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Prunus salicina blooming. Courtesy of Encarnación Ortega Pastor

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How to choose the best pollinizer cultivars for 'Conférence' pear (*Pyrus communis*) in Belgium?

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Abstract

Pear (*Pyrus communis*) is the most important fruit crop in Belgium and 'Conférence' represents 90% of the pear production. 'Conférence' is self-incompatible and requires cross-pollination to develop fertilized fruits. We therefore compared seven pear cultivars ('Dicolor', 'Doyenné du Comice', 'Joséphine de Malines', 'Seigneur Espéren', 'Sweet Sensation', 'Triomphe de Vienne', 'Williams Bon Chrétien') adapted to Belgian conditions in order to identify valuable pollinizer cultivars for 'Conférence'. These cultivars were used as male parent in hand pollination experiments with 'Conférence' and the resulting number of fruits was assessed. Fruit size and weight were measured at harvest. Moreover, to evaluate the pollination efficiency of these cultivars and their attractiveness for bees, we analyzed their pollen and nectar production and composition. Pollen is one of the prime nutrient resources used for bee larvae and nectar represents the major source of sugars for bees. Regarding fruit production, the mean weight and size reached 103-154 g and 50-61 mm respectively depending on the crossings. The largest fruits resulted from the pollination of 'Conférence' with 'Triomphe de Vienne' or 'Doyenné du Comice'. The cultivars differed in their pollen and nectar quantities and compositions. The mean number of pollen grains per anther ranged from 2,200 for 'Dicolor' to 4,600 for 'Williams Bon Chrétien'. Pollen viability was high whatever the cultivar (60-92%). Pollen polypeptide concentration was variable (31-107 μg mg^{-1}) while amino acid concentration was high and stable (346-390 μg mg^{-1}) among cultivars. The nectar sugar content per flower varied between 52 μg for 'Seigneur Espéren' to 255 μg for 'Conférence'. Pear cultivars produced therefore pollen of high quality regarding amino acid content while their nectar production was poor in sugars. Bee attractiveness might differ among cultivars and needs further research to optimize orchard management.

Keywords: fruit production, nectar, pear tree, pollen, pollination

INTRODUCTION

Pear (*Pyrus communis* L.) is currently the main fruit crop in Belgium and 'Conférence' accounts for more than 90% of the production. 'Conférence' is self-incompatible and requires inter-cultivar cross-pollination to develop fertilized fruits (Jacquemart et al., 2006). Production of seedless fruits resulting from parthenocarpy is also observed in 'Conférence' (Quinet and Jacquemart, 2015). However, compatible pollination and seed development increase 'Conférence' fruit size and quality (Warnier, 2000; Quinet and Jacquemart, 2015). It is therefore important to identify efficient pollinizer cultivars for 'Conférence'. We compared seven cultivars as pollen source in 'Conférence' pollination trials, namely 'Dicolor', 'Doyenné du Comice', 'Joséphine de Malines', 'Seigneur Espéren', 'Sweet Sensation', 'Triomphe de Vienne' and 'Williams Bon Chrétien' and we analysed the resulting fruit development. All cultivars are fully compatible with 'Conférence' (i.e., they differ in the *S*-genotype) (Goldway et al., 2009; Quinet et al., 2014). In addition to be compatible with the commercial cultivar, pollinizer cultivars must have concomitant flowering and be attractive to pollinator insects

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(Stern et al., 2007). To assess the potential attractiveness of the studied cultivars to pollinators, we analyzed their nectar and pollen production and composition. Nectar represents the major energy source, while pollen is one of the prime nutrients used for bee larvae (Vanderplanck et al., 2014; Quinet et al., 2016).

MATERIALS AND METHODS

Plant material

Pollen and nectar were collected from flowers of the 8 cultivars in the orchards of the CEF (centre fruitier Wallon) in Wasseiges, Belgium (50°37'58"N, 5°0'57"E) and in the orchard of 'Flore and Pomone A.S.B.L.' in Enines, Belgium (50°41'46"N, 4°56'21"E).

