

POLLINATION, SEED SET AND FRUIT QUALITY IN APPLE: STUDIES WITH *OSMIA LIGNARIA* (HYMENOPTERA: MEGACHILIDAE) IN THE ANNAPOLIS VALLEY, NOVA SCOTIA, CANADA

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Abstract— The orchard crop pollinator *Osmia lignaria* (Hymenoptera: Megachilidae) was evaluated for apple pollination in the Annapolis Valley, Nova Scotia, Canada during 2000-2001. Resulting pollination levels (measured as pollen grains on floral stigmas), percent fruit set, mature fruit weight and seed yield were evaluated against an attempted gradient of *Osmia* bee density. In addition, fruit quality was assessed using two symmetry indices, one based on fruit diameter, the second on fruit height. Pollination levels, percent fruit set and mature fruit quality were much higher than minimums required for adequate crop production, and all but pollination levels showed weak but significant decreases at increased distance from the established nests, suggesting that even at low numbers these bees may have been making significant contributions to apple production. Fruit were typically of better quality in areas of the orchard adjacent to *Osmia* nests, having fewer empty carpels and greater symmetry; fruit quality (i.e., symmetry) is typically most reduced when two or more adjacent carpels are empty. Empty carpels reduce growth in fruit height rather than diameter, suggesting that symmetry indices using fruit diameter are not sensitive enough to evaluate fruit quality. Evidencing this, fruit without mature seeds observed in this study showed high symmetry based on diameter, but were greatly asymmetric with respect to fruit height. Further discussion on *Osmia* bees as apple pollinators and on methods of evaluating apple fruit quality with respect to seed distribution within the apple fruit are provided.

Keywords: *apple pollination, Osmia bees, increased fruit quality, seed distribution*

INTRODUCTION

Pollination is one of the first and most important steps in fruit production, and for almost 90% of angiosperms (Ollerton et al. 2011) this vital ecological service is facilitated through insect vectors, mainly bees (Kevan & Baker 1983; Michener 2007). Many aspects of reproduction for the majority of flowering plants are poorly understood, mainly due to the large number of species of Angiosperms. In contrast, aspects of fruit production in most horticultural crops are relatively well known, as summarized in several reviews (e.g., McGregor 1976; Westwood 1978; Sedgley & Griffin 1989; Sedgley 1990; Free 1993; Delaplane & Mayer 2000). Despite this coverage, there is much about reproduction that is not fully understood in many crops, including apple (*Malus x domestica* Borkh.), which has been cultivated since at least 1000 BC (Morgan & Richards 2002).

The contributions of bees to apple fruit production have been appreciated for a long time (e.g., Brittain 1933; Brittain & Eidt 1933). Often, low yield and/or poor fruit quality are attributed to poor pollination performance by bees due to low numbers (Garratt et al. 2013), unsuitable weather conditions during flowering (Boyle & Philogène 1983; Boyle-Makowski 1987), low pollination efficacy (Robinson

1979; Goodell & Thompson 1997; Schneider et al. 2002), or combinations of these factors (see Bradbury 1929). However, other non-bee related issues such as genetic compatibility among cultivars, orchard structure (Free 1962, Kron et al. 2001a and b) and tree nutrition levels also contribute (Bradbury 1929; Faust 1989).

At least ten pollen grains are needed to produce a full complement of seeds for most apple cultivars (Torchio 1985; Brault & de Oliveira 1995), though due to varying levels of non-viable pollen and genetic incompatibility issues inherent with orchards, many more grains are usually required for seed set (Sheffield et al. 2005); the most promising scenario is when high quantities of viable, compatible pollen are delivered to the stigma(s) by bees. Pollination as such ultimately leads to fertilization and seed development, and influences the number and distribution of seeds within the fruit, which has long been known to influence fruit quality (Brittain 1933; Einset 1939; Abbott 1959; Brault & de Oliveira 1995; Ward et al. 2001; Dražeta 2002) and quantity (Luckwill 1948, 1953a and b, 1957; Brain & Landsberg 1981; Stephenson 1981; Garratt et al. 2013; 2014), thus marketability of the fresh fruit. However, at least for some apple cultivars, pollination equality among stigmas is not necessarily linked directly to fruit quality via seed number and distribution. As recently demonstrated (Sheffield et al. 2005), pollinator efficacy can vary greatly in terms of pollen deposition among the five stigmas, yet still result in high fruit set, and in fruit with a full complement of seeds, ultimately increasing fruit quality (Einset 1939;

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Abbott 1959; Bramlage et al. 1990; Brault & de Oliveira 1995; Keulemans et al. 1996; Currie et al. 2000; Ward et al. 2001).

