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# An immunomarking method to determine the foraging patterns of *Osmia cornifrons* and resulting fruit set in a cherry orchard

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**Abstract** – The foraging patterns of *Osmia cornifrons* (Radoszkowski) (Megachilidae, Hymenoptera) were determined with an immunomarking method and correlated with fruit set in a commercial tart cherry orchard in Pennsylvania. Adults of *O. cornifrons* were self-marked with chicken egg-white protein powder from a dispenser nest box placed at the center of the study orchard at early bloom. Flower samples were collected from randomly selected trees ( $n=30$ ) located at different distances from the nest box. Flowers were analyzed for the presence of immunomarker protein with enzyme-linked immunosorbent assay. Foraging patterns were determined by measuring the distance and direction of marked flowers from the nest box. While marked flowers were found out to 55 m (maximum distance sampled), most marked flowers were found within 35 m from the nest and the percentage of marked flowers declined rapidly beyond that distance. Fruit density per limb cross-sectional area ( $\text{cm}^2$ ) in the study orchard was significantly higher than in the orchard without *O. cornifrons*, indicating the value of *O. cornifrons* as pollinators in increasing fruit yield in tart cherries.

**immunomarking / foraging patterns / *Osmia cornifrons* / self-marking procedure / pollination / pollinator / mason bees**

## 1. INTRODUCTION

*Osmia cornifrons* (Radoszkowski) (Megachilidae, Hymenoptera), commonly known as the horn-faced bee or the Japanese orchard bee, is a univoltine and solitary bee species. It is a manageable and commercially available alternative pollinator of rosaceous tree fruit species (Bosch and Kemp

2002; Mader et al. 2010; Maeta and Kitamura 1974; Sekita and Yamada 1993). In Japan, the development of *O. cornifrons* as a fruit pollinator started in the 1930s and increased in use from 10 % of the total apple production area in 1981 to over 80 % by 1996 (Maeta 1990; Sekita et al. 1996; Batra 1998). Introduced in the eastern United States from Japan in 1977 (Batra 1979, 1998) as an orchard pollinator, it has become naturalized in several states including Pennsylvania where natural emergence coincides with red maple and apricot bloom and continues through the end of

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apple bloom 3–4 weeks later (Biddinger et al. 2009a; Biddinger et al. 2011a, 2011b). It has more recently been introduced as a commercial pollinator of fruit in Korea and China (Xu et al. 1995). While this species nests in abandoned wood beetle galleries and other cavities in the wild, *O. cornifrons* can also be managed by providing nest boxes full of nest cavities (e.g., cardboard tubes, bamboo segments, pre-drilled wooden blocks, etc.) (Mader et al. 2010). These nest boxes can be held at cold temperatures to delay adult emergence and synchronize with later flowering target crops, such as apple and blueberry that bloom towards the end of the natural emergence period (Bosch et al. 2008; Mader et al. 2010).

The maximum distance from which nesting bees are able to find their nests, also known as homing range, is believed to be a good estimate of a bee's maximum foraging range and potential habitat size (Gathmann and Tschamtker 2002; Greenleaf et al. 2007; Guedot et al. 2009). However, homing range depends on various factors, including availability of floral resources. In the case of *O. cornifrons*, it was estimated to be approximately 500 m (Guedot et al. 2009) with effective pollination range of only 40 to 60 m in large orchards (Maeta and Kitamura 1974; Kitamura and Maeta 1969; Yamada et al. 1971). For a similar orchard pollinator, *Osmia cornuta*, adult bees foraged up to 400 m from the nesting sites (Maccagnani et al. 2003). In a similar study on *O. cornuta* foraging in a pear orchard, over 75 % of samples of *O. cornuta* had pear pollen in the fecal matter (Monzon et al. 2004), suggesting strong foraging activity within the orchard where nesting shelters were placed. In the case of *O. cornifrons*, in spite of considerable research in Japan on its ecology and management in commercial tree fruit orchards (Yamada et al. 1971; Yamada et al. 1984, Maeta 1978, Maeta and Kitamura 1974), its foraging behavior in the fruit growing regions of the eastern USA or elsewhere is not well known. Estimating foraging distance of *O. cornifrons* in tree fruit species (e.g., cherry and apple) and their impact on fruit set could be helpful in determining nest density and placement of managed nest aggregations (multiple nest blocks in a nest shelter) in commercial orchards,

as well as the pollination limitations of wild populations flying into commercial orchards from adjacent habitat.