Pollination trials

Pollination was carried out by brushing anthers of the donor flower on the stigmas of emasculated 'Conférence' flowers. Each pollination treatment was performed on ten flowers in 2013 and on 80-130 flowers in 2014. Three days after pollination, ten flowers per treatment were harvested and styles were stained in 0.1% aniline blue solution to determine pollen tube growth (Jacquemart et al., 2006). In 2014, the number of flowers, initiated fruits and fruits at harvest were followed. At harvest, the resulting fruits were analysed to determine their weight, size, length and the presence of seeds.

Pollen and nectar quantity and quality assessment

Ten flowers per cultivar were used to determine the number of pollen grains anther^{-1} (Quinet et al., 2016). Pollen viability was assessed on 10 flowers with fluorescein diacetate (Dafni et al., 2005). The polypeptide (molecular weight >10,000 Da) and amino acid concentrations in pollen were quantified from respectively 5 and 3 mg dry pollen as described in Quinet et al. (2016).

The nectar sampling was carried out on 20-30 freshly opened flowers per cultivar and nectar volumes, sugar concentrations and sugar composition were determined as described in Quinet et al. (2016).

Statistical analysis

ANOVA analyses and Tukey's post hoc tests were performed to evaluate the treatment and cultivar effects on the fruit, pollen and nectar parameters using the SAS Enterprise Guide 7.1. Fruit parameters were weighted by the number of fruits tree^{-1}. Comparisons between proportions were analyzed using chi-square tests. To detect differences in amino acid composition among cultivars, we conducted perMANOVA (Bray-Curtis dissimilarity index, 999 permutations, 'adonis' command) and multiple pairwise comparisons with Bonferroni's adjustment ('betadisper' command) (R-package 'vegan'). Differences were visually assessed using non-metric, multidimensional scaling ordinations (NMDS). Values are presented as means ± standard errors.

RESULTS AND DISCUSSION

Pollinizer efficiency

'Conférence' flowers were used as the female parent in crossings with the pollen of seven compatible cultivars. Self-pollination was performed in order to compare parthenocarpic and fertilized fruits. In 2013, hand pollinations were carried out in the laboratory to check the compatibility among cultivars. Pollen tube growth was observed for all crossings with the exception of self-pollination where 32% of the styles did not show pollen tube growth and arrest of pollen tubes in the first third of the style was observed in 85% of the pistils. In 2014, crossings were realized in the orchards to analyze the resulting fruit production (Figure 1). The number of pollen tubes observed in the styles was lower than in 2013 (10±4% vs. 81±9% of the styles with more than 20 pollen tubes) despite a high pollen viability (60-92%). This difference could be due to the lower temperatures in the orchard (10±6°C during the flowering period) compared to the laboratory (~20°C). Temperature has been pointed out as one of the main factors interacting with flower receptivity, strongly influencing stigmatic receptivity, pollen tube growth and ovule development (Sanzol et al., 2003). Again, pollen tube growth arrest was observed in 85 and 5% of the pistils pollinated with 'Conférence' and 'Dicolor'. Limited pollen tube growth and large callose plugs are considered as incompatibility symptoms (Jacquemart et al., 2006). However, arrest of pollen tube growth in the crossing 'Dicolor' × 'Conférence' would not be due to genetic incompatibility since both cultivars differed by their *S*-alleles. *S*-genotype has not been identified yet in 'Dicolor' but its parental cultivars – 'Forelle' and 'Williams Bon Chrétien' – have different *S*-genotypes than 'Conférence' (Goldway et al., 2009).

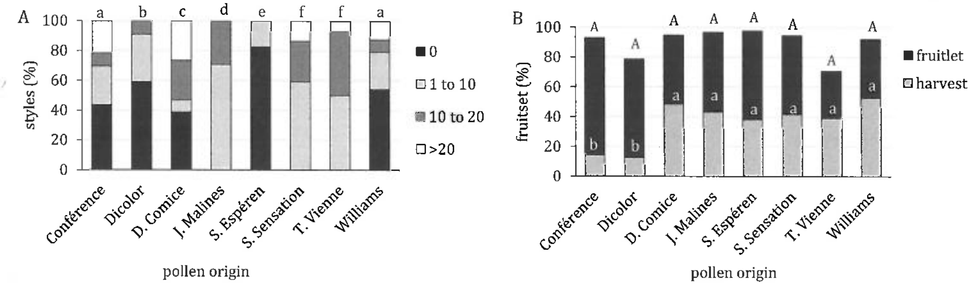

Figure 1. Pollen tube growth and fruit set according to 'Conférence' pollination trials in the field in 2014. A) Percentage of styles with growing pollen tubes, according to numbers of pollen tubes per style; B) fruit set. Fruit set was expressed as the percentage of flowers that initiated fruit two weeks after pollination (fruitlet) and that resulted in a fruit at harvest (harvest). Cultivars followed by different letters are significantly different at P<0.05.