Differences in external floral form are common among apple cultivars (Stott 1972; Ferree et al. 2001) which can lead to inefficient pollination due to its negative influence on bee foraging behaviour (e.g., side-working behaviour) (Robinson 1979; Degrandi-Hoffman et al. 1985; Schneider et al. 2002). Internal morphology, particularly the presence of a compitum (i.e., an area of potential inter-carpel pollen tube growth, see Sheffield et al. 2005) also differs among cultivars, and for some the pollen has to be distributed evenly among stigmas to get a full complement of seeds (Visser & Verhaegh 1987), in others not (Beaumont 1927; Sheffield et al. 2005). As such, it is likely that the intensive cultivar development which has contributed to variation in fruit morphology (Bultitude 1983) has also resulted in modification of external and internal floral form, with resulting implications (e.g., foraging behaviour, pollen tube growth pathways) for seed set and fruit quality (Sheffield et al. 2005). Considering the number of cultivars that have been developed (over 2000, as per Morgan & Richards, 2002), and the trend to develop more, it is important to fully understand all of the factors affecting fruit yield and quality in apple. Unfortunately, pollinator foraging behaviour, floral attractiveness, pollination requirements, pollen tube growth pathways, and other flower-based characteristics of cultivars have seldom, if ever, been considered during new cultivar development though all ultimately influence fruit quality and crop yield.

Between 2000 and 2004, research was done in the Annapolis Valley of Nova Scotia to investigate several aspects of apple pollination, including the evaluation *Osmia lignaria* Say (Megachilidae) as a potentially managed pollinator. The main objectives here are to present findings pertaining to *O. lignaria* as an apple pollinator through quantifying fruit yield (i.e., pollen deposition rates, percent fruit set) and quality (i.e., seed yield, seed distribution within fruit, fruit weight and symmetry) in response to its presence, and to further clarify how seed yield and distribution are linked to fruit quality. Other aspects of *Osmia* management, including methods for increasing bee populations for pollination in subsequent years and winter management strategies for apple growers in the province have previously been reported (Sheffield et al. 2008b and c, respectively).

MATERIALS AND METHODS

Study Site

A 3 ha (the "7 Acre Block") research orchard at the Sheffield Farm (45°07'N, 64°28'W), Agriculture and Agri-Food Canada sub-station in Upper Canada, Kings Co., Nova Scotia, was used during 2000 and 2001. The orchard consisted of alternating double rows of McIntosh and Cortland cultivars, with 6 m spacing between rows. Rows within this site were arranged with a north-to-south orientation, with 4.5 m tree spacing. The orchard was divided into six zones; three in the south half and three in the north. As such, Zone I of the south half was closest (i.e.,

± 10 m) to the *O. lignaria* release sites, Zone 6 of the north half was furthest (i.e., 100 m) from the release sites. Most experiments were restricted to McIntosh trees, as these trees were of production age.

Flowering Phenology

The flowering phenology of both apple cultivars was determined in both years. Each season prior to flowering, a limb on each of 20 randomly selected trees (four to six trees per row) of each cultivar was tagged and the total number of flower clusters and buds determined. Counts of open flowers were done daily until petal fall.

Osmia Release Rates

Wooden nests, measuring 41.5 cm x 41.5 cm x 20.0 cm, were used in this study. Each nest was divided into three 11.0 cm (wide) x 37.0 cm (high) x 19.0 cm (deep) sections, each section contained wooden laminate pieces which fitted together to create 30 rows of nine holes (810 total nesting holes/nest), each with an inner diameter of 8 mm. Each nest was positioned approximately 1.5 m above the orchard floor (Fig. 1), and was oriented to face south for sun exposure. Two such nests were used in 2000; three were used in 2001.

In both years of study, *O. lignaria* nests were placed in the south end of the orchard adjacent to Zone I with the hope of creating a gradient of *Osmia* bees during flowering. In 2000, paper tubes containing un-emerged *Osmia* bees were introduced into nests on May 19, with 150 ♀'s and 300 ♂'s at each of two nests. In 2001, bees were released on May 17, with 172 ♀'s and 435 ♂'s at each nest.



FIGURE 1. Wooden nests used for *Osmia lignaria*. These were placed in the south end of the orchard in an attempt to create a gradient of pollinators.

Apple Pollination

Pollination levels (i.e., total count of pollen grains deposited on five stigmas per flower) throughout the orchard were assessed within the six zones during early, mid and late flowering for both years. For each sampling period, thirty flowers were selected at random from within each of the zones (five flowers from each row pair), and stigmas were collected by snipping the style(s) 3-5 mm below the stigmatic surface(s), and then mounted on a glass slide in

basic fuschin gelatin stain (Dafni 1992). Pollen grains were counted on each of the five stigmatic surfaces per flower, to a maximum of 100 grains per stigma (i.e., 500 grains per flower). Pollination levels among the six zones were analyzed using two-fixed factor Analysis of Variance (ANOVA), testing for differences between flowering periods, zones, and the interaction of these factors. Data were transformed using the square root transformation for count data with zeros (Zar 1999) prior to analysis.