In the past, researchers have used a variety of techniques to quantify the movement of different insect species. Most of these techniques (e.g., mark–release–recapture using trace elements) had numerous drawbacks, particularly related to their high cost of implementation or extensive technical expertise needed for their operation (Akey et al. 1991). Moreover, some of these techniques require capturing and killing foraging bees. In large colonies of social bees using non-reproductive workers as foragers consequences to the colony are likely insignificant, but the capturing and killing of foraging solitary bee females also means killing potential offspring. Recently, a highly effective protein-based marking (immunomarking) method for studying dispersal of insect fauna was developed (Hagler et al. 1992; Hagler 1997; Hagler and Jackson 1998). In this method, experimental insects or their habitat were marked with a vertebrate-specific protein marker, and the recaptured insects were examined for the presence of the marker antibodies using a highly sensitive enzyme-linked immunosorbent assay (ELISA) procedure. In the early phase of research and development of this marking technique, costly rabbit IgG and chicken IgG proteins were widely used as marker proteins in mark–release–recapture studies (Hagler et al. 1992; Hagler 1997; Blackmer et al. 2004; Hagler and Naranjo 2004; Buczkowski and Bennett 2006). These markers were costly, however, so recently, inexpensive protein markers such as chicken egg whites, bovine milk protein, and soy milk protein have been employed for mark–release–recapture studies (Jones et al. 2006; Hagler and Jones 2010).

The immunomarking (protein-based) mark–release–recapture method has been used in determining dispersal and movement of a wide range of insect pests of fruit crops including adult pear psylla (*Cacopsylla pyricola*), codling moth (*Cydia pomonella*) (Jones et al. 2006), and glassy-winged sharpshooter, *Homalodisca vitripennis* (Blackmer et al. 2006).

Hagler et al. (2011a, 2011b) used a self-marking mechanism to apply unique fluorescent powders or protein markers to *Apis mellifera* as they left the hive to determine foraging ranges by capturing individual bees. Our study used this technique to mark *O. cornifrons* females as they foraged from a nest shelter placed in the center of a tart cherry orchard. *O. cornifrons* are much more wary and energetic in the field than *A. mellifera* (Biddinger, personal observation) and are capable of visiting up to 15 flowers per minute (Maeta and Kitamura 1974). This makes them difficult to capture in the field. So, rather than capturing the bees, we collected flowers at set distances from the nest box to test for the presence of the marker to see where the bees had visited. By looking at the percentage of flowers marked, we hoped to quantify pollination range limits at which a single nest box of *O. cornifrons* could effectively pollinate the crop (without killing the bees or disrupting their behavior).

The information related to foraging ranges of *O. cornifrons* is critical in developing management guidelines (e.g., how far apart to place nests) for using *O. cornifrons* as a managed pollinator of tree fruits. *O. cornifrons* is abundant in forest edges adjacent to fruit orchards in some parts of Pennsylvania and many fruit growers are relying on it and other unmanaged populations of on non-*Apis* bees to pollinate cherries, apples, and other tree fruit (Ritz et al. 2012; Biddinger et al. 2010, 2011a; Joshi et al. 2011). Understanding its foraging range in flowering orchards is also necessary to understand how much and how far pollination services may be provided by bees nesting in wooded areas adjacent to orchards.

Specifically, we investigated (1) the appropriateness of a self-marking system for *O. cornifrons*; (2) the relationship between the proportion of immunomarked flowers, distance and direction from the nest and, thus, the foraging patterns of *O. cornifrons* in a tart cherry orchard during early stage of bloom; and (3) the relationship between the proportion of marked flower samples and the fruit density per limb cross-sectional area (cm<sup>2</sup>) of sampled trees as a measure of yield over the marked foraging area and as a measure of pollination efficiency when these trees

were compared to cherry trees of a control orchard block without *O. cornifrons*.

## 2. MATERIALS AND METHODS

### 2.1. Study orchards

Field and laboratory studies were conducted in Adams County, Pennsylvania in a commercial and a research tart cherry, *Prunus cerasus* L., orchard located adjacent to and on the Pennsylvania State University Fruit Research and Extension Center during the 2011 growing season. The nest box with the protein marker dispenser was placed in a 7.4-ha commercial Montmorency cherry orchard on Mahaleb rootstock that was planted in 2004 at a spacing of 4.9 m by 7.2 m (287 trees/ha). A 0.25-ha control orchard planted in 2006 with the same tart cherry variety, on the same rootstock and with the same row spacing was located on the Penn State Fruit Research and Extension Center. The control block was 600 m away from the commercial orchard and from the nearest *Osmia* nesting habitat. Timed counts (30 min) of bees visiting cherry flowers in each cherry block found equivalent levels of *A. mellifera* in both blocks with commercial hives located equidistant from both blocks. The timed counts of bees were performed by randomly walking through the whole area of the study and control orchard blocks. In the year of the study, honey bees were not brought into the station until after cherry bloom for the beginning of apple bloom, so neither cherry block had stocked honey bees. Other pollinator species (several species of *Andrena*) were found infrequently along the borders of the commercial cherry block, but not in the control block on the research station. During the study period, meteorological data (wind speed, wind direction, and air temperature) were recorded by a Weather Station of the Penn State Fruit Research and Extension Center located adjacent to the study orchard.

