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Research paper

# Cultivar placement affects pollination efficiency and fruit production in European pear (*Pyrus communis*) orchards



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#### ABSTRACT

European pear (Pyrus communis) requires insect pollination among compatible cultivars for fruit production. However, most commercial orchards have a limited number of cultivars arranged in monotypic blocks or rows. This can result in insufficient inter-cultivar pollination. We hypothesise that limitations in pollen transfer among cultivars could be explained by both insect behaviour and orchard design. We compared insect activity and pollination efficiency in two European pear cultivars, in orchards with different designs: (i) cultivars alternated in the same row or (ii) cultivars in separate rows. To assess limitations in pollen transfer, we also compared hand pollination with compatible pollen versus open pollination by insects. Insect visitors mainly foraged on neighbouring trees within a row, with few movements across rows (1%). Honey bees (Apis mellifera) and bumble bees (Bombus terrestris) visited significantly more flowers per tree (8.5 vs. 3) and more trees (2.1 vs. 1.3) than solitary bees (Andrena spp.) and hoverflies. Insect visitors deposited large amounts of pollen (~500 pollen grains) on flower stigmas regardless of the insect type. Cultivar placement affected inter-cultivar pollination; less incompatibility signs were observed when cultivars alternated in the same row (5%) than when cultivars were in separate rows (38%). We observed limitations in pollen transfer as open pollination resulted in significant reduced fruit set, compared with hand pollination, in 'Conférence' (21% vs. 30.7%) and 'Doyenné du Comice' (7.2% vs. 16.8%). The foraging behaviour of the insects limited thus inter-cultivar pollen transfer in the orchards with cultivars in separate rows. Cultivars used for pollination (pollinizers) should be planted in the same rows as the main cultivar to increase inter-cultivar pollination.

#### 1. Introduction

Insect pollination increases the productivity of about 75% of crop species (Klein et al., 2007). Pollinators are particularly important in orchards, as most fruit trees exhibit self-incompatibility (Delaplane and Mayer, 2000) and require pollination between compatible cultivars to ensure fertilization and fruit production (Delaplane and Mayer, 2000; Stern et al., 2007). Honey bee (Apis mellifera) colonies are commonly used to facilitate pollination in orchards, but other insects, such as bumble bees (Bombus spp.), solitary bees (Andrena spp., Osmia spp., Lasioglossum spp., Colletes spp., etc.), and non-bee insects (like syrphids), also contribute to pollination in fruit trees (Blitzer et al., 2016; Mallinger and Gratton, 2015; Martins et al., 2015; Park et al., 2016; Rader et al., 2016). Pollinator diversity improves overall pollination leading to higher fruit production (Blitzer et al., 2016; Garibaldi et al., 2013; Mallinger and Gratton, 2015; Martins et al., 2015). Different pollinator species might complement each other in terms of spatial or temporal distribution and foraging behaviour, and thus affect pollination efficiency (Brittain et al., 2013; Martins et al., 2015).

To facilitate inter-cultivar pollination, growers plant compatible pollinizer cultivars along with the commercial cultivar of interest. The pollinizer cultivars need to produce viable, compatible pollen and flower at the same time as the main cultivar (Webster, 2002). Most commercial fruit tree orchards consist of a limited number of cultivars planted in monotypic blocks or rows to facilitate management (Kron et al., 2001a). In such orchards, pollen must be dispersed between rows or blocks for effective pollination (Kron et al., 2001a). However, gene flow studies have shown that pollen dispersal mainly occurs between neighbouring trees (Jackson, 1996; Kron et al., 2001b) due to the foraging behaviour of honey bees. Honey bees most often forage within a tree and then move to the next tree within the same row (Brittain et al., 2013; Cranmer et al., 2012; Free, 1960). This foraging pattern tends to transfer mostly incompatible pollen, due to the placement of the commercial cultivar trees within the same row (Brittain et al., 2013) and limits inter-cultivar pollination. Thus, the placement of cultivars in the orchard, as well as the diversity and behaviour of pollinator insects,

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must be considered when attempting to improve pollination efficiency in fruit trees.

Most pear cultivars require insect pollination due to gametophytic self-incompatibility (Delaplane and Mayer, 2000; Goldway et al., 2009; Quinet et al., 2014) though some, such as 'Conférence', have a natural tendency for parthenocarpy resulting in seedless fruits (Deckers and Schoofs, 2002; Nyéki and Soltész, 1998; Quinet and Jacquemart, 2015). Fertilization and seed set affect fruit size and quality, and thus the marketability of the fruits (Monzón et al., 2004; Stern et al., 2007; Theron, 2011). Honey bees are the most important pollinators of pear, although other bee species, such as bumble bees (Bombus terrestris) and solitary bees (Osmia cornuta), have also been found to be efficient pollinators (Benedek et al., 1998; Calzoni and Speranza, 1996; Delaplane and Mayer, 2000; Maccagnani et al., 2003; Monzón et al., 2004; Stern et al., 2004; Zisovich et al., 2012). The number of honey bees per tree positively correlates with yields in several pear cultivars and insect density recommendations vary between 6 and 15 bees  $tree^{-1} min^{-1}$  (Stern et al., 2007).