Fruit Set and Total Seed Yield

Percent fruit set was determined in the orchard along the six linear transects (i.e., tree row pairs) running south to north. On three McIntosh trees per transect per zone, three limbs were tagged (nine limbs in total) and the total number of flower buds counted. During the calyx stage of fruit development (2-3 weeks following petal fall), developing fruits on each of the tagged limbs were counted to determine percent fruit set. At maturity for both years, five fruits from each of ten randomly selected McIntosh trees per transect per zone were collected and placed into cold storage until processing. Various measurements of each fruit were recorded, including weight (g), maximum and minimum diameter (mm), and maximum and minimum height (mm). Fruit quality was assessed using two symmetry indices; Symmetry Index A was calculated as minimum fruit diameter/maximum fruit diameter; Symmetry Index B was calculated as minimum fruit height/maximum fruit height. As such, a perfectly symmetric fruit would result in values near zero for both indices. Individual fruits were then cut along the equatorial axis, and the number of seeds/carpel and total seeds per fruit determined. Differences in percent fruit set and seeds per fruit among the zones and rows were compared using ANOVA. Percent fruit set data were arcsine transformed prior to analysis to obtain homoscedasticity of variance (Zar 1999).

The impact of seed distribution on total fruit quality was also examined on pooled data for both years. Following seed counting, individual fruit were assigned to one of eight possible categories (Fig. 2) based on presence (I) or absence (O) of seeds within each of the five carpels (fruit with only four carpels were removed from the analysis): A) I, I, I, I, I (i.e., a fruit with a full complement of seeds); B) I, I, I, I, O; C) I, I, I, O, O; D) I, I, O, O, O; E) I, O, O, O, O; F) I, O, I, O, I; G) I, O, I, O, O; and H) O, O, O, O, O (i.e., no seeds observed). ANOVA on arcsine transformed data was

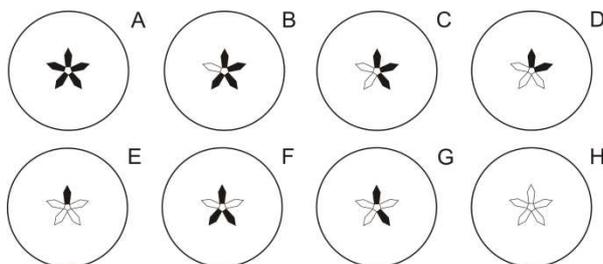


FIGURE 2. Carpel categories used to represent all combination of seed distribution among the five carpels of McIntosh apples.

used to detect differences in the two symmetry indices (see above) for each carpel category. Chi-square analysis with multiple comparisons of proportions (Zar 1999) was used to examine differences in the proportion of fruit assigned to each carpel category among zones; expected values were calculated based on no differences among zones. Additionally, the relationship between seed number and fruit weight was linearly regressed, and weights of apples in each carpel category were compared using ANOVA.

For comparative purposes, 100 fresh market McIntosh apples were purchased from a local grocery store in 2001 to establish quality fruit standards. All fruit were weighed, and the same variables (i.e., maximum and minimum height and diameter, seed count and distribution) were measured.

RESULTS

Flowering Phenology

In both years, flowering for both cultivars began in the third week of May (Fig. 3). Peak bloom for both cultivars occurred between five and eight days following the commencement of flowering. By June 5 of both years, petal drop was virtually complete for both cultivars, although flowering was observed within the orchard at low levels for several days beyond this date.

Pollination Levels

Pollen deposition levels for whole flowers (i.e., five stigmas combined) were high throughout the orchard at all stages of bloom for 2000 (early $\bar{x} = 110.1 \pm 149.0$; mid $\bar{x} = 184.5 \pm 162.4$; late $\bar{x} = 296.3 \pm 134.8$), but significant differences were observed among stages of flowering ($F_{3,522} = 85.8, P < 0.001$), being lowest in early bloom (Fig. 4A), intermediate during mid-bloom (Fig. 4B) and highest during late bloom (Tukey's HSD test, $P = 0.05$) (Fig. 4C).

Differences observed among the zones were also significant ($F_{3,522} = 3.94, P = 0.002$), but only during the mid and late stages of flowering did the levels of pollination follow the expected gradient response of decreased pollen deposition with increased distance from the *Osmia* nesting

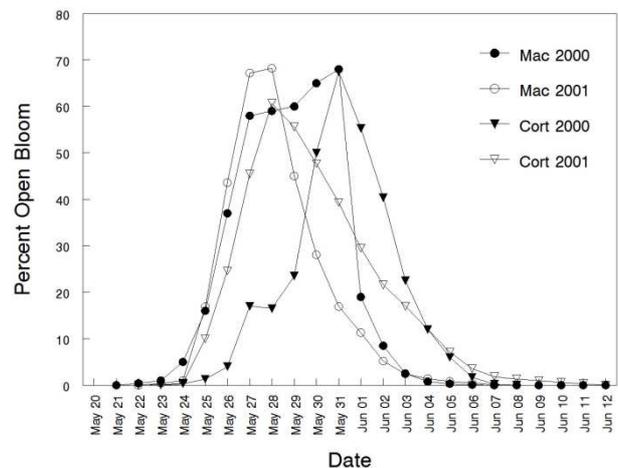


FIGURE 3. Flowering phenology of McIntosh (Mac) and Cortland (Cort) apple cultivars during 2000 and 2001.