### 2.2. Study insects

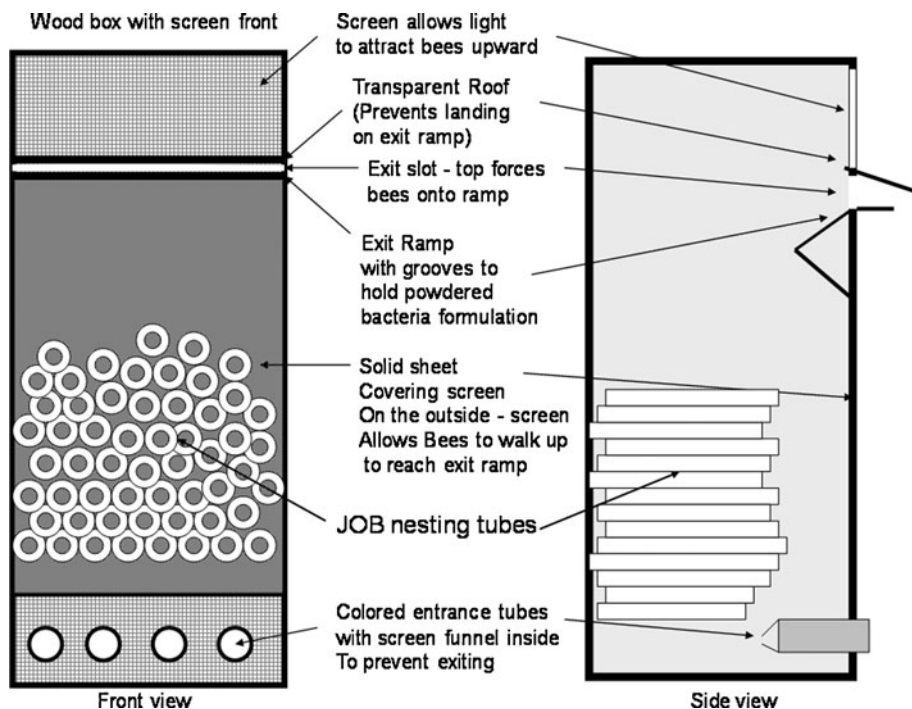
The overwintering adults of *O. cornifrons* (1,200 loose cocoons extracted from nest tubes of which a subsample reared to eclosion in cages, indicated a 59:41 ratio of males to females, and about 10 % mortality in the

cocoon phase) used in this study were obtained by trapping wild populations of *O. cornifrons* from several nearby Adams County orchards the previous spring using *Osmia* BinderBoards® (Pollinator Paradise, Parma, ID, USA). Binderboards® are laminated wooden blocks with 49 holes (8 mm diameter and 150 mm depth) lined with paper straws. The nests were left in the orchards all season from mid-April through October and then gathered into a screened insectarium in October to overwinter under ambient conditions. The number of *O. cornifrons* released for this study was based on recommended number of females needed to pollinate 1 ha of apples in Japan (Yamada et al. 1971).

### 2.3. Nest placements

A nest box with dispenser (for the powdery chicken egg white protein formulation), previously tested to use *O. cornifrons* as a delivery system for a powdered formulation of *Bacillus subtilis* for the biological control of fireblight in apple and pear orchards (Biddinger et al. 2009b, 2010), allowed the exiting bees to self-mark (Figure 1). This simple wooden structure was modified

from a design by Maccagnani et al. (2006) who used it to test fireblight biological control using *Bombus* as the dispersing agent, but we modified it to accommodate the much smaller *O. cornifrons*. Three empty BinderBoard® wooden blocks, each with 49 holes (8 mm) lined with paper straws for nesting were placed within the main body of the nest dispenser. Loose *O. cornifrons* overwintering cocoons ( $n=1,200$ ) were placed inside the nest box shelter in two emergence cardboard boxes each with two 10 mm emergence holes. A transparent plastic exit ramp with a removable piece of 10-mm-thick plexiglass with six shallow channels (4 mm deep) was loaded manually with the fine egg white powder in the early morning before flight and again at mid-day. To force the bees to move through the channels and pick up the egg white powder rather than directly flying out of the ramp opening, a piece of clear acetate, used for overhead projectors, was taped over the opening, sloping down to the edge of the ramp to force bees to exit through the constricted opening formed by the acetate and grooved plexiglass plate. This plexiglass plate could be moved freely in and out of a groove formed by aluminum channel plates on each side and rested upon another piece