Although pear trees rely on bees for pollination, their flowers are not as attractive as those of other fruit trees, due to the low volume of nectar secreted per flower (< 3  $\mu$ l) and its low sugar concentration (< 25%) (Delaplane and Mayer, 2000; Farkas et al., 2002; Monzón et al., 2004; Quinet et al., 2016b). The low attractiveness of pear nectar may be compensated for by the attractiveness of the pollen (Delaplane and Mayer, 2000; Quinet et al., 2016b). Pear pollen is rich in polypeptides (~6%), amino acids (~35%), and phytosterols (~4%) (Quinet et al., 2016), suggesting that bees visit pear flowers mainly for the pollen (Díaz et al., 2013; Stern et al., 2007).

Limitations in pollen transfer are the principal reason for poor yield in pear orchards (Webster, 2002). Although some studies investigated insect activity on pear trees (Calzoni and Speranza, 1996; Maccagnani et al., 2003; Mayer and Lunden, 1997; Monzón et al., 2004; Stern et al., 2004; Zisovich et al., 2012), the impact of cultivar placement in orchards has received little consideration. We hypothesise that limitations in pollen transfer among cultivars could be explained by both insect behaviour and orchard design. We studied insect diversity and foraging behaviour in pear orchards with different placements of the pollinizer cultivar (Fig. 1). We addressed the following questions: (i) which insects visit pear flowers and what is their foraging behaviour and pollination efficiency; (ii) what is the extent of pollination limitation in the studied orchards and does it affect fruit production; and (iii) does cultivar placement and the overlap of flowering times of the different cultivars limit inter-cultivar pollination?

#### 2. Material and methods

#### 2.1. Sites and plant materials

This study was conducted annually between 2011 and 2016 in four pear orchards that belonged to the Centre Fruitier Wallon (CEF) in Merdorp (50°38′31″N; 5°0′15″E) and Wasseiges (50°37′58″N; 5°0′57″E), central Belgium (Fig. S1). This region, called Hesbaye, is dominated by openfields (cereals, sugar beet) and the orchards were located at less than 1 km from the villages and surrounded by fields. 'Conférence' was used as the main commercial cultivar and 'Dovenné du Comice' was planted as the main pollinizer cultivar. The orchards covered a total area of 2 ha of pear trees planted at a  $3.75 \text{ m} \times 1.5 \text{ m}$  spacing. Two different cultivar placements (each covering approximately 1 ha) were compared: i) the pollinizer cultivar was planted in the same row as the main cultivar at a mean ratio of 1 pollinizer tree to 16 'Conférence' trees or ii) the pollinizer cultivar and the main cultivar were planted in separate rows with about 1 row of pollinizer trees to 5 rows of 'Conférence' trees (Fig. S1). All other orchard management practices were identical to those for commercial production. Each year, at the beginning of the flowering period, 1-2 honey-bee (Apis mellifera) hives (~40,000 individuals/hive) and 1 bumble-bee (Bombus terrestris) hive (multihive, Biobest, Westerlo, Belgium;  $\sim$  450 individuals/hive) were introduced per ha of pear trees (Fig. S1) as hive density recommendations ranged from 1 to 5 hives/ha for pear tree orchards (Stern et al., 2007).

#### 2.2. Insect behaviour, fidelity, and pollen transfer efficiency

Flower visitations were recorded on sunny days during the whole flowering period between 10:00 h and 16:00 h in 2013-2016. Insects were observed during a total of 19 days over the four years (four days in 2013, eight days in 2014, three days in 2015 and four days in 2016) and observations were performed in all orchards each day. The mean temperature in the orchards during the observation periods was 12.2  $\pm$  2.4 °C and the maximum temperature reached 18.9  $\pm$  3.4 °C. Visitors were classified as honey bees (Apis mellifera), bumble bees, solitary bees, hoverflies, other Diptera (Diptera excluding Syrphidae), and other minor visitors. Observations were performed along transect walks. When an individual was observed on a flower it was followed until it flew out of the field of vision of the observer. The period during which each individual was followed was called a 'sequence'. The number of trees, inflorescences, and flowers visited per sequence and the time spent per sequence, tree, inflorescence, and flower were recorded. The relative position of the successive trees visited in a sequence was also assessed.

To investigate the amount of pollen carried per insect, 10 individuals per category (honey bee, bumble bee, and solitary bees) were collected on pear flowers in 2016 on the same day in the same orchard and identified to the species level. Solitary bees (*Andrena* spp.) were separated according to their gender (4 males and 6 females), as differences were observed between the males and females. The pollen grains present on the different insect body parts (excluding the pollen loads) were counted as described in Quinet et al. (2016a). We distinguished the pollen grains of pear from pollen of other plant species. To further assess the fidelity of the insects to pear trees, insect visiting pear flowers were caught in 2014 and 2015 (all insect were collected on a same day for a same year) and one of their pollen loads was sampled (a total of 22 honey bees, 10 bumble bees, and 7 solitary bees were caught) and pollen grains were identified as described in Quinet et al. (2016b).

