosh apples in 2002.

Discussion

Wintering success in insects is a complex symphony of internal physiological adaptations and behaviour modifications prior to, during and following the winter. In addition to these organism-level considerations, many ecological factors play significant roles in dictating insect survival (Danks 1978, 1991; Leather et al. 1993). Surviving the winter is therefore tied to several aspects of an insect's life history, and the most complete understanding of its ability to overwinter successfully comes from taking all of these aspects into consideration, as they do not necessarily work in isolation of one another (Danks 1978; Leather et al. 1993). In addition, understanding the complexity of wintering mechanisms which act on insect populations is critical to developing strategies for management which involve, by necessity, a wintering period.

Considering this, several factors may have contributed to the slight but significantly higher survival of bees reared in Nova Scotia than those from Utah. The first and possibly most important involved the comparative total length of the adult dormant period, in particular the pre-wintering period. In Utah, *O. lignaria* is released for apple pollination from late-April to mid-May, and adult activity usually ceases by mid-June (Torchio 1985; Bosch and Kemp 2000, 2001). However, in Nova Scotia bees are released approximately one month later as apple bloom typically occurs from late-May to mid-June (Fig. 7). In terms of development, populations reared under ambient conditions in Utah reach the adult wintering stage by August/September (Rust 1995; Bosch and Kemp 2000, 2001) which

again is earlier than in Nova Scotia. The bees from Utah were not imported into Nova Scotia until late-October/November, as bees had to be at least in the prepupal/pupal stages prior to shipment, and due to delays with importing procedures. The result was a much extended pre-winter storage period for this population compared to the one reared in Nova Scotia.

Bosch and Kemp (2003, 2004) report significant weight loss and mortality when pre-wintering duration is extensive for *Osmia lignaria* and the closely related *O. cornuta* (Latreille), and suggest an optimum range of between 10 and 30 days following adulthood for both species under commercial storage conditions. The pre-wintering period is apparently a critical one for fat body depletion, and placing bees within cold storage within the suggested time frame greatly reduces the rate of loss in *O. lignaria* (Bosch et al. 2000; Bosch and Kemp 2001, 2004).

The extended pre-wintering period also lengthened the overall wintering period for this population. Kemp et al. (2004) reported that after approximately three months of wintering under controlled conditions, *O. lignaria* enters a postdiapause transitional period, indicated in that study by increased oxygen consumption rates. Bosch and Kemp (2001, 2003, 2004) provide detailed accounts of several aspects of winter storage for *O. lignaria* and *O. cornuta*, and for the later species, maximum survival and longevity were found in populations wintered for 90-150 days (Bosch and Kemp 2004). In general, excessive prolonged storage (and/or excessive warm winter temperatures) causes additional depletion of the winter reserves (i.e., fat body) beyond a critical level, as the bees enter the postdiapause transitional period (Kemp et al. 2004). For longer storage periods, colder temperatures (i.e., 0°C) are favourable to even slightly warmer ones (i.e., 4°C) (Bosch and Kemp 2003). The depletion of these resources during the postdiapause transitional period occurs at a faster rate than during the winter, and bees held longer than one month beyond the recommended duration show increased mortality (Bosch and Kemp 2001) (bees may even become active while in cold storage at 4°C during the post diapause transitional period – some male *O. lignaria* have been observed chewing and emerging when they are held beyond the typical release time). Thus, winter survival is influenced mainly by fat body depletion via increased metabolism which can be assessed by measuring respiration rates and weight loss. A similar effect from prolonged storage has been reported in another solitary commercial pollinator, the alfalfa leafcutter bee, *Megachile rotundata* (Richards et al. 1987), although bees of the genus *Megachile* overwinter as prepupae.

Despite the extended pre-wintering/wintering period of the Utah population, survival was higher than expected (>85%) based on findings reported by Bosch and Kemp (2004), but those authors report a similar rate of mortality (16.6%) for bees held at 4°C for 210 days (Bosch and Kemp 2003). The slight discrepancy between the findings of that study (Bosch and Kemp 2004) and the present one is probably due to the standard by which survival was measured. Bosch and Kemp (2004) recognized that survival is more than just getting through the winter, and thus measured longevity of emerged bees which gives some indication of the bees ability to fly, feed and mate following the winter. In the present study, bees were considered survivors if they actually emerged, or if inspection of nesting tubes revealed bees which had chewed out of the natal cocoons even if they never emerged from the nesting tube (mainly due to obstruction).