**Figure 1.** *Osmia* nest box used in the study (Source: Penn Fruit News). *JOB* Japanese orchard bee (*O. cornifrons*).

of plexiglass of the same dimensions that was permanently affixed to the outside of the nest box. This allowed quick and easy removal and filling of the grooves with the egg white powder without disturbing the bees. The entrance tubes at the bottom of the nest box were made of PVC tubing with an inside diameter of 9 mm and were marked with different dark colors to help females “remember” the correct entrance hole from their initial orientation flight and return directly to a specific tube opening. In the fireblight biocontrol study, Biddinger et al. (2010) observed that 95 % of the bees exited the nest properly using the ramp and were exposed to powder, but only about 50 % returned to enter the lower tubes properly. The other half used the exit grooves to re-enter. Proper exit behavior and contact with the biocontrol product was by far the most important aspect of the dispenser. In the present study, the nests with *O. cornifrons* cocoons were placed in the study orchard approximately 2 weeks prior to immunomarking in order to allow for emergence, mating, and nest establishment prior to cherry bloom. Adult bees were observed foraging on weeds in the ground cover prior to cherry bloom.

#### 2.4. Marking of bees

*O. cornifrons* adults were self-marked with chicken egg whites (Deb-El Foods Corporation, Elizabeth, NJ, USA). Egg white marker was placed in the grooves of the plexiglass plate 30 h prior to flower sample collection. Early bloom and the short exposure period was chosen to maximize the possibility of bees moving to fresh flowers, for flowers to be sampled before senescence, and to minimize secondary movement of the marker to new flowers by other pollinators such as honey bees and the few Andrenid bees that were present. Excluding night hours when the bees do not fly, flight was possible during the 19 h of daylight, but 3 h were below the 10 °C threshold for flight. Of the remaining 16 h, wind speed above 10 km/h with wind speed gusts of over 24 km/h minimized flight for about 10 h, so actual optimal foraging time before the flower samples were collected was approximately only 6 h.

#### 2.5. Sample collection and handling

Sampling points ( $n=30$ ) at different distances were randomly selected in three concentric zones (15, 35, and

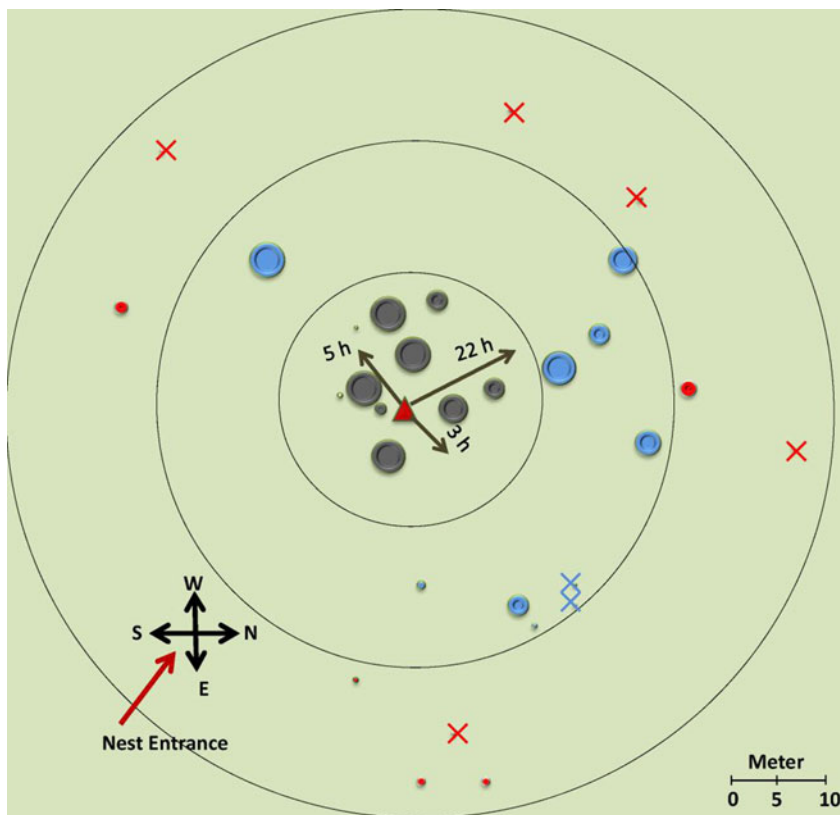
55 m radius from the *Osmia* nest box) in the study orchard (Figure 2). The closest distance sampled was 4.95 m from the nest box. In each zone, ten randomly selected trees were sampled comprising single-tree replications. From each replicate tree we randomly collected 50 samples (five cherry blossoms/sample) totaling 250 blossoms per tree. Thus a total 1,500 samples (7,500 blossoms) (500 samples/concentric zone) were collected from 30 trees for analysis. In all samples, each blossom was individually plucked from trees with the help of a flat-tip straight forceps (made of stainless steel). Blossoms were randomly selected and were collected from the interior and exterior canopy of sample trees. Forceps were cleaned in alcohol (70 %) when moving to new trees and only the base of the flower was touched while collecting to avoid cross-contamination.

