Impact of enhanced Osmia bicornis (Hymenoptera; Megachilidae) 1 populations on pollination and fruit quality in commercial sweet 2 cherry (Prunus avium (L)) orchards

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Impact of enhanced *Osmia bicornis* (Hymenoptera; Megachilidae) populations on pollination and fruit quality in commercial sweet cherry (*Prunus avium* (L)) orchards

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Short Title: *Osmia bicornis* pollination of *Prunus avium.*

Abstract

The impact on pollination of supplementing wild pollinators with commercially reared *Osmia bicornis* in commercial orchards growing the self-fertile sweet cherry variety "Stella" was investigated in each of two years. The quality characteristics used by retailers to determine market value of fruit were compared when insect pollination was by wild pollinators only, or wild pollinators supplemented with *O. bicornis* released at recommended commercial rates. No effect of treatment on the number of fruit set or subsequent rate of growth was recorded. However, supplemented pollination resulted in earlier fruit set when compared to pollination by wild pollinators alone and offered the potential benefit of a larger proportion of the crop reaching optimum quality within a narrower time range, resulting in more consistent produce. Retailers use five key quality criteria in assessment of market value of cherries (the weight of individual fruit, width at the widest point, fruit colour, sugar content and firmness). Price paid to growers depends both on meeting the criteria and consistency between fruit in these characteristics. In both years, the commercial criteria were met in full in both treatments, but harvested fruit following supplemented pollination were consistently larger and heavier compared to those from the wild pollinator treatment. In the year where supplemented pollination had the greatest impact on the timing of fruit set, fruit size and sugar content were also less variable than when pollination was by wild species only. The implications for the commercial use of *O. bicornis* in cherry orchards are considered.

Keywords

Solitary bee/ Sweet Cherry/ Fruit set/ Fruit quality/ *Osmia bicornis*/ Pollination

Introduction

Insect pollination is a key ecosystem service, with estimates valuing it at £430 million per annum within UK agricultural systems alone (Vanbergen, Heard, Breeze, Potts, & Hanley, 2014). Insect pollinators (including wild bees) account for 35% of global crop pollination (Garibaldi et al., 2013) but a decline in bee populations has been observed over the last 60 years (Potts et al., 2010) and linked to a range of drivers (Neumann & Carreck, 2010; Potts et al., 2010). Although the lack of coordinated monitoring programmes results in their being only limited firm evidence for widespread losses in most pollinator groups, the strongest conclusions can be drawn from data generated in Europe and North America where well studied groups displaying a decline include honey bees and bumblebees. Fragmentary evidence for other groups is available, however, and quantitative synthesis of local scale studies has revealed a wide scale pattern of loss of pollinator richness and abundance (Ricketts et al., 2008; Winfree et al., 2009) that collectively suggests that a widespread decline is occurring in many regions of the world (Potts et al., 2010). The decline is more severe amongst specialist feeding species with many generalist feeders less affected due to their association with a wider range of plant species (Neumann & Carreck, 2010; Potts et al., 2010). It has been suggested that wild pollinator decline has had a greater impact on pollination of high value fruit crops such as top fruit orchards than on other crops, and to address shortfalls in pollination services in orchards wild pollinators are commonly supplemented with commercially managed species such as *Apis mellifera* (Allsopp, de Lange, & Veldtman, 2008; Breeze, Bailey, Balcombe, & Potts, 2011, Garibaldi et al., 2013). Against a background of increasing costs associated with such managed bees and the decline in wild pollinators (Allsopp et al., 2008; Breeze et al., 2011; Potts et al., 2010), interest in the potential commercial use of alternative organisms to supplement pollination is rising.

Solitary bees of the genus *Osmia* have been shown to be an effective alternative to existing commercial pollinators in several fruit crops. *Osmia cornifrons* is used for commercial apple and cherry pollination in China (Batra, 1978) and Japan (Bosch &

Kemp, 2002; Sekita, 2000; Sekita & Yamada, 1993) and *Osmia lignaria* is used in orchards in the USA (Morales-Ramos, Rojas, & Shapiro-Ilan, 2013). In Europe use of *Osmia cornuta* was developed successfully for orchard pollination, and this was followed by its introduction to California in the 1980s to pollinate almond crops (Torchio & Asensio, 1985). Use of *O. cornuta* as a pollinator for blackberry (*Rubus fruticosus* L.) has also been investigated in confined environments in Italy, and heavier berries bearing more drupelets were produced when *O. cornuta* were used than in systems relying on self or wind pollination (Pinzauti *et al*., 1997). *Osmia bicornis* (Linnaeus, 1758) (previously *Osmia rufa*) has been used in European orchards from the 1970s, since being developed as a pollinator for fruit trees (including sour cherries) and other crops such as strawberries and oilseed rape (Hansted, Grout, Toldam-Andersen, & Eilenberg, 2014; Sedivy & Dorn, 2014). Although positive effects on both yield and quality (compared to background pollination by wild pollinators alone) have been reported when this species is released in later flowering fruit crops, more work is needed to determine its efficacy in earlier flowering crops such as cherries.

O. bicornis is a widely distributed univoltine, polylectic species, ranging from Scandinavia to the Mediterranean (Lhomme, 2014; O'Toole, 2000). It is active in Europe from March onwards in most years (O'Toole, 2000; Raw 1972), and commercial rearing techniques ensure the availability of adults for release sufficiently early in the year to pollinate earlier flowering orchard crops, such as cherries, at a time when fewer alternative wild pollinators are available (Gruber, Eckel, Everaars, & Dormann, 2011).