To assess the amount of pollen deposited per insect on the stigmas after a single flower visit, inflorescences were bagged before anthesis in 2016. The flowers were then unbagged at anthesis at the exception of some inflorescences remained bagged as controls. A total of 55 visited flowers were harvested after a single visit and the visitor was classified as honey bee, bumble bee, or solitary bee. Ten unvisited flowers were harvested as controls. The stigmas were crushed separately on microscope slides in 10  $\mu$ l acid fuchsin (2 g/L) to stain the pollen grains and the number of pollen grains was counted under a light microscope (Ernst Leitz Wetzlar). To estimate the amount of pollen deposited by insects, we calculated the differences between the amount of pollen deposited on visited and unvisited flowers.

#### 2.3. Assessment of pollen limitation

Open pollination and hand pollination with compatible pollen were compared to estimate the limitations in pollen transfer in 'Conférence' annually between 2011 and 2016 and in 'Doyenné du Comice' annually between 2014 and 2016. Each year, a total of 80–90 inflorescences on 2-year-old wood branches were selected per cultivar (6 trees per cultivar and 10–15 inflorescences per tree) and after treatment, fruit development was assessed on these inflorescences from flowering (April) until harvest (September). Half of the inflorescences were left un-manipulated to assess open pollination. For hand pollination, flowers were emasculated, hand pollinated with compatible pollen, and bagged during the flowering period to avoid insect pollination. 'Doyenné du Comice' pollen was used to pollinate 'Conférence' flowers and





### PEAR ORCHARD

Cultivar flowering (flowering overlap)
Orchard design (placement of pollinizer trees)

## POLLINATORS

- Insect diversity and abundance
- Insect foraging behaviour
- Insect pollination efficiency

(pollen transport and deposit on stigmas)

POLLEN LIMITATION

Inter-cultivar

pollination

#### **Open pollination** >< Hand pollination

- pollen tube growth
- fruit and seed sets
- fruit size
- pollinisation limitation index

Fig. 1. Summary of the main objectives and experiments of the study.

'Conférence' pollen was used to pollinate 'Doyenné du Comice' flowers.

The number of flowers, fruitlets, and fruits at harvest were recorded in April, May, and September, respectively. At harvest, fruit size and number of viable and aborted seeds per fruit were quantified on 30 fruits per cultivar and treatment. The pollination limitation index (PL) was calculated for fruit and seed production. PL was determined as the ratio between the relative number of open-pollinated fruits or seeds (Po) and hand-pollinated fruits or seeds (Ps) (PL = 1 - (Po/Ps); Larson and Barrett, 2000). Values range from 0 to 1, with PL = 0 indicating no pollen limitation.

To determine pollen tube growth and incompatibility, 10 flowers per treatment (open and hand pollinations) were harvested 3 days after pollination and the styles were stained in 0.1% (v/v) aniline blue solution and examined by fluorescence microscopy (Eclipse E400; Nikon, Amsterdam, The Netherlands) according to Jacquemart et al. (2006). Incompatible pollen tubes have limited growth and produce large callose plugs (Jacquemart et al., 2006).

#### 2.4. Flowering period overlap between cultivars and cultivar placement

We observed the flowering period of selected inflorescences in both cultivars and recorded the number of flowers at anthesis each day throughout the flowering period.

To investigate the effect of cultivar placement (cultivars alternating in the same row vs. cultivars in separate rows) on inter-cultivar pollination, about 40 'Conférence' flowers were randomly harvested per orchard at the end of the flowering period in 2013 and 2014. Pollen tube growth and incompatibility were observed as previously described on a total of 335 flowers.

#### 2.5. Statistical analyses

All analyses were conducted in SAS Enterprise Guide 7.1, except for

the principal component analyses (PCA), which were conducted in R version 3.0.2 (R Development Core Team 2013) using the 'FactoMineR' package (Husson et al., 2015). The normality of the data was estimated using Shapiro–Wilk tests, and homoscedasticity was verified using Levene's tests. The data were transformed when required to ensure normal distributions. Log-transformations were performed for insect behaviour parameters (sequence duration, trees visited per sequence, flowers visited per tree, visitation time per flower) and insect pollination efficiency parameters (pollen grains on insect body and pollen grains deposited on stigma per insect). The data are presented as means  $\pm$  standard errors unless indicated otherwise.

The number of observed insects was compared between cultivars using a mixed linear model with the year and orchard design as random factors. Insect diversity (proportion of individuals per insect type) was estimated between cultivars and between orchard design using chisquare tests. Insect behaviour parameters (sequence duration; number of trees, inflorescences, flowers visited per sequence; number of inflorescences and flowers visited per tree; visitation time per tree, inflorescence, flower) were visualized using PCA analysis and compared with mixed linear models with insect type and cultivar as fixed factors and the year and orchard design as random factors. The amount of pollen on the insect body and the proportion of pear pollen were compared among insect types and body parts using two-way analyses of variance (ANOVA). Finally, the amount of pollen deposited after a single visit was compared among insect types using one-way ANOVA. Post-hoc analyses were performed using Tukey's tests to investigate the differences among insect types.