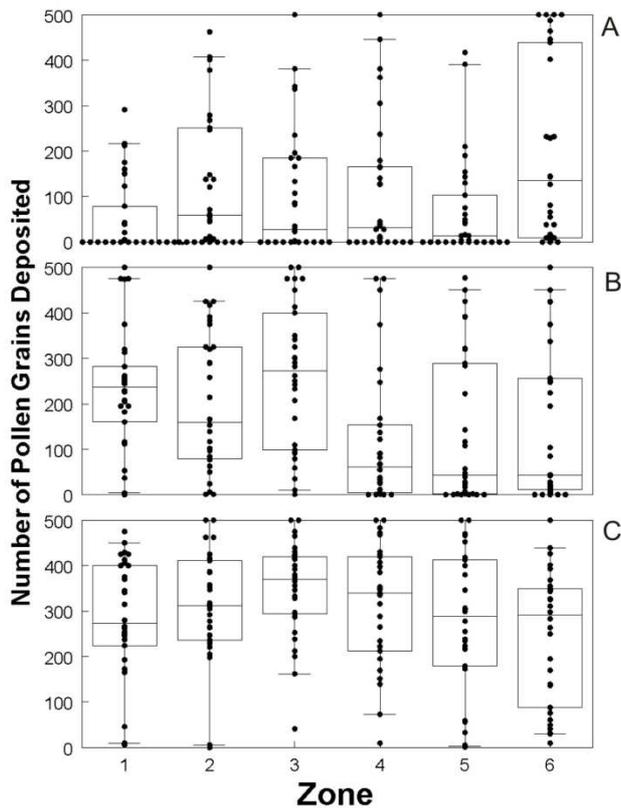


FIGURE 4. Pollen deposition per whole flower (box and whisker plot, central horizontal line showing mean, box = 25th and 75th percentiles, whiskers = 5th and 95th percentiles) during A) early, B) mid, and C) late flowering of 2000. Zone 1 is closest to *Osmia* nests, Zone 6 most distant. Significant differences were observed among stages of flowering but not among the zones; a significant interaction between stage of flowering and zone was observed (see text).

sites (Fig. 4). A significant interaction between stage of flowering and zone was observed ($F_{10,522} = 7.4$, $P < 0.001$). A large proportion of the stigmas from the early stage of flowering were not pollinated (Fig. 4A).

In 2001, pollination levels were also high (early $\bar{x} = 353.4 \pm 165.4$; mid $\bar{x} = 366.7 \pm 176.7$; late $\bar{x} = 405.2 \pm 150.4$), and significant differences were observed among stages of flowering ($F_{2,522} = 4.98$, $P = 0.007$), again being lowest in early and mid-bloom, which did not differ (Fig. 5A), and highest at late bloom (Tukey's HSD test, $P = 0.05$) (Fig. 5C). No differences were observed among the zones ($F_{5,522} = 0.82$, $P = 0.537$). Throughout the flowering period, a large proportion of the stigmas were pollinated (Fig. 5), and a gradient response of decreased pollination levels at increased distance from the *Osmia* nests was not supported during any stage of bloom, nor was an interaction of flowering period and zone observed ($F_{10,522} = 0.82$, $P = 0.609$).

Fruit Set and Seed Yield

Average percent fruit set across the orchard was moderately high in 2000 ($\bar{x} = 25.1\% \pm 9.13$) and 2001 ($\bar{x} = 16.7\% \pm 3.73$). In 2000, differences in percent fruit set were observed among the zones ($F_{5,318} = 5.64$, $P < 0.001$);

percent fruit set was highest in Zone 1, and significantly greater than all but Zone 2 (Tukey's HSD test, $P = 0.05$) (Fig. 6A, open bars). In 2001, a trend similar to 2000 was observed at increased distance from bee nests, fruit set being highest nearest to the bee nests in Zone 1 ($F_{5,318} = 2.50$, $P = 0.031$) (Fig. 6A, hatched bars).

Weak negative relationships between seed yield and increased distance from nesting sites were observed in both years (2000: $R^2 = 1.6\%$, $P < 0.001$; 2001: $R^2 = 2.3\%$, $P < 0.001$); seed yield in the zones closest to the nests were significantly greater than those away from the nesting sites in both 2000 ($F_{5,1694} = 6.74$, $P < 0.001$, Tukey's HSD test $P = 0.05$; Fig. 6B, open bars) and 2001 ($F_{5,1694} = 11.11$, $P < 0.001$, Tukey's HSD test, $P = 0.05$; Fig. 6B, hatched bars).