All samples of cherry blossoms were collected into plastic sample bags with mesh dividers (Agdia Inc., IN, USA), and were immediately placed in a cooler containing ice in field. These samples were brought to the laboratory and were kept in a -80 °C storage facility (Thermo LabSystems, MA, USA). ELISA was used to determine the presence of protein marker chicken egg albumin (CEA).

#### 2.6. Laboratory assays

Standardization of bioassays for *O. cornifrons* marked flowers was conducted in a set of preliminary laboratory studies, and the ELISA procedure (Jones et al. 2006; Hagler and Jones 2010) described below was optimized for extracting and quantifying the immunomarker from flower samples collected from cherry trees.

Extractions of CEA from cherry blossoms were performed with 10 mL of TBS with 1 % EDTA at 27 °C for 3 min. Eighty microliters of sample were then transferred to ELISA plates (Plates, Nunc-Immuno Plate MaxiSorp Surface (NUNC Brand Products, A/S, Roskilde, Denmark)). All samples and controls were run in triplicate. Negative controls were prepared from unmarked cherry blossoms sampled from a control orchard. Incubation was done for 1 h at 37 °C. Plates were washed 5× with PBST using a Dynex multi plate washer (Thermo LabSystems). Plates were blocked with 200 µL of PBS-BSA (1% BSA) at pH 7.4 (Sigma) and incubated overnight at 4 °C. After blocking, plates were washed 5× with PBST. All reagents were prepared in



**Figure 2.** Map of the study area where red triangle is *O. cornifrons* nest dispenser box and circles represent sampled trees. Diameter of each circle represents protein marking (%) found on that tree ranging from 0.01–100 % and X represents zero protein marking. Arrows indicate the wind direction and associated number is averaged duration of wind in that direction. Red arrow is showing the bee entrance direction. Sample points in dark gray, blue, and red color represent sampled trees in concentric zone of 15 m (inner), 35 m (middle), and 55 m (outer) radius, respectively from the nest box.

filtered double-distilled water (Thermo Nanopure system). Anti-CEA prepared in rabbit at 1:8,000 dilution (Sigma No.C-6534) prepared in PBS–BSA (1 %)+1.3 ppm Silwet L77 (1.3  $\mu\text{L}/\text{mL}$ ) was added per well and incubated for 1 h at 37  $^{\circ}\text{C}$ . Plates were washed 5 $\times$  with PBST. Goat anti-rabbit IgG (Sigma No. A-6154) conjugated to hydrogen peroxidase prepared 1:12,000 dilution in PBS–BSA (1%)+(1.3  $\mu\text{L}/\text{mL}$ ) 80  $\mu\text{L}$  per well was added and incubated for 2 h at 37  $^{\circ}\text{C}$ . Plates were washed 5 $\times$  with PBST. Eighty microliters per well of substrate (TMB Substrate) were incubated at 15 and 30 min and read with microplate reader (Multiskan Ascent, Thermo Labsystems) at 650 nm. Blossom samples were considered

marked when the absorbance reading was twice the negative check.

## 2.7. Horticultural measurements in study orchard

### 2.7.1. Measurement of percent bloom

Estimates of percent bloom were determined at the time of immunomarked *O. cornifrons* release as well as at the time of flower sample collection. Percent bloom on the day of release of immunomarked *O. cornifrons* was determined by counting fully opened flowers and

flower buds until  $n=100$  was reached and then calculating the proportion of fully opened flowers on five randomly selected limbs of all 30 sample trees. Similarly, percent bloom at the time of flower collection was calculated from all trees in the same manner.