In the UK both self-fertile and self-sterile varieties of sweet cherry (*Prunus avium* (L.)) flower in late March or early April. Rapid ovule degeneration occurs during flowering and the majority of self-fertile varieties that are grown in commercial orchards (e.g. Stella) are thought to benefit from supplementary pollination by insects (Delaplane & Mayer, 2000; Lane, 1979). Early pollination has been shown to influence fruit set, and it has been suggested that flowers pollinated later in the blossom period may show a reduction in eventual fruit quality (Mayer, Rathbone, & Miliczky, 1987; Ughini & Roversi, 1993). Thus pollinators that are actively foraging during the short flowering period may play an important role in maintaining yield or quality of produce (Delaplane & Mayer, 2000; Lane, 1979). The early flowering period of cherry trees coincides with activity of a restricted range of wild pollinators, amongst which some *Bombus spp*, *Andrena spp* and *Osmia spp* are thought to be of key importance (Guler & Dikmen, 2013 Kirk & Howes, 2012). These early flying wild species can increase fruit set and yield, even when honey bee colonies are placed in the orchards (Holzschuh, Dudenhöffer, & Tscharntke, 2012).

Successful use of *O. bicornis* in commercial orchards is due in part to their morphology and, resulting from their nesting behaviour, the ease with which bees can be released and retained in a local area (Hansted et al., 2014; Sekita, 2000). Mason bees, such as *O. bicornis*, collect pollen on the scopa, which is located on the ventral surface of the gastrum. The location of the scopa increases the potential for contact with plant reproductive structures (Kuhn & Ambrose, 1984) and pollen is easily dislodged resulting in effective transfer between flowers (Raw, 1972). Pollination can thus be achieved by fewer floral visits than is required by social bee species (Klein et al., 2012). The utility of *O. bicornis* is also enhanced because it requires a high number of foraging trips for provisioning of larval nest cells, and these trips are commonly completed within a short foraging range (Gathmann & Tscharntke, 2002). These factors, coupled with the ability to fly in adverse weather (Güler & Dikmen, 2013; Stone & Willmer, 1989), increases the potential of the species to act as an effective early season commercial pollinator.

Building on previous research, the crop production industry is currently considering the potential of *O. bicornis* as a pollinator in oilseed rape, cherry orchards and soft fruit crops such as strawberry (Bilinski & Teper, 2004; Gruber et al., 2011; Teper & Bilinski, 2009; Wilkaniec & Radajewska, 1996), and it has been shown that *Osmia spp.* can be effective pollinators of crops in both confined and open environments (Pinzauti et al., 1997; Sedivy & Dorn, 2014). Little information is available on the impact of *O. bicornis* in UK cherry (*Prunus avium*) production systems, however, and there is active debate regarding its potential efficacy. Research is therefore required to support optimisation of pollination services in this crop. This study investigates the hypothesis that supplementing pollination by *O. bicornis* release will increase the quality and yield of fruit in commercial cherry orchards.

Materials and Methods

Experiments were conducted in a mature commercial sweet cherry orchard (*Prunus avium*) in North Herefordshire, UK (SO583502), established in 2000 using the self-fertile cultivar "Stella" (RHS, 2016). The orchard is on south facing slopes with well-draining red Herefordshire soil, slightly acid loamy and clayey (Soilscapes, 2016), at 200m above sea level, and with a density of 1900 trees per hectare yielding a mean of 20 tonnes fruit per hectare. Trees were covered with open ended 100m polythene tunnels (poly-tunnels), each containing 2 rows separated by a narrow (2m) grass strip with occasional herbaceous flora including *Taraxacum* and *Ranunculus spp*. Normal commercial husbandry practice included opening tunnels during spring and summer, allowing access for pollinating insects during the flowering period.

Experimental design and treatments

A randomised design of four blocks each containing two treatment plots was established during early spring of 2015. The experiment was replicated in 2016. Each treatment plot occupied the central portion of a poly-tunnel and contained 50 trees (two rows of 25 trees). Fourteen days prior to bud burst (growth stage 2: 23rd March 2015, 21st March 2016; Chapman & Catlin, 1976) the polythene sides of each plot were replaced with an insect-proof mesh covering. Mesh walls were also constructed to seal both open ends, thus facilitating release and containment of known numbers of *O. bicornis*.

Within each block, in one treatment plot (control) insect pollination relied on the wild pollinators trapped when mesh walls were constructed. In the second, wild pollinating insects were supplemented with commercially reared *O. bicornis* released at the standard rate (2 adult bees/tree) and timing recommended for cherry orchards by the supplier (Mason Bees Ltd., Shropshire, UK). Cocoons were removed from nest cells in early November the previous year and maintained under conditions of continuous dark and at a temperature varying between 2-4 ºC until being moved to experimental plots. Two standard weatherproof release boxes (18 x16 x 8cm) were set at 1.5m above the soil surface according to normal commercial practice. Boxes were positioned at distances equivalent to approximately one-third and two-thirds along the length of each of the plots, with an exit slit facing towards the South to allow escape of adult bees. Fourteen days before commencement of bud burst, fifty *O. bicornis* cocoons were placed in each release box and the adults allowed to emerge (60:40 female to male ratio). To confirm the total number of adult *O. bicornis* that were active during flowering in each plot, empty cocoons were counted at 7-day intervals until all had emerged. Cocoons from which adult bees failed to emerge within the expected time period were removed and replaced.

With the exception of the treatment-specific procedures, all crop husbandry activities were identical in all plots and followed normal commercial practice for the orchard.

Assessments

Temperature – Temperatures within each treatment plot were recorded with a handheld digital thermometer at each assessment visit (TPI Digital Pocket Thermometer, Crawley UK). Three measurements were recorded in each treatment/replicate at each visit, at 10:00, 13:00 and 15:00.

Abundance of wild pollinators - The abundance of wild pollinating fauna was established by taking sweep net samples in all plots on each of five days during the flowering period of the orchard. Sampling was conducted while walking at a standard speed (circa 2 ms^{-1}) along the full length of the central strip between the 2 rows of cherry trees, before the catch was transferred to a sealed plastic bag and returned to the laboratory where it was stored in a freezer at $-20^{0}C$ until processing. To take account of diurnal activity cycles of different pollinators, sampling was undertaken during three periods (08:00-10:00, 11:30-13:30, 15:00-17:00), and was replicated on each of five days during the blossom period. Counts were only taken on days when temperatures were favourable for pollinator activity (>12°C), based on the temperature assessments described above.