Fruit weight weakly increased linearly with the number of seeds per fruit ($R^2 = 5.1\%$, $P < 0.001$) (Fig. 7), and increased numbers of seeds had a positive effect on both symmetry indices, though was much stronger in Symmetry Index B (compare Figs. 8A and C). The mean value for Symmetry Index A was highest when seeds were present in all but one (Category B), but did not differ significantly from categories C, D and H; categories E, F and G were

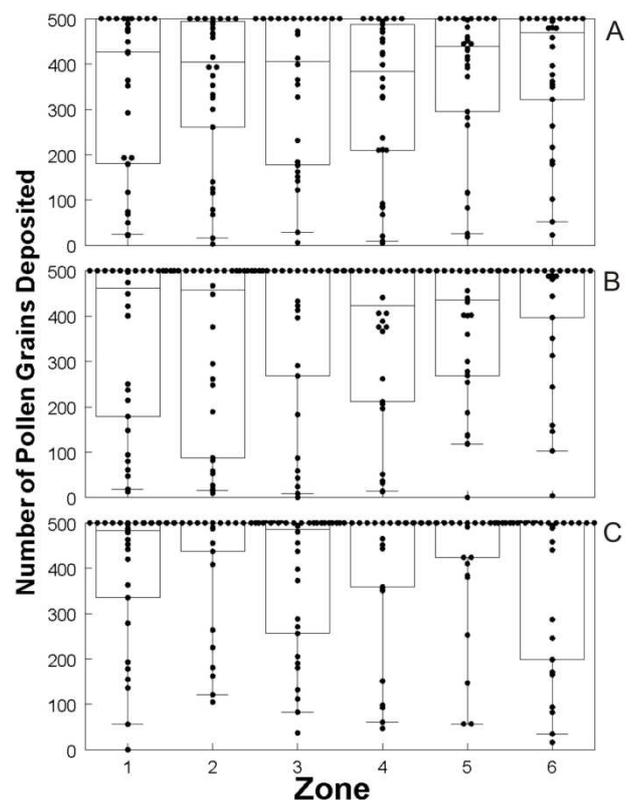


FIGURE 5. Pollen deposition per whole flower (box and whisker plot, central horizontal line showing mean, box = 25th and 75th percentiles, whiskers = 5th and 95th percentiles) during A) early, B) mid, and C) late flowering of 2001. Zone 1 is closest to *Osmia* nests, Zone 6 most distant. Significant differences were observed among stages of flowering but not among the zones; a significant interaction between flowering period and zone was not observed (see text).

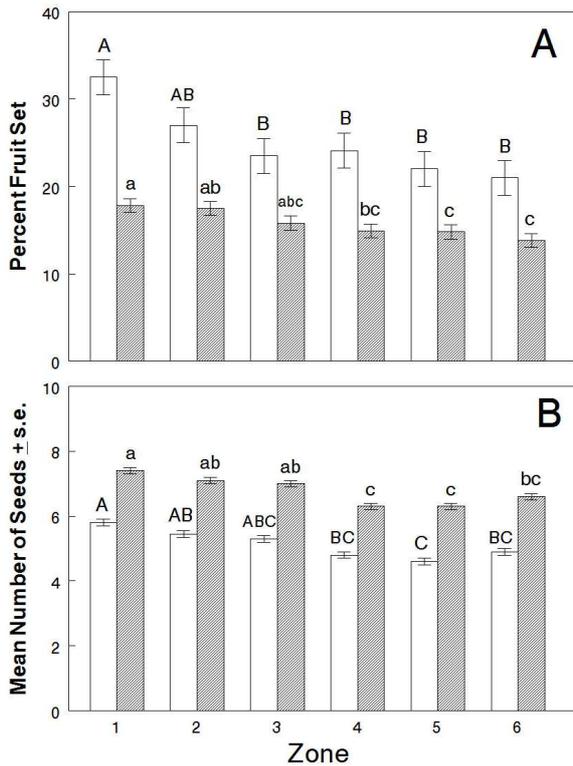


FIGURE 6. a) Percent fruit set in each zone, and b) seed yield per fruit in each zone; open bars 2000, hatched bars 2001. Bars sharing uppercase (2000) and lowercase (2001) letters within each graph are not significantly different (Tukey's HSD test, $P=0.05$). Zone 1 is closest to *Osmia* nests, Zone 6 most distant.

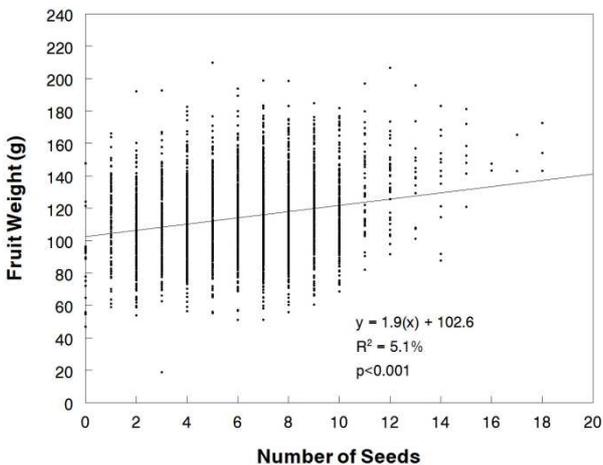


FIGURE 7. Apple fruit weight (grams) versus the number of seeds per fruit.

significantly lower ($F_{6,3166} = 10.0, = 6; P < 0.001$, Tukey's HSD test, $P = 0.05$) (Fig. 8B). Symmetry Index B showed a much greater response to seed presence and distribution; fruits with 3 or more adjacent empty carpels were of much inferior quality (Fig. 8D) ($F_{6,3166} = 113.28, P < 0.001$, Tukey's HSD test, $P = 0.05$). No fruit with a full complement of seeds (i.e., Category A) were observed.