To assess fruit production, fruit parameters (fruit size, total and viable seed sets, fruitlet set and fruitset at harvest) were analysed through mixed linear models to compare the effects of cultivars and pollination treatments (fixed factors) using the year and the number of fruits per tree as random factors. Linear regressions between fruit size and seed set were performed for each cultivar and Pearson correlations



Fig. 2. Insect visits in the pear (*Pyrus communis*) orchards. (a) Proportions of insects visiting the flowers of 'Conférence' and 'Doyenné du Comice'. (b–c) Principal component analysis of insect behaviour on 'Conférence' and 'Doyenné du Comice'. (b) Variable graph: number of flowers, inflorescences and trees visited per sequence (flowers, inflo, trees); number of flowers visited per inflorescence (flowers/inflo); visitation time per flower, inflorescence, tree (time per flower, inflorescence, tree); sequence duration (see Table 1 for details) (c) Individual graph showing the mean values per insect type and visited cultivar.

were calculated. Differences in fruit production, fruit size, and pollen tube growth between the pollination treatments were visualized for each cultivar using PCA analysis. Mixed linear models were used to investigate the impact of orchard design (fixed factor) on pollen tube growth (year as random factor).

#### 3. Results

#### 3.1. Insect foraging behaviour and pollination efficiency

More than 800 insects were observed on the pear flowers over 31.8 h of observation. The number of insects observed per hour did not significantly differ over the 4 years of observation ( $F_{3,23} = 2.15$ , p = 0.1220) and ranged from 14.90  $\pm$  8.80 in 2015 to 32.17  $\pm$  22.70 in 2014. The number of insects observed per hour was significantly lower on 'Conférence' than on 'Doyenné du Comice' flowers

 $(20.37 \pm 2.20 \text{ vs. } 40.93 \pm 11.56, F_{1,22} = 4.94, p = 0.0368)$  and insect diversity varied according to the cultivar (Fig. 2a,  $\chi^2 = 13.12$ , p = 0.0107). Cultivar placement did not affect insect diversity ( $\chi^2 = 7.089, p = 0.214$ ). Honey bees (*Apis mellifera*) were the dominant visitors, but more honey bees were observed on 'Doyenné du Comice' (68%) than on 'Conférence' (46%) flowers (Fig. 2a). Flowers were also visited by bumble bees (15%), hoverflies (14%), and solitary bees (6%). Most of the bumble bees were *Bombus terrestris* individuals coming from the hives and the solitary bees mainly included Andrenidae species (*Andrena haemorrhoa, Andrena fulva, Andrena cinerea, Andrena tibialis*, etc.). These Andrenidae are ground nesting and, as most solitary bees, have rather small foraging range (< 1km) (Gathmann and Tscharntke, 2002).

Insect behaviour differed among the insect species and the visited pear cultivars (Fig. 2b,c). Axis 1 of the PCA accounted for 74.34% of the variance and mainly separated the insect types (Fig. 2b,c). Honey bees and bumble bees visited flowers for longer sequences and visited about two times more trees and four times more inflorescences and flowers per sequence than solitary bees and hoverflies (Table 1). Apidae species also visited two to four times more flowers and inflorescences per tree than solitary bees and hoverflies (Fig. 2b, c, Table 1). The visitation time per flower and inflorescence was two-fold longer for solitary bees and hoverflies than for honey bees and bumble bees on 'Conférence'. On 'Doyenné du Comice', the visitation time per flower was longer for hoverflies as compared to Apidae (Table 1). PCA axis 2 explained 17.19% of the variance and separated solitary bees and bumble bees according to the cultivar (Fig. 2b,c).

Whatever the cultivar, the insects mainly foraged on trees within the same row; only 1% (honey bees and bumble bees) of the observed insects visited flowers on trees in adjacent rows (Fig. 3). Within rows, insects preferentially foraged on neighbouring trees; we observed insects flying a two- to three-tree distance in only 2% of our observations. The maximum number of successive trees visited per sequence was nine for honey bees, six for bumble bees, four for hoverflies, and two for solitary bees.

Between 3000 and 12,000 pollen grains were counted per insect body (Fig. 4a) and the pollen charge on the insect body depended on the species ( $F_{3,91} = 23.28$ , p < 0.001). More pollen grains were transported by bumble bees than by honey bees. Regarding solitary bees (Andrena spp.), female individuals transported three times more pollen on their body than male individuals (Fig. 4a). Pollen numbers differed by body part ( $F_{3,91} = 5.79, p < 0.0001$ , Fig. 4a). Most of the pollen was observed on the legs (32-42%, excluding pollen loads) regardless of insect type. Honey bees and bumble bees also transported large amounts of pollen on their heads (20% and 17%, respectively) while solitary bees mainly transported pollen on their upper thorax (20-26%). Less pollen was observed on the abdomen in all insect types (Fig. 4a). More than 90% of the pollen on the insect bodies and in pollen loads was pear pollen for all insect types (Fig. 4b,c). The amount of pollen deposited after a single flower visit did not differ among insect species and averaged 500 pollen grains ( $F_{2,52} = 0.51$ , p = 0.6044, Fig. 4d).