As available resources precluded identification of all individuals to species, the insects caught were recorded under six categories, wild (non-*O.bicornis*) solitary bees, bumblebees (*Bombus* spp.), honey bees (*Apis mellifera*), hoverflies (Syrphidae), “other” diptera, and “other” insects (Hymenoptera (mainly parasitoids, Coleoptera and Neuroptera). The assessment therefore recorded the groups found in each sample that potentially contributed to wild pollination, but as counts included both pollinating and some non-pollinating species it is likely that they overestimated the cumulative contribution of wild pollinators to cherry pollination in the treatment tunnels.

Fruit set and fruit drop - Prior to the start of bud burst (growth stage 2, Chapman & Catlin, 1976), 10 trees were selected at random in each treatment/replicate (five from each row), and a branch from mid-canopy level was selected for assessments and labelled. The number of buds on the distal 50cm portion of each labelled branch was counted. After the end of all flowering (growth stage 7, Chapman & Catlin, 1976) the number of developing fruit was counted, with further counts of fruit being taken on 5, 10, 16, 23 June and 1 July in 2015, and 7, 12, 19, 24 June and 3 July 2016. The last count of fruit was taken at the commencement of ripening.

Fruit growth - The terminal fruit cluster from labelled branches was identified and the width at the widest point of each individual fruit was measured with digital callipers (Sealey, Suffolk UK). Measurements were repeated at weekly intervals (2015: 5, 10, 16, 23 June, 1 July; 2016: 7, 12, 19, 24 June and 3 July).

Fruit quality – Fruit quality assessments were taken within 2 days of the harvest date for the orchard (10th July 2015; 9th July 2016) with a minimum of 40 fruit sampled from each plot. Fruit were harvested by commercial pickers, placed carefully in labelled punnets and returned immediately to the on-site cold storage facility. Five quality measurements were made for each individual fruit (weight), width at the widest point, fruit colour, sugar content and consistency (firmness), using the standard equipment and approaches used in commercial quality assessment procedures for determining market value (Sainsbury's Supermarkets Ltd, 2015). Weight was assessed using a 50g spring scale (Pesola Light-Line, Schindellegi, Switzerland), width using the callipers described above, fruit colour on the industry standard scale of 1 (light fruit) to 7 (dark fruit) using the standard commercial colour guide (Centre Technique Interprofessionel des Fruits et Legumes, Paris France), and sugar content (percentage brix) by piercing the skin of the fruit and squeezing the juice onto the receptor of an Atago digital refractometer (Atago, Tokyo, Japan). Fruit consistency was assessed using a digital firmness penetrometer (Agro Technologie, Serqueux, France) by averaging two measurements of fruit consistency taken at the widest point of the cherry (separated by 180 Degrees). In each measurement consistency was recorded as the pressure required to penetrate the flesh of the cherry and expressed (according to normal commercial practice) as percentage of the maximum pressure that could be exerted by the penetrometer, which corresponded to a pressure of 806g (Agrosta, 2015). Penetrometer assessments were only made in 2015 due to an equipment failure in 2016.

Statistical analysis

Statistical analysis was conducted using R version i 386 3.2.3 (R core team, 2012). All data was checked for normality and Log transformations applied where necessary. Factor reduction was conducted allowing for the removal of non-significant terms and interactions in order to reach the minimum adequate model for all statistical tests conducted. During factor reduction, ANOVA between models was conducted to verify that the validity of the statistical model was not affected.

Temperature data consisted of a continuous response variable with categorical explanatory variables, thus a two-way analysis of variance (ANOVA) was utilised.

Due to the low numbers of insects recorded in assessments of wild pollinators, paired *t*-tests were used in comparisons of both numbers caught in different treatments, and to investigate differences between the overall numbers caught in 2015 and 2016.

The number of buds and number of fruit set per unit length of branch was count data and thus was analysed using GLM with Poisson error structure. Where residual deviance was found to be greater than the degrees of freedom a Quasi-Poisson error structure was applied. The proportion of fruit set was analysed with a GLM with a Binomial error structure.

Impact of treatment on cherry development (fruit size over time) consisted of both a continuous and categorical response variable, due to this an ANCOVA was used for analysis.

For fruit quality post-harvest assessments, data for width, weight, firmness and brix were all subjected to ANOVA and Tukey post hoc test to assess the impact of treatment.

Fruit colour data was collected on an ordinal scale and differences between treatments were investigated using a Kruskal-Wallis one-way analysis of variance and post-hoc Dunn test.

For all post-harvest quality assessments a Fisher's *F*-test was conducted to investigate whether the variability of fruit differed between treatments.

Results

For all assessments, block and plot were found to be non-significant in both years and therefore removed in both factor reduction and creation of the minimum adequate model.

Temperature - During the creation of the minimum adequate model, treatment was found to be non-significant and thus removed from analysis. Thus, there was no difference in temperature between treatment blocks. Temperature varied significantly between dates in both 2015 (F = 2.87, d.f. = 4, 91, $p < 0.05$) and 2016 (F = 700.90, d.f. = 1, 69, $p < 0.001$) reflecting the transition from spring to summer. Higher temperatures were recorded in 2016 than 2015 (F = 279.05, d.f. = 2,160, $p < 0.001$).

Wild pollinators - Very few wild pollinators from any of the six groups (wild (non-*O. bicornis*) solitary bees, bumblebees (*Bombus* spp.), honey bees (*Apis mellifera*), hoverflies (Syrphidae), "other" diptera, and "other" insects), were recorded in sweep net samples taken in either year (Table 1). The results of paired t-tests show no significant differences between treatments in the numbers of insects caught in either year (2015: t = -1.67, d.f. = 3, $p > 0.05$; 2016: t = -1.71, d.f. = 3, $p > 0.05$). Although very low in both years, insect counts were significantly higher in 2016 compared to 2015 (t = 5.41, d.f. = 7, p <0.001).