### 2.7.2. Fruit density measurement in the study orchard

The fruit density per limb cross-sectional area ( $\text{cm}^2$ ) of sample trees in the study orchard was compared with sample trees ( $n=30$ ) of a control orchard block at the time of fruit harvest. Sample trees in control orchards were randomly selected from all directions from the center and in similar concentric zones as study orchard. At that time, three limbs were randomly selected from each sample tree, the total number of fruit per limb was counted and the limb cross-sectional area was measured (Schupp and Greene 2002). Limb cross-sectional area and fruit density were determined as follows:

$$\text{Limb cross-sectional area} = \frac{(\text{Limb circumference})^2}{4\pi} \quad (1)$$

$$\text{Fruit density} = \frac{\text{Total number of fruits}}{\text{Limb cross-sectional area (cm}^2\text{)}} \quad (2)$$

## 2.8. Statistical analysis

The relationship between distance from the point of release (i.e., nest box) of *O. cornifrons* and the proportion of immunomarked cherry flower samples collected from different sampling points (i.e., cherry tree) was determined by using a linear regression analysis. This regression analysis was also used to analyze the data related to the percent bloom at the time of sample collection and distance from the nest box. A polynomial regression analysis (SigmaPlot® 11, Systat Software, Inc., Chicago, IL) determined the relation between the distance of sample trees from the nest box and the fruit density per limb cross-sectional area ( $\text{cm}^2$ ). A linear regression analysis was used to determine the relation between fruit density per limb cross-sectional area ( $\text{cm}^2$ ) and the proportion of

protein marked samples. A *t* test compared fruit density per limb cross-sectional area ( $\text{cm}^2$ ) of study orchard and control orchard block.

## 3. RESULTS

### 3.1. Foraging of *O. cornifrons* in cherry orchard at the time of bloom

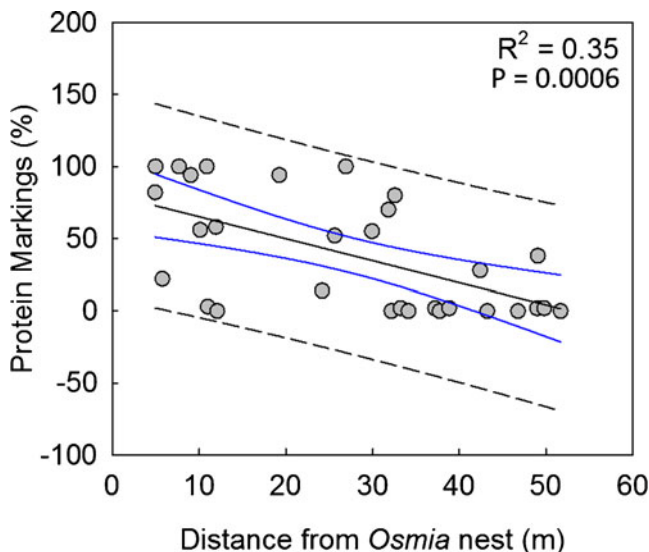
Percent bloom at the time of sample collection was evenly distributed throughout the study orchard and did not show any significant relationship with distance from *O. cornifrons* nest box ( $P=0.42$ ,  $R^2=0.024$ , slope=0.038, intercept=6.76). During the study, the average wind direction was northwest for 22 h of the 30-h foraging period (Figure 2).

The proportion of protein marking decreased with increasing distance from *O. cornifrons* nest box (illustrated by circles whose diameter corresponds to the proportion of protein marking in Figure 2). Trees at the medium distance (16–35 m radius) from the nest box of *O. cornifrons* showed greatest variability in protein marking (Figure 2). The proportion of protein-marked flowers from trees at increasing distances from the nest linearly decreases ( $R^2=0.35$ , slope=1.52, intercept=80.38,  $P=0.0006$ ; Figure 3). There was a sharp decrease in the proportion of protein markings beyond 35 m from the nest box (Figure 3).

### 3.2. Relationship between distance from nest box, proportion of immunomarked flower samples, and fruit density

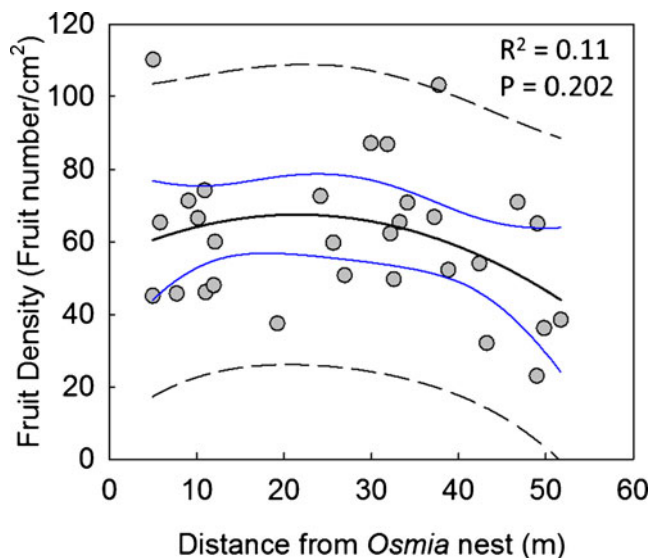
There was no statistically significant declining trend ( $P=0.20$ ,  $R^2=0.11$ ) in the mean fruit density (in terms of mean number of fruit per limb cross-sectional area) with increasing distance from *O. cornifrons* nest box (Figure 4). There was no trend in average fruit density within 40 m from *O. cornifrons* nest box; however, a decline in fruit density after 40 m from *Osmia* nest box was recorded (Figure 4). The mean fruit density of sampled trees in the study orchard did not show