#### 3.2. Limitations of pollen transfer with insect pollination

Fruit size was positively correlated with the number of seeds per fruit in both 'Conférence' (R = 0.38, p < 0.0001) and 'Doyenné du Comice' (R = 0.22, p = 0.0130), showing that fertilization was required to produce fruits of commercially viable size (Fig. 5 a, d).

The PCA showed that fruit production differed between open and hand pollination in both cultivars (Fig. 5 b–c, e-f). In 'Conférence', hand pollination resulted in higher fruit set at harvest (30.7% vs. 21%,  $F_{1,15} = 1.55$ , p = 0.048), total seed set (52.3% vs. 27.1%,  $F_{1,405} = 59.08$ , p < 0.0001), and fruit size (57. 6 ± 0.4 mm vs. 56.0 ± 0.4 mm,  $F_{1,405} = 5.66$ , p = 0.0179) compared to open pollination. The rates of fruit abortion were 79% and 64% for open and

#### Table 1

Insect behaviour on 'Conférence' and 'Doyenné du Comice' pear (Pyrus communis) flowers.

Insect	n	Sequence duration (s)	Trees per sequence	Flowers per tree	Flowers per inflorescence	Visitation time per flower (s)
'Conférence' Honey bees Bumble bees Solitary bees Hoverflies	80 33 23 17	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{l} 2.58 \ \pm \ 0.22^{a} \\ 2.23 \ \pm \ 0.26^{ab} \\ 1.1 \ \pm \ 0.07^{b} \\ 1.56 \ \pm \ 0.22^{ab} \end{array}$	$\begin{array}{rrrr} 7.16 \ \pm \ 0.65^{ab} \\ 9.41 \ \pm \ 2.10^{a} \\ 3.34 \ \pm \ 0.63^{bc} \\ 1.84 \ \pm \ 0.29^{c} \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
'Doyenné du C Honey bees Bumble bees Solitary bees Hoverflies Insect type Cultivar	omice' 107 9 6 19	$\begin{array}{l} 96 \ \pm \ 8^{ab} \\ 95 \ \pm \ 25^{ab} \\ 50 \ \pm \ 26^{b} \\ 69 \ \pm \ 19^{b} \\ F_{3,264} = \ 7.47, \ p \ < \ 0.0001 \\ F_{2,264} = \ 0.12, \ p = \ 0.8897 \end{array}$	$\begin{array}{l} 2.05 \ \pm \ 0.12^{ab} \\ 1.67 \ \pm \ 0.24^{ab} \\ 1.00 \ \pm \ 0.00^{b} \\ 1.47 \ \pm \ 0.21^{b} \\ F_{3,279} = 10.55,  p \ < \ 0.0001 \\ F_{2,279} = 0.78,  p = 0.4616 \end{array}$	$\begin{array}{l} 5.94 \ \pm \ 0.42^{abc} \\ 11.46 \ \pm \ 4.91^a \\ 4.5 \ \pm \ 1.77^{abc} \\ 2.76 \ \pm \ 0.6^c \\ F_{3,279} = 15.38,  p \ < \ 0.0001 \\ F_{2,279} = 1.97,  p = 0.053 \end{array}$	$\begin{array}{rrrr} 1.44 \ \pm \ 0.04 \ ^{a} \\ 1.81 \ \pm \ 0.36 \ ^{a} \\ 2.58 \ \pm \ 0.81 \ ^{b} \\ 1.56 \ \pm \ 0.24 \ ^{a} \\ F_{3,279} = \ 4.40, \ p = \ 0.0048 \\ F_{2,279} = \ 5.85, \ p = \ 0.0032 \end{array}$	$\begin{array}{l} 9.16 \ \pm \ 0.43^{\rm b} \\ 9.24 \ \pm \ 1.77^{\rm bc} \\ 9.41 \ \pm \ 1.05^{\rm bc} \\ 16.51 \ \pm \ 2.02^{\rm ac} \\ F_{3,264} = 9.99, p \ < \ 0.0001 \\ F_{2,264} = 6.94, p = 0.0012 \end{array}$

'Sequence' refers to the period of visitation during which the insect was followed (until it flew out of the observer's field of vision).

Values followed by different letters for the same cultivar are significantly different according to Tukey's tests (p < 0.05).