Table 1 Here

Fruit set

Bud counts –There were more buds per branch in 2015 than in 2016 (t = 11.97, d.f = 233, p <0.001), but there were no significant differences between treatments in the number of buds in either year (2015: t = 0.24, d.f. =152, p >0.05; 2016: t = 1.056, d.f. = 79, p >0.05, Figure 1A, B).

Figure 1 Here

Proportion fruit set – In 2015 the proportion of buds from which fruit was set was lower than in 2016 (z = -29.61, d.f. = 233, p <0.001). Differences between treatments were not consistent between years. In 2015, the proportion of buds from which fruit was set was not significantly different between wild pollinator and *Osmia* supplemented treatments (z = 0.19, d.f. =152, p >0.05) (Figure 1C), but in 2016 the proportion of fruit set was lower in *Osmia* supplemented treatments (z = -8.76, d.f. = 79, p <0.001) (Figure 1D).

Fruit counts – The results for fruit counts mirrored those for fruit set. In 2015 total fruit count was lower than in 2016 (t = -6.59, d.f. = 233, p <0.001) and total fruit counts were not significantly different between wild pollinator and *Osmia* supplemented treatments (t = 0.19, d.f. =152, $p > 0.5$) (Figure 1E). In 2016 total fruit count was found to be lower in *Osmia* supplemented treatments compared to the treatment with wild pollinators only (t = -2.60, d.f. = 79, $p < 0.05$) (Figure 1F).

Fruit growth

Following log transformation to normalise the residuals of the data, fruit size increased as a function of time (t = 46.0, d.f. = 392, $p < 0.001$), but no significant differences were found between treatments in the slopes of the lines describing the growth in width of cherries with time (Figure 2A). This interaction was therefore removed from the minimum adequate model for both 2015 and 2016.

Figure 2 Here

There was, however, another significant effect of treatment on cherry development in 2015 (F = 8.94, d.f. = 1, 392, $p < 0.01$), with the intercepts of the regression line for *Osmia* supplemented treatments occurring earlier than that of the wild pollinator treatments (t = 225.8, d.f. = 392, $p < 0.001$), indicating that the mean time of commencement of fruit growth (following fruit set) was earlier in the *Osmia* supplemented treatments (Figure 2A). As pollination could only commence when flowers opened, which occurred at the same time in each treatment, the earlier mean time for commencement of fruit growth in the *Osmia* supplemented treatment suggests that pollination/fertilisation was completed within a shorter time period when the bees were present.

A similar outcome was recorded in 2016 (Figure 2B). A significant effect of treatment on cherry development was recorded (F = 100.56, d.f. = 1,637, $p < 0.001$), with the intercept for the *Osmia* supplemented treatment occurring significantly earlier than in the wild pollinator treatment (t = - 165.71, d.f. = 637, $p < 0.001$). All fruit widths increased as a function of time (t = 37.56, d.f. = 637, $p < 0.001$) (Figure 2).

Post-harvest assessments

For all postharvest assessments block and plot were found to be non-significant in both years and were removed in factor reduction. Due to only two treatments being available, two tailed t-tests were utilised for analysis.

Weight and width - Fruit weight (2015: t = 5.66, d.f. = 935, $p < 0.001$; 2016: t = 3.46, d.f. = 633, $p < 0.001$) and width (2015: t = 5.12, d.f. = 934, $p < 0.001$; 2016: t = 5.81, d.f. = 633, $p < 0.001$) were both found to be higher in the *Osmia* supplemented treatment in both 2015 and 2016 (Figure 3). In 2015, however, there was no difference between treatments in the variability of individual cherry weight (F = 0.96, d.f. = 501, 434, $p > 0.05$) or width (F = 1.16, d.f. = 501, 433, $p > 0.05$). In 2016, the variability of fruit weight did not differ between treatments (F = 0.92, d.f. = 306, 385, $p > 0.05$), however width was found to be significantly more variable for fruit from the wild pollinators treatment compared to the fruit from the *Osmia* supplemented treatment (F = 1.35, d.f. = 306, 385, p = < 0.01).

Figure 3 Here

Sugar content and consistency - The cherries from all treatments met commercial requirements for sugar content (Sainsbury's Supermarkets Ltd, 2015), but Brix did not vary as a function of treatment in 2015 (t = 1.39, d.f. = 934, p >0.05) or 2016 (t = 1.16, d.f. = 633, p >0.05) (Figure 4). Likewise firmness did not vary as a function of treatment in 2015 (t = -1.28, d.f. = 937, p >0.05). Cherries from the wild pollinators and *Osmia* supplemented treatments were found to be equally variable for both sugar content and consistency in 2015 (Sugar content: F = 0.99, d.f. = 502, 432, p >0.05; consistency: F = 0.88, d.f. = 502, 435, p >0.05). In 2016 however, sugar content was found to be more variable for fruit in the treatment with wild pollinators alone (F = 16.92, d.f. = 306, 385, p <0.001).

Figure 4 Here

Colour - In 2015 and 2016 the colour of cherries varied between treatments (2015: H = 14.85, d.f. = 1, p <0.001; 2016: H = 13.22, d.f. = 1, p <0.001). Fruit from *Osmia* supplemented treatments were darker in colour than those harvested from the wild pollinator treatments in 2015, with this reversed in 2016 (Figure 5). However, fruit colour scored lower (overall lighter) in 2015. The variability in colour of cherries did not differ between treatments in either 2015 or 2016 (2015: F = 0.90, d.f. = 502,435, p >0.05; 2016: F = 1.00, d.f. = 306, 385, p >0.05) and all required quality standards were met.

Figure 5 Here

Discussion

The value of a sweet cherry crop at harvest is determined by yield, and quality characteristics of the produce (including weight, size, colour, sugar content, and firmness of the fruit), but simply meeting the set quality criteria is not sufficient to command the highest prices. The consistency between fruit in key quality factors is also an important consideration in commercial quality grading procedures determining the price paid to growers (Sainsbury’s Supermarkets Ltd, 2015). In this study, all the quality characteristics of cherries from trees subjected to wild pollinator only treatments, and those exposed to both *O. bicornis* and wild pollinators, were within the ranges required by retailers.