Despite the low number of pollen tubes observed for some crossings (Figure 1A), 71 to 93% of the flowers settled fruitlets (χ^2=7.38, df=7, P=0.39) (Figure 1B). Fruit set at harvest was lower for the crossing with 'Conférence' and 'Dicolor' compared to the others (χ^2=42.86, df=7, P<0.0001) (Figure 1B). Similar fruit set (40-58%) was previously reported for cross-pollination of 'Conférence' with 'Triomphe de Vienne' and 'Doyenné du Comice' (Warnier, 2000). According to the standards, fruit set above 10 and 25% are considered as good and very good, respectively (Kemp et al., 2008). High fruit set was thus obtained whatever the crossings in our trials. Most fruits (82-100%) resulting from pollination with 'Conférence', 'Dicolor' and 'Seigneur Espéren' were seedless while seeds were present in more than 75% of the fruits for the remaining crossings. The mean number of viable seeds per fruit ranged from 0 to 4.3 (Table 1). Similar number of seeds fruit^{-1} were reported in other 'Conférence' pollination trials (Kemp et al., 2008).

Fruit size and weight varied according to pollen source, 'Triomphe de Vienne' gave the biggest fruits and 'Seigneur Espéren' gave the smallest (Table 1). 61 and 54% of the fruits resulting respectively from the crossings with 'Doyenné du Comice' and 'Triomphe de Vienne' had a diameter higher than 60 mm while this percentage dropped below 40% for the other crossings. Warnier (2000) reported a similar percentage of 'Conférence' fruits bigger than 60 mm in its pollination trials with 'Doyenné du Comice' (71%) and 'Triomphe de Vienne' (52%) compared to un-pollinated fruits (19%). 'Conférence' could also be compatible with other *Pyrus* species. Kemp et al. (2008) recommended four ornamental *Pyrus* accessions as polliziners for 'Conférence' based on fruit and seed set ability. However, they did not investigate the fruit size and weight at harvest.

Table 1. Fruit parameters of 'Conférence' pears derived from hand pollination trials.

Pollen source	n	Weight (g)	Size (mm)	Length (mm)	Number of seeds Viable	Aborted
Conférence	22	119±10^{ab}	52.5±1.6^{ab}	103.3±7.4^{a}	0.2±0.1^{c}	2.2±0.6^{ab}
Dicolor	8	119±2^{ab}	51.6±1.2^{ab}	97.2±6.8^{a}	0±0^{c}	0±0^{b}
D. Comice	28	142±7^{ab}	60.1±0.9^{ab}	98.3±2.6^{a}	3.7±0.4^{ab}	4.8±0.4^{a}
J. Malines	20	132±8^{ab}	57.8±1.7^{ab}	97.3±2.2^{a}	1.6±0.3^{bc}	5.4±0.7^{a}
S. Espéren	11	104±9^{b}	50.10±2.6^{b}	93.7±3.8^{a}	0.2±0.1^{c}	1.5±0.6^{ab}
S. Sensation	13	106±16^{b}	52.3±3.0^{ab}	92.0±6.4^{a}	4.30±0.6^{a}	3.2±0.3^{ab}
T. Vienne	35	154±8^{a}	61.3±1.1^{a}	99.9±1.8^{a}	3.5±0.3^{ab}	5.1±0.4^{a}
Williams	18	123±8^{ab}	56.6±1.3^{ab}	96.9±3.1^{a}	2.7±0.4^{ab}	5.4±0.4^{a}
		$F_{7,141}$=3.33 P=0.0026	$F_{7,20.3}$=7.74 P=0.0001	$F_{7,16.4}$=0.56 P=0.7802	$F_{6,54.8}$=34.7 P<0.0001	$F_{3,50.7}$=4.87 P=0.0005

Floral resources

Nectar and pollen production and composition were analysed in the eight cultivars to investigate their potential attractiveness for pollinator insects.