Fig. 3. Proportion of insects visiting pear (*Pyrus communis*) flowers on the same tree (white), on different trees within the same row (grey) or on different trees on adjacent rows (black) during a 'sequence' according to insect type.

hand pollination, respectively. Pollen limitation indices showed that pollen limitation was detected for both fruit set (PL<sub>fruit</sub> = 0.32) and seed set (PL<sub>seed</sub> = 0.48). In 'Doyenné du Comice', hand pollination increased fruit set at harvest (16.8% vs. 7.2%,  $F_{1,8} = 13.60$ , p = 0.0061). According to pollen limitation indices, pollen limitation was observed for fruit set (PL<sub>fruit</sub> = 0.57) but not for seed set (PL<sub>seed</sub> = 0).

# 3.3. Effect of cultivar placement and flowering overlap on inter-cultivar pollination

The duration of flowering for both cultivars was about 10 days (Fig. 6 a, b). 'Doyenné du Comice' flowered slightly later than 'Conférence' and the overlap in flowering between the two cultivars varied according to the year. Over the six years of observations, flowering overlap averaged 7.5  $\pm$  1.5 days and ranged from two days in 2016–10 days in 2012. The number of insects visiting flowers was higher when both cultivars were simultaneously in bloom (Fig. 6 a, b).

Cultivar placement affected inter-cultivar pollination as observed by pollen tube growth (Table 2). Pollen tube growth was observed in 98% of the flowers although the number of pollen tubes per style varied according to the orchard. The percentage of flowers showing signs of incompatibility (limited pollen tube growth and large callose plugs) was 7.6 times higher in the orchards where rows of 'Doyenné du Comice' and 'Conférence' trees alternated compared to the orchards where both cultivars were in the same rows. Cultivar placement did not affect insect diversity ( $\chi^2 = 7.089$ , p = 0.214) or the number of trees visited per insect ( $F_{3,268} = 1.46$ , p = 0.1907).



honey bees bumble bees solitary bees

**Fig. 4.** Insect pollination efficiency and fidelity on pear flowers. (a) Pollen transfer capacity according to insect body parts (excluding pollen loads). (b–c) Insect fidelity. (d) Efficiency in pollen deposition after a single visit. Values followed by different letters are significantly different according to Tukey's tests (p < 0.05) for the number of pollen grains on insect bodies in (a), proportion of pear pollen on insect bodies in (b) and pollen loads in (c), number of pollen grains deposited on stigmas per flower in (d).

#### 4. Discussion

#### 4.1. Insect behaviour limits pollen transfer between rows

Honey bees, bumble bees, solitary bees, and hoverflies were observed visiting the pear flowers in our trials. The addition of bumble



Fig. 5. Effect of pollination treatment on fruit production of (a–c) 'Conférence' and (d-f) 'Doyenné du Comice' pear (*Pyrus communis*) cultivars. (a, d) Correlation between the number of seeds per fruit and fruit size. Note that the axis scales differed depending on the cultivar. (b–c, e–f) Principal component analysis (PCA) of pollen tube growth, fruit set, seed set, and fruit size after open pollination and hand pollination. (b, e) The year was added as a supplemental variable.

bees or solitary bees can improve fruit yield in pear orchards (Monzón et al., 2004; Zisovich et al., 2012). Studies in apple and almond also showed that an increase in the diversity of pollinator species improved pollen dispersal (Blitzer et al., 2016; Brittain et al., 2013; Mallinger and Gratton, 2015; Martins et al., 2015). In our study, pollen was dispersed mainly within rows. Most of the observed insects foraged within the

same tree or on neighbouring trees within the same row. We observed only 1% across-row foraging regardless of the insect species. Other studies in pear, apple, and almond reported that honeybees tend to forage on a small number of neighbouring trees per sequence (Free, 1960; Kron et al., 2001a, 2001b; Monzón et al., 2004; Stern et al., 2007, 2004). Such behaviour may limit inter-cultivar pollen transfer when



**Fig. 6.** Overlapping flowering and insect visitations between 'Conférence' and 'Doyenné du Comice' in the pear (*Pyrus communis*) orchards in (a) 2013 and (b) 2014. Flowering phenology expressed as the percentage of flowers at anthesis; insect visits expressed as the number of insects observed on pear flowers per hour of transect walk (data are presented for dates where observations were performed).

cultivars are planted in separate rows. Insect observations are limited by the fact that bee movements between rows are harder to observe, thereby potentially underestimating pollen movement (Free, 1960; Kron et al., 2001a). Moreover, honey bees have been found to disperse pollen through in-hive pollen transfer in addition to transfer from tree to tree (Free and Williams, 1972; Kron et al., 2001b). Contacts in the hive transfer pollen among individuals and compatible pollen could be dispersed in the orchard at the next flight (Degrandi-Hoffman et al., 1986). In addition, Brittain et al. (2013) reported that honey bees increased their proportion of movement between tree rows in almond orchards when other pollinator species were present. The exact mechanism for these increased between-row movements remains unknown, although it could be due to competition for floral resources (Brittain et al., 2013). Zisovich et al. (2012) reported that adding bumblebees to honeybees in pear orchard had no influence on honeybee activity. Such comparison was not performed in our study. We could not exclude that the high levels of managed pollinators compared to wild pollinators observed in the studied orchards may have displaced wild pollinators towards other source of pollen. Competition between visitors may also be lower in pear than in almond orchards, as pear flowers are less attractive to insects than apple, almond, or cherry flowers (Díaz et al., 2013; Quinet et al., 2016b; Stern et al., 2007), which may explain the minimal between-row movements.