Very low numbers of naturally occurring insects from the main pollinator groups were recorded during flowering in the experiments conducted in both years, possibly reflecting the earlier flowering time of cherry trees which does not coincide with the main emergence period of most insects in the UK (Leather et al., 1995), and illustrating the importance of the core self-fertilisation in this variety. Slightly higher numbers were recorded in 2016 than in 2015, potentially linked to the higher ambient temperatures during the flowering period in that year. The low numbers present reduced the risk of the experiment being saturated by pollinators (i.e. achieving the maximum potential pollination irrespective of treatments imposed). Importantly, no significant differences were found between the numbers of these alternative pollinator species between treatments, which coupled with the low numbers present, gives confidence that they did not significantly affect the conclusions relating to the impact of *O. bicornis*.

Significant differences between treatments in some key characteristics were found. In both years fruit from the *Osmia* supplemented treatment were larger and heavier at harvest than those produced in the treatment with wild pollinators alone (Figure 3A, B). In 2015, no differences in fruit count were recorded between treatments (Figure 1E), indicating that the *Osmia* supplemented treatment resulted in a higher overall weight of cherries per unit branch length. This effect on total yield per unit branch length did not, however, occur in 2016, because fruit count was higher in the treatment with wild pollinators alone (Fig 1F) counteracting the impact of larger individual fruit weight in the *Osmia* supplemented treatment (Fig 3B). We therefore found consistent effects on quality of individual fruit but not on total yield.

The rate at which the fruit grew following fruit set did not differ between treatments in either year. In both years, however, differences between treatments in the mean timing of fruit set were recorded. Trees in *Osmia* supplemented plots completed fruit set earlier than those with wild pollinators alone. Flowering commenced at the same time in both treatments, but pollination occurred more rapidly after bud burst in supplemented pollinator plots, and fruit set from all flowers on a tree was completed during a shorter time window, particularly in 2016 (Figure 2).

The shortening of the pollination window established in this study will result in greater synchronisation of cherry development within the crop, and it has been suggested that in other crops this contributes to the production of more uniform fruit size and quality at harvest (Freihat, Al-Ghzawi, Zaitoun, & Alqudah, 2008; Hasegawa, Matsushita, & Kitajima, 2003; Stephenson, 1980). This study provides supporting evidence as improved developmental synchrony of sweet cherries from pollinator supplemented plots can be linked to fruit uniformity through significantly lower variability in fruit size and sugar content. However, significant effects on sugar content were only recorded in the year in which the largest differences between treatments in the length of the pollination window occurred (2016), and further work is required to establish both the factors influencing reliability of this outcome and its economic importance. In addition to improved market value, growers have commented that benefits are also accrued if synchronisation results in a larger proportion of the crop being ready for harvest within a narrow time range, reducing the number of passes pickers need to make and associated labour costs.

An increase in fruit quality has been reported from a variety of crops when *O. bicornis* contributes to pollination, partly a result of the mechanical action by which pollination is achieved increasing the amount transferred (Klatt et al., 2014; Kuhn & Ambrose, 1984; Wilkaniec & Radajewska, 1996). Higher levels of pollen deposition have been shown to increase fruit set and quality in some *Prunus* species, and the high pollination efficiency established by other studies may have contributed to the shortening of the pollination window when *O. bicornis* was released (Kuhn & Ambrose, 1984; Wilkaniec & Radajewska, 1996; Zhang, Tateishi, & Tanabe, 2010). The importance of pollen deposition may be amplified in “Stella” cherries, as other self-fertilising crop species, such as blueberries, have been shown to require higher levels of pollen grain deposition on the pistil when compared to cross pollinating varieties (Parrie & Lang, 1992). If a similar higher level of pollen grain deposition on the pistil is beneficial to self-fertile varieties of sweet cherries, then the very low numbers of wild pollinators present in this study make it unlikely that this would be achieved without the supplementary *O. bicornis*, explaining the differences in fruit quality recorded between treatments.

Although significant differences between treatments in fruit colour were recorded, they were not consistent between years, suggesting other factors may have influenced the findings. In 2015 fruit colour (an indicator of ripening) was darker in *Osmia* supplemented plots compared to those with only wild pollinators (Figure 5). Treatments were harvested simultaneously, suggesting that the earlier completion of fruit set in *Osmia* supplemented treatments resulted in optimal harvest time being slightly earlier. However, results from 2016 suggested over-ripening of cherries in the wild pollinator treatment compared to those in the *Osmia* supplemented treatment. Fruit firmness (as measured using a penetrometer) is also, in part, related to degree of ripening if harvest is late, but did not vary as a function of treatment in 2015 (Figure 4). Further work is required to improve understanding of factors influencing these important quality characteristics to support decisions on time of harvesting.

In conclusion the release of *O. bicornis* in cherry orchard plots significantly increased the quality of fruit produced by shortening the pollination window, resulting in greater size and uniformity, important fruit quality characteristics, compared to pollination by wild insects alone. Although the impact of *O. bicornis* pollination in other UK crops such as strawberry (Klatt et al., 2014) and apples (Garratt & Truslove, 2013) is more clearly established, the commercial potential of *Osmia* as a pollinator of cherries continues to be debated (Hansted et al., 2014). Further research is required to investigate yield and quality responses in both self–fertile and non-self-fertile *P. avium* varieties to support cost benefit analyses for its commercial use, and to enable comparisons with alternative managed pollinators. The effect of pollination treatment on shelf life of the crop, a key characteristic for both growers and retailers, also warrants investigation.

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Table captions

Table 1: The abundance of wild pollinators in the cherry orchard in 2015 and 2016. Figures for each treatment/insect category are the mean of 60 assessments (three standard sweep net samples taken in each of four treatment replicates, on each of five days) taken during the flowering period of the orchard. Insects were recorded under six categories, wild (non-*O.bicornis*) solitary bees, bumblebees (*Bombus* spp.), honey bees (*Apis mellifera*), hoverflies (Syrphidae), "other" diptera, and "other" insects (Hymenoptera (mainly parasitoids, Coleoptera and Neuroptera).