Body size has been also indicated as a factor affecting wintering success in

O. lignaria. Tepedino and Torchio (1982) found that smaller than average offspring of both sexes of *O. lignaria* have a greater likelihood of dying during the winter, and that mortality in females (the larger sex) is higher than in comparable weighted males. In this study, variability was observed within the weight range of both sexes (Fig. 4). In a study comparing winter mortality of two distinct populations of *O. lignaria*, Rust (1995) found that mortality was significantly higher in bees from Reno, Nevada than those from Logan, Utah when both populations were wintered in the later, more northern location. The differences in mortality in that study (Rust 1995) were almost entirely attributed to the differences in winter temperatures (both average monthly temperatures and extreme minima) normally observed between the two locations. Obviously, winter temperatures are a major factor limiting the geographic ranges of many species of organisms, and local populations of a given species may show some localized differences in cold-hardiness. However, another factor might have been body size (i.e., weight) differences between males and females from both populations as per Tepedino and Torchio (1982). The significantly lighter bees (based on the reported emergence weights) from Nevada had significantly higher winter mortality than those from Utah, but in that study bees from Nevada were trap nested in tubes of 5, 6, 7, 8, and 9 mm diameter versus the uniform 7 mm tubes used in Utah (Rust 1995). Bee body size is limited in the smaller sized tubes (Tepedino and Torchio 1989), a characteristic of cavity-nesting species (Roulston and Cane 2000), and this may have contributed significantly to the mortality differences observed. Other factors such as dietary history differences (food plant type and quality) between the two populations also may have contributed to the observed body size differences, as could the time when eggs were laid; size of progeny becomes smaller as the season progresses (Tepedino and Torchio 1989). In the present study, there were significant weight differences between populations (Nova Scotia reared bees were lighter), but this did not seem to influence mortality, as the heavier Utah bees had higher mortality.

Despite the slight but significant differences in survival observed between populations, high survival was obtained under both controlled and ambient outdoor conditions (Fig. 2), even with the extended pre-wintering period in the Utah population and with minimum outside temperatures approaching the supercooling points of wintering bees. For the time being, *O. lignaria* must be imported into eastern Canada to obtain adequate numbers for apple pollination. Importation should always be done in the late summer or early autumn when bees have reached the immature non-feeding stages (i.e., prepupal and pupal), but prior to the critical pre-wintering period indicated by Bosch and Kemp (2004). Once bees have reached the adult wintering stage, exposure to elevated pre-winter temperatures should be avoided (Kemp et al. 2004) due to increased winter mortality from excessive fat body depletion (Bosch et al. 2000). Conditions during transit may be variable and temperatures above 5°C may actually increase mortality, or cause bees to emerge prematurely. Consistent or gradually fluctuating temperatures promote survival, while rapid fluctuations in daily minimum and maximum temperatures can be detrimental (Leather et al. 1993).

Bosch and Kemp (2001, 2003, 2004) stressed the importance of monitoring the populations for determining the development stages prior to wintering, a suggestion even more relevant for importation of bees into a different geographic location. For winter storage

of bees imported into Nova Scotia, the life stage of the shipment should be determined, and when possible, cocoons should be placed immediately into controlled storage facilities at 0°C. Subsequent generations reared in Nova Scotia may be kept outside; ambient temperatures in the province are suitable for winter storage as long as bees are placed in sheltered areas to avoid extreme low temperatures beyond the supercooling capacity. In general, wintering *O. lignaria* in unheated but sheltered conditions is a viable and affordable alternative to expensive controlled storage facilities for apple producers in Nova Scotia. *O. cornifrons* also showed high survival under different winter storage conditions, with comparable survival in controlled and ambient outdoor conditions in Iowa, USA (Wilson and Abel 1996).

Based on one year, emergence of *O. lignaria* populations wintered under ambient conditions in Nova Scotia corresponds favourably with the flowering period of apple (Fig. 7), as 2-3 days are required for the majority of females to mate and establish nests (Bosch and Kemp 2003). Prior to apple flowering, dandelion (*Taraxacum sp.*) serves as a main food source in Nova Scotia (Sheffield 2006). However, since mating and nest establishment would occur during pre-flowering of the crop, caution must be used with spraying to control pre-flowering pests as death of males and females within the crop system could occur. Predictable emergence and tighter synchrony with crop flowering remains the main benefit of controlled storage (Bosch and Kemp 2003).

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