Differences in behaviour among pollinator insects may affect pollination. Bees prefer collect pollen rather than nectar from the pear flowers (Free, 1960; Mayer and Lunden, 1997; Stern et al., 2004; Quinet et al., 2016b). We observed that honey bees and bumble bees visited more flowers per tree and more trees, compared with solitary bees and hoverflies, but solitary bees and hoverflies spent more time per flower than honey bees and bumble bees. Previous studies on pear reported a similar number of flowers visited per tree (7-9) and time spent per flower (9-10s) for honeybees (Free, 1960; Monzón et al., 2004; Zisovich et al., 2012). Some authors reported that honey bees visited more pear flowers per minute than bumblebees (Mayer and Lunden, 1997) while others showed the opposite (Zisovich et al., 2012); such differences were not observed in our study. Flower visitation time depended on the species for solitary bees. For example, Andrenid species (Andrena sp.) spent more time per flower (Martins et al., 2015) compared to Osmia cornuta (Monzón et al., 2004). Visiting more flowers will increase pollination as long as the bees deposit enough pollen on each flower. However, pollination efficiency does not always increase with increasing visitation time. Pollination efficiency primarily depends on the amount of pollen deposited on the flower stigmas and on the source of the pollen. Several studies reported that, compared with honey bees, bumble bees and solitary bees transport and deposit more pollen on the stigmas (Brittain et al., 2013; Free and Williams, 1972; Jacquemart et al., 2006; Maccagnani et al., 2003; Monzón et al., 2004; Thomson and Goodell, 2001; Zisovich et al., 2012). A similar trend was observed in our study for bumblebees and female Andrena individuals as compared with honey bees although the difference was not significant. Regardless of bee type in our study, the individuals transported large amounts of pollen on their bodies and deposited about 500 pollen grains per flower (for 10 ovules). About 35% of the pollen transported by honey bees and bumble bees was observed on the head and lower thorax, the body parts that are the most in contact with the stigmas when visiting pear flowers. Therefore, the amount of pollen was likely not a limiting factor for pollination. Monzón et al. (2004) and Park et al. (2016) also reported that bees carried sufficient amounts of pollen per flower for adequate fertilization in pear and apple trees as long as stigma contact occurred and the pollen was compatible.

In our study, bees mainly or exclusively transported pear pollen regardless of species. Pear flowers were the main floral resource in the surrounding area during early Spring as the orchards were mainly surrounded by open fields and apple trees flowered somewhat later than pear trees (Quinet et al., 2016b). However, some herbaceous ruderals were observed in the orchards during pear blooming and ornamental flowering plants were present in gardens in the vicinity of the orchards. The availability of alternative foraging resources in the surrounding area may have a strong influence on the pollinator fidelity. Pollinators displayed a high fidelity for pear flowers in some studies (Benedek et al., 1998; Maccagnani et al., 2003; Zisovich et al., 2012) while in other studies, visitors mainly foraged on other plant species (Faoro and Orth, 2015; Free and Williams, 1972). Bees are efficient pollinators in pear as long as they transfer pollen between compatible cultivars.

4.2. Limitations in insect pollination resulted in reduced fruit production in 'Conférence' and in 'Doyenné du Comice'

Limitations in insect pollination were previously reported in pear

Table 2

Effect of cultivar placement on the percentage of 'Conférence' flowers with pollen tube growth and incompatibility after open pollination.

Pollinizer	Num	ber of pollen tubes per	Signs of incompatibility			
arrangement	n <sup>1</sup>	0	1–10	10–20	> 20	
in the row different rows ANOVA1	171 164	$\begin{array}{l} 2.1 \ \pm \ 0.6^{a} \\ 2.8 \ \pm \ 0.7^{a} \\ F_{1,333} = \ 0.57, \\ p = \ 0.4516 \end{array}$	20.1 $\pm$ 1.7 <sup>a</sup> 9.7 $\pm$ 1.3 <sup>b</sup> F <sub>1,333</sub> = 23.36, p < 0.0001	$\begin{array}{rrrr} 31.2 \ \pm \ 0.8^{a} \\ 22.1 \ \pm \ 1.5^{b} \\ F_{1,333} = 14.91, \\ p \ < \ 0.0001 \end{array}$	$\begin{array}{rrrr} 47.0 \ \pm \ 2.4^{\rm b} \\ 65.4 \ \pm \ 2.3^{\rm a} \\ {\rm F}_{1,333} = \ 29.81, \\ p \ < \ 0.0001 \end{array}$	$5.4 \pm 1.2^{b}$ $38.3 \pm 2.2^{a}$ $F_{1,333} = 176.8, p < 0.0001$

Values followed by different letters for the same condition are significantly different according to Tukey's tests (p < 0.05).