Figure captions

Figure 1:- Mean (± standard error) number of buds in 2015 (A) and 2016 (B), proportion of fruit set in 2015 (C) and 2016 (D), and mean total number fruit set in 2015 (E) and 2016 (F) on 50cm lengths of branch in *Osmia* supplemented and wild pollinator treatments. Treatments sharing the same letter did not vary significantly from each other (p >0.05)

Figure 2:- Increase in fruit width (mm) with time (sample week) in 2015 (A) and 2016 (B). + / —— = *Osmia* supplemented treatment; x / --- = wild pollinator treatment.

Figure 3:- Mean (± standard error) fruit weight (g) in 2015 (A) and 2016 (B), and mean fruit width (mm) in 2015 (C) and 2016 (D), on 50cm lengths of branch in *Osmia* supplemented and wild pollinator treatments. Treatments sharing the same letter did not vary significantly from each other (p >0.05)

Figure 4:- Mean (± standard error) post-harvest sugar content (% Brix) in 2015 (A) and 2016 (B), and consistency (pressure required to penetrate the fruit expressed as percentage of maximum pressure exerted by the penetrometer) in 2015 (C), on 50cm lengths of branch in *Osmia* supplemented and wild pollinator treatments. Treatments sharing the same letter did not vary significantly from each other ($p > 0.05$)

Figure 5:- Mean (± standard error) post-harvest colour measurements (Industry standard scale) in 2015 (A) and 2016 (B), on 50cm lengths of branch in *Osmia* supplemented and wild pollinator treatments. Treatments sharing the same letter did not vary significantly from each other ($p > 0.05$)

Table and Figures

Table 1:

Year	Treatment	Wild solitary bees	*Bombus* spp.	*Apis mellifera*	Syrphidae spp.	Other Diptera	Other insects
2015	*Osmia* supplemented	0	0	0.03	0.08	0	0
	Wild pollinator	0.02	0.02	0.07	0.23	0	0
2016	*Osmia* supplemented	0.12	0.03	0.2	0.08	2.48	0.83
	Wild pollinator	0.18	0.02	0.35	0.13	1.88	1.0

Figure 1:

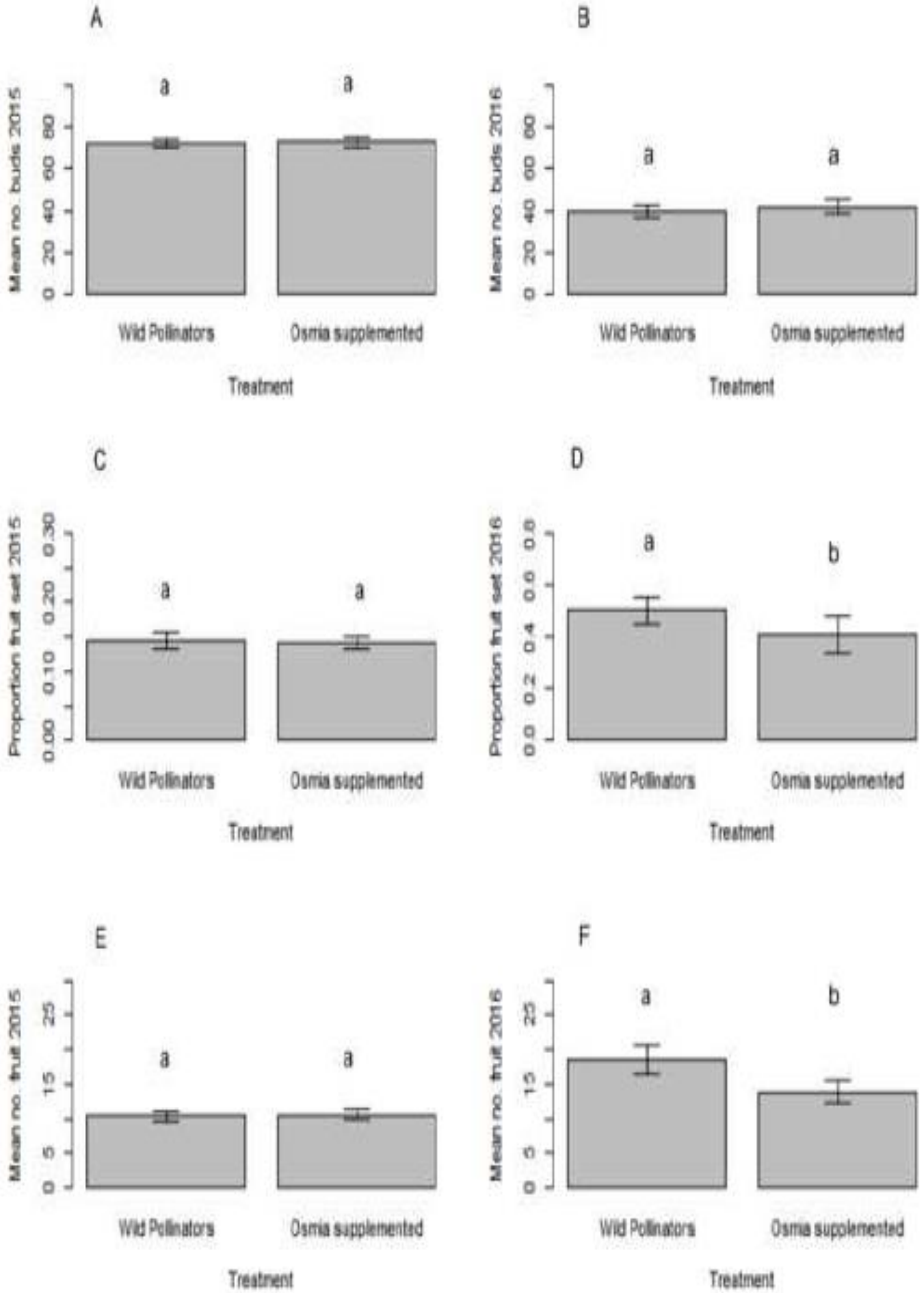
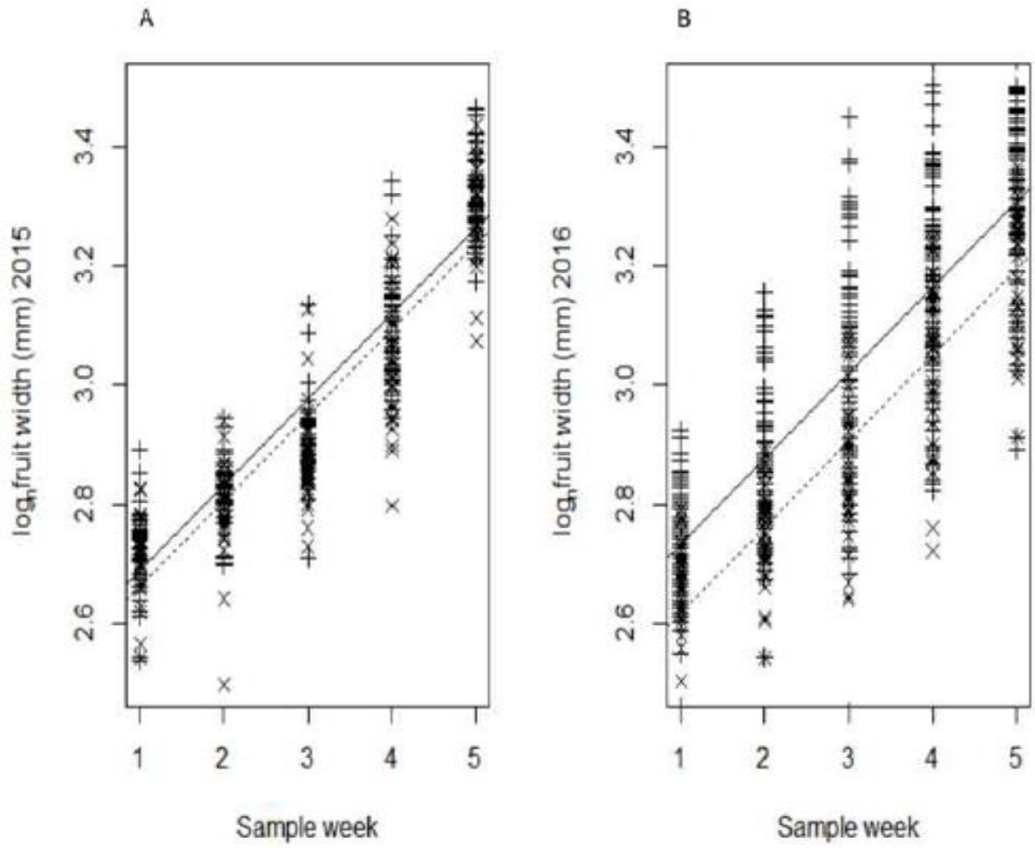