The presence of seeds had a strong influence on outward fruit growth from the carpel containing them (Fig. 8), especially in cases where carpels with seeds were adjacent (compare Figs. 2 and 8). As expected, when two or more adjacent carpels were without seeds, the influence on fruit growth was most significant; single empty carpels were not a great factor influencing asymmetric fruit growth (Figs. 8b and d). Seed distribution patterns had a significant effect on fruit weight ($F_{6,3166} = 4.63, P < 0.001$, Tukey's HSD test, $P = 0.05$), being greatest in category B, E-G (Fig. 9).

The highest quality fruit (i.e., with only one empty carpel; Category B) made up the largest proportion (i.e., 0.58) of the fruit collected within the orchard. A significantly higher frequency of these fruits was found in zones closest to *Osmia* nesting sites ($\chi^2_{0.05,5} = 61.3$; Fig. 10B). The poorest quality fruits were most abundant in areas of the orchard furthest from *Osmia* nests, though values were only significant for Category E ($\chi^2_{0.05,5} = 19.4$; Fig. 10E) and H ($\chi^2_{0.05,5} = 12.0$; Fig. 10H).

Commercially purchased apples were of high quality, with both symmetry indices being greater than 0.9 (Symmetry Index A $\bar{x} = 0.96 \pm 0.03$ s.d.; Symmetry Index B $\bar{x} = 0.90 \pm 0.04$ s.d.), with $\bar{x} = 7.07 \pm 3.1$ s.d. seeds per fruit. The majority of fruit had a full complement of seeds (i.e., 56%, Carpel Category A) or one (i.e., 22%, Category B) to two empty carpels (8% Category C), though all carpel categories (Fig. 2) were represented.

DISCUSSION

Since the 1940's studies of indigenous (non-*Apis*) bees in North America have increased, with the intentions of developing some of the more promising species for crop pollination (see Torchio 1990; Batra 2001; Strickler & Cane 2003). Megachilid bees, particularly members of the genus *Osmia*, have shown great potential as managed pollinators of spring flowering tree fruit crops (e.g., Torchio 1976, 1982a and b, 1984a and b, 1985; Maeta 1990; Bosch & Kemp 1999, 2001, 2002, Vicens & Bosch 2000; Wei et al. 2002; Ladurner et al. 2004; Bosch et al. 2006; Gruber et al. 2011; Sedivy & Dorn 2014). Results from Nova Scotia are also promising (see Sheffield et al. 2008b and c), but the contributions of *O. lignaria* as a pollinator of apple reported here are somewhat obscured because of the inability to isolate their specific impact in the presence of many other native pollinators in Nova Scotian orchards (Sheffield et al. 2003, 2008a, 2013). During both years of study, pollination levels (Figs. 4 and 5) were over ten times greater than required to produce a sustainable crop as only ten viable/compatible pollen grains are actually needed to produce a full complement of seeds for most cultivars (Torchio 1985); even if a large proportion of the counted pollen would have been non-viable, pollination would have been more than adequate to compensate. In addition, for McIntosh flowers, sufficient pollination of only one stigma can provide full fertilization among the carpels and result in fully formed fruit (Sheffield et al. 2005).