**Figure 3.** Foraging of *O. cornifrons* as quantified from the proportion of protein marking in flower samples collected from 30 trees (sampling points) at different distances within a 55-m radius area from *O. cornifrons* nest placed at the center of a commercial tart cherry orchard in Adams County, Pennsylvania. *Inner solid lines* represent mean and 95 % confidence interval and the *outer dashed lines* represent the prediction band.

any trend with proportion of protein markings on sampling distance point ( $R^2=0.02$ , intercept= 57.65, slope=0.076,  $P=0.42$ ).



**Figure 4.** Relationship between the mean fruit density [number of fruits/limb cross-sectional area (cm<sup>2</sup>)] and different distances (sampling points) within 55 m radius area from *O. cornifrons* nest box. *Inner solid lines* represent mean and 95 % confidence interval and the *outer dashed lines* represent the prediction band.

### 3.3. Fruit yield in *O. cornifrons* pollinated orchard and control orchard block

The study orchard with the *O. cornifrons* nest box showed significantly greater fruit density on the trees that were sampled for protein marking (mean=60.57 fruits/cm<sup>2</sup> of tree limb, SD=19.89,  $P=0.000$ ,  $t=7.146$ ,  $n=30$  trees) than the control orchard block without *O. cornifrons* nest (mean=29.11 fruits/cm<sup>2</sup> of tree limb, SD=13.63,  $n=30$  trees).

## 4. DISCUSSION

The results of this study showed that the foraging range and behavior of *O. cornifrons* adults in a cherry orchard can be quantified by using an immunomarker. In a manner similar to studies which have successfully used *Osmia* sp. as a vector to disperse bacterial biological control agents of fire blight (Biddinger et al. 2009a, 2010; Maccagnani et al. 2006), *O. cornifrons* adults were able to acquire the fine egg white particles on their bodies and disperse these particles to cherry flowers at levels high enough to be detected. Wind direction appears to influence the distribution pattern of immunomarking in this study. Wind plays a crucial role in the dispersal of insects in field environment (Schneider 1962), especially for a relatively small-bodied bee attempting to return to a nest flying into a stiff wind. Though, in our study, the *O. cornifrons* nest was sheltered from the wind with a plastic shelter from all directions except the front (facing southeast), that wind direction and speed affect the foraging behavior of *O. cornifrons* in an orchard is evident from the majority of the marked blossoms being detected downwind from the nest box (Figure 2). Average wind duration for N-W direction was 22 h of the 30-h foraging period with most of the foraging taking place in the 4 h that wind speed was below 10 kmh. Therefore, effect of wind speed and direction on *O. cornifrons* dispersal as quantified in terms of the proportion of immunomarker found in the flower samples in field environment needs further investigation.

Our results suggest that the protein marking decreased with increasing distance from *O.*

*cornifrons* nest box (Figure 2). Results from the marked bloom samples show a decline in fruit yield at those same distances after 40 m and indicate the effective foraging range of approximately 500 *O. cornifrons* females in a commercial tart cherry orchard to be about 35–40 m under the relatively short foraging period allowed in this experiment and under the relatively cool, windy conditions that prevailed. This range estimate correlates well with visual observations made for *O. cornifrons* in Japanese apple orchards of 40–60 m from the nests, as well as the recommendation to place nests 100 m apart (Yamada et al. 1971; Maeta and Kitamura 1974). Several other methods of studying dispersal tend to overestimate foraging distances, but the immunomarking method may underestimate them, as the number of flowers visited in a foraging bout increases, the amount of immunomarking on the bee's body will decrease. So these bees may visit other flowers in the study orchard beyond 35–55 m, while no longer depositing immunomarking powder. However, this immunomarking dilution effect needs further investigations.