A
Mean no. buds 2015
0 20 40 60 80
a
a
Wild Pollinators
Osmia supplemented
Treatment
B
Mean no. buds 2016
0 20 40 60 80
a
a
Wild Pollinators
Osmia supplemented
Treatment
C
Proportion fruit set 2015
0.00 0.10 0.20 0.30
a
a
Wild Pollinators
Osmia supplemented
Treatment
D
Proportion fruit set 2016
0.0 0.2 0.4 0.6 0.8
a
b
Wild Pollinators
Osmia supplemented
Treatment
E
Mean no. fruit 2015
0 5 15 25
a
a
Wild Pollinators
Osmia supplemented
Treatment
F
Mean no. fruit 2016
0 5 15 25
a
b
Wild Pollinators
Osmia supplemented
Treatment

Figure 2:



A
B
fruit width (mm) 2015
fruit width (mm) 2016
Sample week
Sample week

Figure 3:

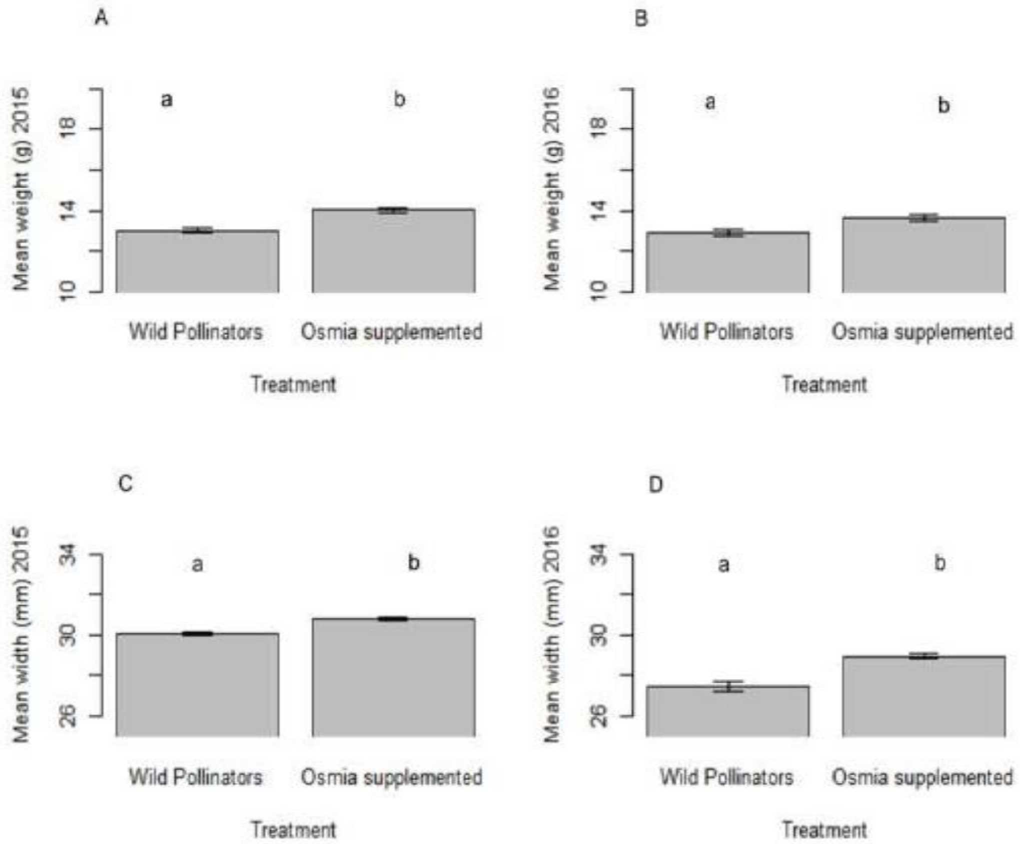
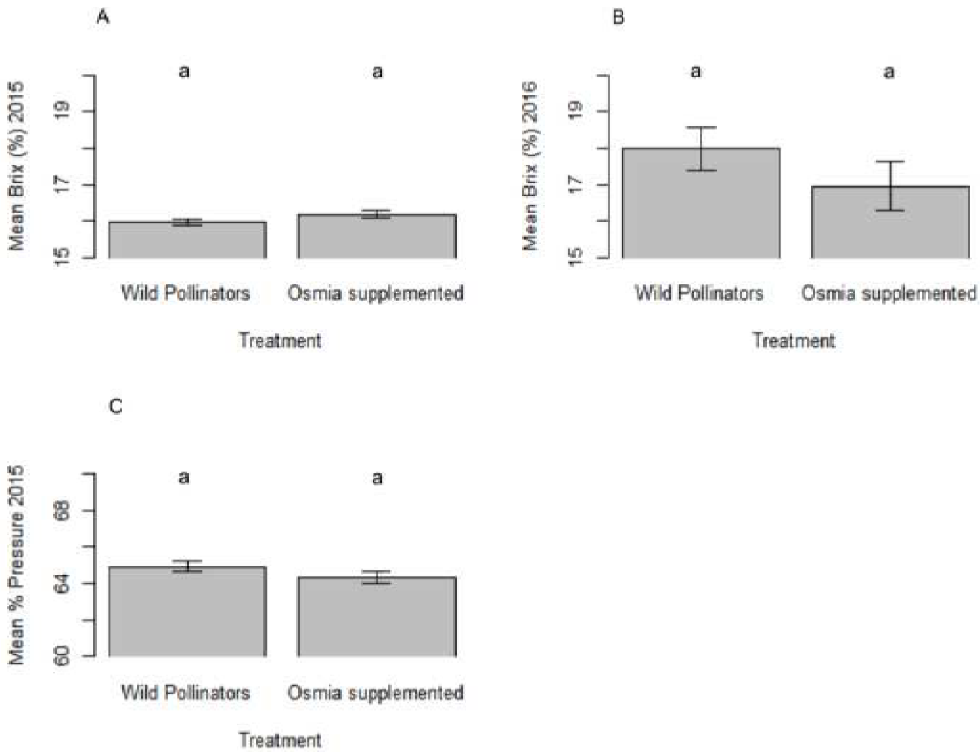
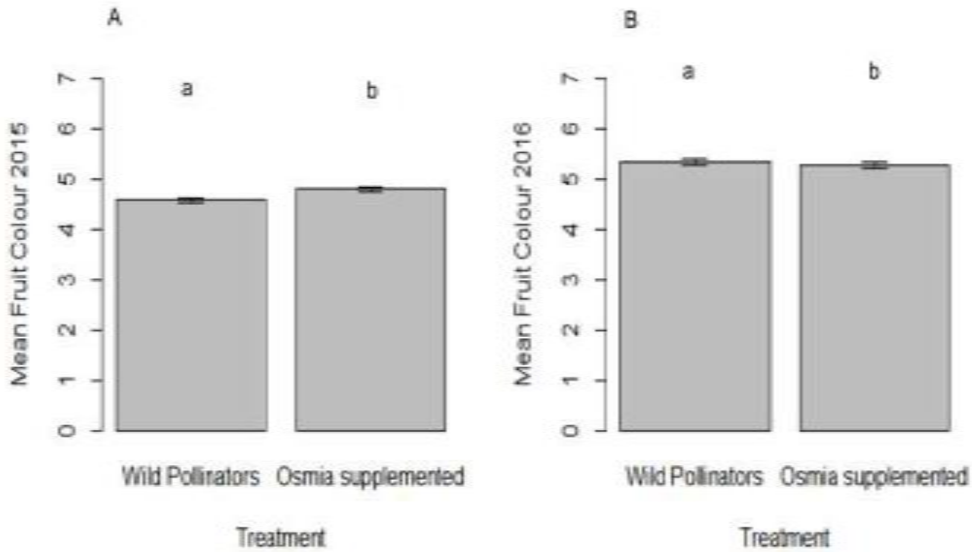

A
Mean weight (g) 2015
10
14
18
a
b
Wild Pollinators
Osmia supplemented
Treatment
B
Mean weight (g) 2016
10
14
18
a
b
Wild Pollinators
Osmia supplemented
Treatment
C
Mean width (mm) 2015
26
30
34
a
b
Wild Pollinators
Osmia supplemented
Treatment
D
Mean width (mm) 2016
26
30
34
a
b
Wild Pollinators
Osmia supplemented
Treatment

Figure 4:



A
a
a
Mean Brix (%) 2015
15
17
19
Wild Pollinators
Osmia supplemented
Treatment
B
a
a
Mean Brix (%) 2016
15
17
19
Wild Pollinators
Osmia supplemented
Treatment
C
a
a
Mean % Pressure 2015
60
64
68
Wild Pollinators
Osmia supplemented
Treatment

713 **Figure 5:**



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