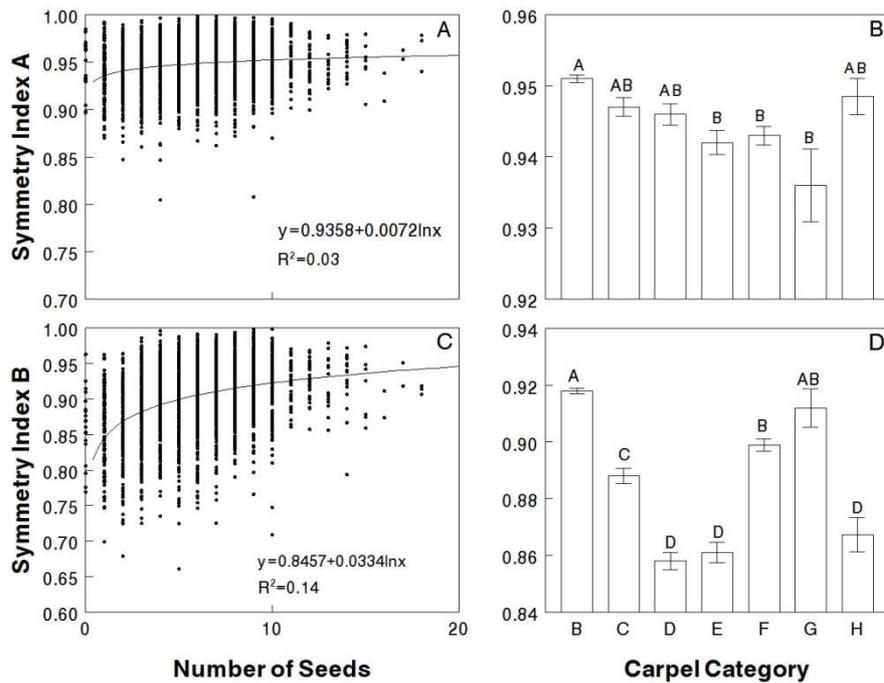


FIGURE 8. A) Symmetry Index A and C) Symmetry Index B versus the number of seeds per fruit, and bar graphs of the mean (\pm s.d) of B) Symmetry Index A and d) Symmetry Index B for each carpel category; bars sharing letters are not significantly different (Tukey's HSD test, $P = 0.05$).

Unfavourable weather conditions, particularly a wet, cool early flowering period in 2000 probably contributed to pollination levels being less for this period than in later periods (Fig. 4), and in the same period in 2001 (Fig. 5) due to decreased pollinator activity. However, more *O. lignaria* females were released in 2001, which may also account for higher pollination levels in that year. Ideally, pollination, fruit set and subsequent fruit development of the king flower (i.e., central flower of each blossom cluster, which is the first to open) is desired for crop production as it results in the largest apple due to apical dominance (Bangerth 1989; Ferree et al. 2001). As recently observed by Losada & Herrero (2013), the stigma receptivity period of the king

flower is typically significantly shorter than for the lateral flowers. These authors attribute these differences to a fail-safe mechanism within a single inflorescence; under early flowering conditions which are favourable for pollinator flight and pollination (e.g., 2001; Fig. 5a), the king flower will have an advantage and likely set strong fruit, though lateral flowers, with a longer receptivity period, will increase the chances of fruit set when conditions for pollination are not optimal during the early flowering period (Losada & Herrero 2013) (e.g., Fig. 4a). However, in both years, fruit set was much higher than the 5-10% required for a full, economically viable crop (Roland 1978), reaching levels in which chemical thinners would have been applied under commercial settings. Apical dominance, and the resulting influence on fruit weight was not studied here, but ultimately could have contributed to the large variations in weight versus seed number observed here (Figs. 7 and 9).

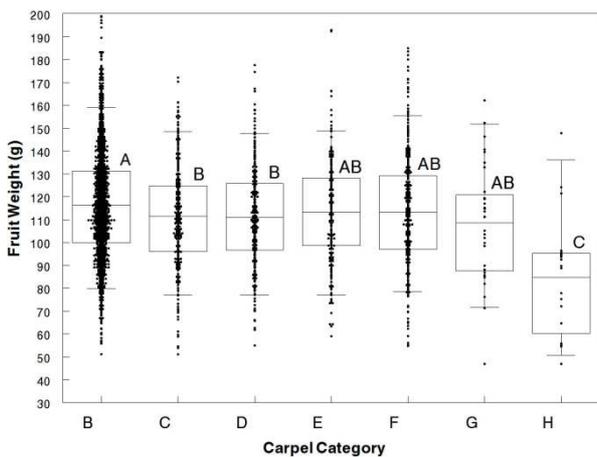


FIGURE 9. Apple fruit weight (box and whisker plot, central horizontal line showing mean, box = 25th and 75th percentiles, whiskers = 5th and 95th percentiles) versus carpel category. Boxes sharing letters are not significantly different (Tukey's HSD test, $P = 0.05$). Dots added to show number of fruit per carpel category.

Although fruit set and seed yield levels in this study cannot be directly attributed to the presence and or activity of *O. lignaria* within this site, especially since these bees were infrequently observed within the orchard, the slight but significant trends observed could be indicative of their effectiveness even at low numbers (Bosch & Kemp 2001, Torchio 2003), as were used here. As indicated by Torchio (1985), *O. lignaria* females are so efficient as apple pollen collectors that reduced fecundity may occur when bees are released within isolated orchards (i.e., those without other floral resources), hence recommended release rates of only 100 females/ha. Supporting this, Ladurner et al. (2004) found that a single female *Osmia cornuta* Latrielle (a closely related Old World species) can provide sufficient pollination for 5 apple trees.