<sup>1</sup> n: number of flowers per treatment.

and other fruit trees (Garratt et al., 2014; Kron et al., 2001b; Monzón et al., 2004; Webster, 2002). Limitations in inter-cultivar pollen transfer occurred in our study due to the presence of incompatible pollen on flower stigmas. Pollen deposit on flower stigma was not limited as pollen tube growth was observed under open pollination, but incompatibility was higher with open pollination than with hand pollination. The limitation in compatible pollen transfer reduced seed set and fruit size in 'Conférence' and reduced fruit set in 'Doyenné du Comice'. This discrepancy could be explained by the ability of 'Conférence' to produce parthenocarpic fruits, which does not occur in 'Dovenné du Comice' (Deckers and Schoofs, 2002: Nvéki and Soltész, 1998; Ouinet and Jacquemart, 2015). Under open pollination, 49% of the fruits produced by 'Conférence' were parthenocarpic fruits that were seedless and had a smaller size than fertilized fruits. In 'Doyenné du Comice', fruit set relies exclusively on compatible pollination and fertilization (Monzón et al., 2004; van den Eijnde, 1996). Commercialsized fruits occur in 'Doyenné du Comice' with a minimum of two to three seeds (Monzón et al., 2004). The observed decrease in fruit set and fruit size observed under open pollination in our study was thus mainly explained by the transfer of incompatible pollen among flowers.

# 4.3. Differences in flowering periods between cultivars and cultivar placement limit inter-cultivar pollination

Synchronization of flowering between the pollinizer cultivars and the main cultivar is crucial for efficient pollination and successful fertilization in fruit production (Quinet et al., 2016b; Zisovich et al., 2012). Pear flowering lasts only a few days and all cultivars do not flower at the same time (Quinet et al., 2016b; Zisovich et al., 2012). Pear trees flower in early Spring in Belgium and pear flowering started between April 1st and April 30th across our 6 year-study according to the weather conditions. Pear flowering date is thus likely to be affected by climate change in the next years. However a time lag was observed between the flowering dates of the different cultivars whatever the years (Quinet et al., 2016b). We observed that 'Conférence' began to flower before 'Doyenné du Comice' and that the overlap between the flowering periods varied between two and ten days, depending on the year. van den Eijnde (1996) reported that, in The Netherlands in 1992, 'Conférence' started also to bloom before 'Doyenné du Comice' and observed a flowering overlap of about 5 days. Thus, it could be advantageous to add an early-flowering pollinizer in addition to 'Doyenné du Comice', in 'Conférence' orchards to maximize pollination. 'Triomphe de Vienne' is a suitable candidate, as it is compatible with 'Conférence' and typically begins to flower just before 'Conférence' (Quinet et al., 2016b, 2014; Warnier, 2000). Planting both early- and late-flowering pollinizers is recommended so that pollen is available throughout the entire flowering period of the commercial cultivar (Ramírez and Davenport, 2013).

The placement of the pollinizer trees within the orchard is also important for improving pollination efficiency. We observed that intercultivar pollination decreased when pollinizer and main cultivar trees were planted in different rows. Pollen transfer was higher within-row than across-row, due to the insect behaviour observed in our study. The insect foraging behaviour explains the limitation in inter-cultivar pollen transfer when cultivars are organised in rows or blocks. It is worth mentioning that further research would be needed to estimate the optimal number of pollinators required within the intra-row treatment. Gene flow studies in other rosaceous fruit trees showed that most pollen was dispersed within 10-15 m of the tree (Jackson, 1996; Kron et al., 2001b, 2001a). To optimize pollination, orchards should be designed to bring compatible pairs of cultivars as close as possible (Jackson, 1996). However, commercial orchards are usually planted in monotypic blocks or rows to facilitate management (Kron et al., 2001a). Alternating cultivars within the rows in a checkerboard pattern may cause difficulties for harvesting (Jackson, 1996) as fruits of different cultivars could be mixed during harvest. Moreover, if the pollinizer and the main cultivar mature at different times, several harvest operations would be required. Thus, orchard design requires a compromise between optimal inter-cultivar pollen transfer and ease of management. The proportion of pollinizer trees must be sufficient to ensure cross-pollination while allowing an easy orchard management.

#### 4.4. Conclusion

Our results showed that although bees are efficient pollinators, limitations in inter-cultivar pollination restricted fruit production. These limitations were mainly due to orchard design and insect behaviour. Most pollinators foraged on trees within the same row, limiting pollen transfer between rows. From a pollination point of view, orchards should be designed to bring compatible cultivars as close together as possible (Jackson, 1996; Kron et al., 2001b; Ramírez and Davenport, 2013) as opposed to planting different cultivars in separate blocks or rows. Pollinizer cultivars should flower concurrently with the main cultivar. The proportion of pollinizer trees in the orchard required to maximize pollination needs further investigation and would depend on the abundance and diversity of insect pollinators. Although we did not observe differences in pollination efficiency between the bee species in our study, the relative importance of different insect species as pollinators of pear deserves further investigation.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eja.2017.09.015.

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