The slightly lower foraging range we found compared to the Japanese studies may be due to the limited foraging time we allowed our bees during this study compared to the entire bloom period for the Japanese studies or due to differences in cherry and apple as a crop. Such short range foraging of *O. cornifrons* in cherry orchard could be due to the presence of more blossoms per tree than other fruit trees such as apple. In general, *Osmia* bees may fly long distances while foraging, but when sufficient pollen resources/host flowers are present near their nesting sites (such as in this study) they may prefer foraging close to their nesting sites instead of flying longer distances (Zurbuchen et al. 2010, Radmacher and Strohm 2010). Besides number of blossoms on a tree, differences between the aforementioned studies could be due to tree planting/density and bee shelter location within the orchards.

In previous studies, foraging distance was estimated by calculating homing ranges, the maximum distance from the nest that a bee can find its way home. However, this overestimates

foraging range. Greenleaf et al. (2007) reviewed 96 published records for 62 bee species that evaluated maximum foraging/homing distances, and found a highly significant positive relationship between the bee body size and various methods of measuring homing/foraging limits. Foraging range for a bee, however, is dependent on multiple factors such as the spatial and temporal distribution and availability of resources during its life cycle. For example, while the homing range of *O. cornuta* (Latreille) was estimated at 1,800 m, when placed in an orchard during peak bloom with abundant floral resources, most females forage within 100–200 m of their nests (Vincens and Bosch 2000). The homing range of the smaller *O. cornifrons* (Radoszkowski) in Japanese apple orchards was likewise found to be approximately 500 m, but likewise, its effective pollination range in large orchards was estimated to be only 40 to 60 m from the nesting site (Maeta and Kitamura 1974; Kitamura and Maeta 1969; Guedot et al. 2009). The foraging ranges of several stingless bee species have likewise been shown to be approximately 300 m shorter than their homing distance (van Nieuwstadt and Ruano Iraheta 1996).

The *O. cornifrons* nest box remained in the orchard throughout the 7-day bloom period thus the yield data should have been an estimate of the full pollination potential of that nest beyond the 30-h test period. Our study found the number of cherries per limb cross-sectional area to be over twice as high on trees in the *Osmia* test orchard than in a control orchard very similar in age, size, and management and where equivalent levels of honey bees were present. While comparing these two orchard blocks, any minor differences between blocks should have been minimized by the horticultural method of relating fruit number to the scaffold limb cross-sectional area (Schupp and Greene 2002). This control block was isolated by distance from habitat containing wild populations of *O. cornifrons* and none had been released, so the effects on fruit number is directly attributable to the additive effect of having *O. cornifrons* supplementing the level provided by honey bees alone. Similarly, a related species, *Osmia lignaria* Say doubled cherry yields in Utah over a 5-year period over that of *A. mellifera* Utah (Bosch and

Kemp 2006). They attributed this to shorter foraging range that forced it to concentrate on fruit trees within the targeted orchard, a greater fidelity to fruit trees when foraging, longer foraging period due to its lower light and temperature thresholds, and in its greater effectiveness in transferring pollen (Torchio 1981; Kuhn and Ambrose 1984).

Other factors contributing to higher fruit density (yield) in the study orchard could be the abundance of additional native pollinators, but net collections of bees in both the test and control orchard during bloom found that while honey bee numbers were fairly abundant and similar in both blocks, solitary bees and bumble bees were not found in the areas sampled of either cherry block.

This study demonstrates an immunomarking method to mark bees and quantify where they have been by measuring the residues they leave on flowers is viable and a powerful tool in understanding the foraging behavior and limits of bees. Our data based on the marking and yield data suggest that the effective pollination range of 500 *O. cornifrons* females from a single nest box is only about 40 m rather than the 500 m estimate based on homing range. This translates into an effective foraging/pollinating area of slightly more than 0.5 ha for each nest and we would recommend placing nests at a slightly closer range of 80 m apart rather than 100 m apart as recommended for apple pollination currently in Japan. Future research into optimizing release rates of female *O. cornifrons* in nests may find the optimal number of bees needed for commercial pollination of various fruit crops in the eastern USA. This number may vary by crop. For example, since tart cherry needs 20–75 % of flowers set for a commercial crop and has more flowers per tree than apple, which needs only 2–8 % set, the number of *Osmia* necessary for pollination might be much higher in cherry (Chaplin and Westwood 1980).

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**Technique d'immunomarquage pour déterminer les modes d'approvisionnement d'*Osmia cornifrons* et la production de fruits en résultant, dans un verger de cerisiers**

**Technique de marquage / approvisionnement / processus d'auto-marquage / pollinisation/ guêpe maçonne / pollinisateur**

**Eine Immuno-Markierungsmethode zur Bestimmung des Sammelverhaltens von *Osmia cornifrons* und der daraus resultierende Fruchtansatz in einer Kirschplantage**

**Immunomarkierung / Sammelverhalten / *Osmia cornifrons* / Selbst-Markierung / Bestäubung / Bestäuber**

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