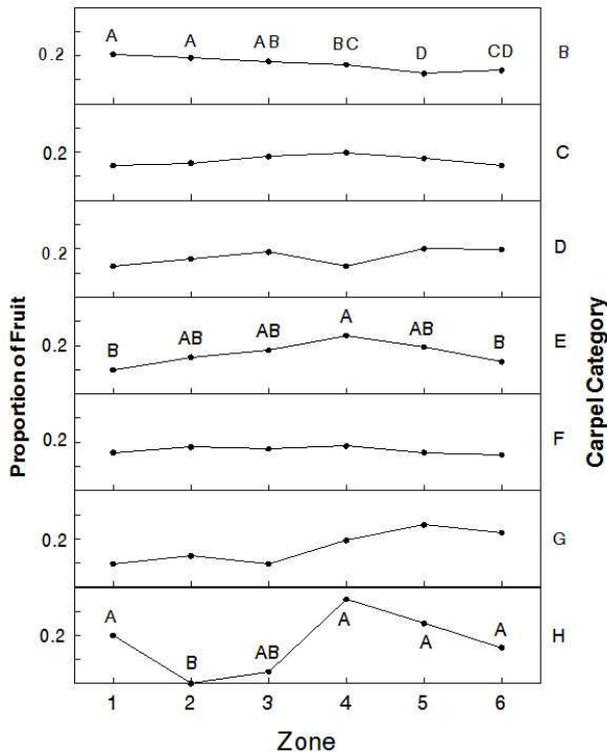


FIGURE 10. The proportions of fruits in each zone representing each carpel category (right Y-axis; see Fig. 2). Zone 1 is closest to *Osmia* nests, Zone 6 most distant. Points within a single graph sharing letters are not significantly different (multiple comparisons for proportions, $q_{0.05,6}=4.033$); points in graphs without letters are not significantly different.

Ultimately, it is not pollination levels *per se*, but the resulting seed distribution within the apple fruit and the respective hormones they produce (Abbott 1959; Luckwill 1948, 1953a and b, 1957; Nitsch 1970) which dictates fruit quality (i.e., symmetry). Hormones produced by the developing seeds in each carpel influence growth in tissues surrounding adjacent empty carpels. It is only when two or more adjacent carpels are empty (Fig. 2) that decreased outward fruit growth from that area occurs, resulting in fruit asymmetry. This was demonstrated most clearly with Symmetry Index B for the carpel categories D, E and H (Fig. 8d), suggesting that fruit growth in response to seed presence occurs primarily on a single plane – outward horizontally from the carpels and contributing to increased fruit diameter, thus influencing Symmetry Index A much more than B (Fig. 8). As such, fruit quality assessments based on symmetry indices which measure fruit diameter or width (e.g., Symmetry Index A, and see Brault & de Oliveira 1995; Matsumoto et al. 2012; Garratt et al. 2013, 2014) are not necessarily always reflective of quality. For this same reason, using empty carpels as an indicator of fruit asymmetry (e.g., Ladurner et al. 2004) is also not necessarily reflective of fruit quality due to the compensatory effect of seeds in adjacent carpels, unless multiple adjacent carpels are empty; many marketable apples of high quality have empty carpels. As such, methods of evaluating fruit symmetry based on multiple planes (e.g., Currie et al. 2000), attributed back to

seed presence and distribution within the fruit may offer more accurate, albeit more difficult to calculate, representation of the fruit.

Despite there not being a direct link between pollination levels and seed set/distribution, apple fruit quality followed a pattern related to *Osmia* nesting site location. In fact, for apple, qualitative (i.e., pollen viability, pollen compatibility) pollination is much more important than quantitative aspects (i.e., deposition levels); despite having 50 times more pollen deposited than required, seed yield and distribution was seldom maximized in this study. The highest quality fruit observed in this study, those with one empty carpel (Category B), were the most abundant, comprising almost 60% of the fruit examined. Fruit of this category also weighed the most (Fig. 9) and were proportionally the greatest in areas adjacent to *Osmia* nests (Fig. 10B). Fruit with more empty carpels were most often observed in the half of the research orchard furthest from the nesting sites (Fig. 10), supporting that *Osmia* bees may have been making significant contributions to fruit quality at low numbers. In studies such as this, it is often difficult to isolate the effects of target pollinators when unmanaged wild bees are also present within the orchards. Almost 50 species of bees visit apple flowers in Nova Scotia (Sheffield et al. 2003, 2008a), and many use the surrounding natural habitat for nesting and foraging sites (Sheffield et al. 2013). Their presence in the wooded periphery of the orchard may explain the high pollination levels throughout the orchard (Figs. 4 and 5). In addition, honey bees were very abundant within the orchards although no colonies were brought in to supplement pollination. However, studies to date demonstrate that *O. lignaria* is an exceptional pollinator of apple and several other rosaceous tree fruit crops (Torchio 1976, 1982a and b, 1984a and b, 2003; Bosch & Kemp 1999, 2001, 2002; Monzon et al. 2004). Evidence of pollen use by female *O. lignaria* in the study site indicates that it did contribute to apple pollination as it collected high proportions of apple pollen (Sheffield et al. 2008b). The resulting aspects of fruit quantity and quality assessed in the present study also lend credit to the claims of *Osmia* bees as excellent managed pollinators of tree fruit crops.

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