1. Nectar.

'Conférence', 'Doyenné du Comice' and 'Sweet Sensation' produced more sugar content in nectar flower^{-1} than the other cultivars and the lowest sugar contents were observed in 'Dicolor' and 'Seigneur Espéren' (Table 2). Differences in nectar sugar content was due to differences in both nectar volume and sugar concentration (Table 2). 'Doyenné du Comice' produced higher volume of nectar flower^{-1} with a low sugar concentration while 'Dicolor' flowers produced low nectar volume more concentrated. All cultivars produced hexose dominant or hexose-rich nectar (Table 2) and sucrose was present in the nectar of all cultivars except 'Seigneur Espéren'.

Table 2. Nectar parameters of the eight studied pear cultivars.

Cultivars	Volume (µL)	Sugar concentration (%)	Sugar content flower^{-1} (mg)	Sucrose/hexoses
Conférence	1.62±0.18^{ab}	14.8±1.1^{bc}	0.26±0.03^{a}	0.063±0.01^{a}
Dicolor	0.22±0.07^{d}	25.7±1.1^{a}	0.07±0.01^{c}	0.05^{a}
D. Comice	2.12±0.37^{a}	11.2±1.0^{c025}	0.25±0.04^{ab}	0.024±0.008^{a}
J. Malines	1.34±0.20^{bc}	13.1±1.2^{bc}	0.15±0.03^{abc}	0.065±0.03^{a}
S. Espéren	0.27±0.06^{d}	18.3±1.47^{b}	0.04±0.01^{c}	0±0^{a}
S. Sensation	1.21±0.18^{bc}	12.7±1.1^{bc}	0.24±0.05^{ab}	0.022±0.012^{a}
T. Vienne	0.87±1.5^{bcd}	13.0±1.1^{bc}	0.11±0.02^{bc}	0.041±0.03^{a}
Williams	0.57±0.09^{cd}	10.5±0.6^{c}	0.09±0.02^{bc}	0.003±0.002^{a}
	$F_{7,154.8}$=17.44 P<0.0001	$F_{7,197}$=9.22 P<0.0001	$F_{7,197}$=6.23 P<0.0001	$F_{7,18}$=1.11 P=0.4013

Whatever the cultivar, nectar production and sugar content were low compared to other fruit species (Stern et al., 2007). Farkas et al. (2002) also reported low nectar production (1.1-8.3 µL flower^{-1}) and sugar concentration (7.1-17.7%) in pear cultivars. Pear cultivars secreting nectar continuously are more attractive to insect compared to cultivars without nectar (Farkas and Orosz-Kovács, 2003).

2. Pollen.

The number of pollen grains anther^{-1} ranged from 2,200 for 'Dicolor' to 4,600 for 'Williams' (Table 3). Sharifani and Jackson (2004) compared pollen production in pear species and observed that *Pyrus communis* cultivars produced more pollen than the wild species.

Table 3. Pollen parameters of the eight studied pear cultivars.

Cultivars	Pollen grains anther^{-1}	Viability (%)	Polypeptides (µg mg^{-1})	Amino acids (µg mg^{-1})
Conférence	2545±146^{c}	86.5±1.9^{a}	77.7±7.7^{ab}	362±9^{a}
Dicolor	2205±174^{c}	66.4±1.2^{cd}	79.4±6.9^{ab}	389±22^{a}
D. Comice	3416±216^{b}	92.1±0.7^{a}	51.9±6.7^{bc}	346±11^{a}
J. Malines	3341±245^{b}	67.7±2.0^{cd}	31.1±1.9^{c}	ND[1]
S. Espéren	3533±192^{b}	71.0±1.3^{c}	46.8±1.2^{c}	387±4^{a}
S. Sensation	2685±235^{c}	60.3±1.9^{d}	107.3±13.8^{a}	390±14^{a}
T. Vienne	2677±163^{c}	79.6±0.6^{b}	50.6±3.2^{bc}	349±4^{a}
Williams	4615±162^{a}	72.8±1.9^{c}	75.1±11.2^{ab}	ND[1]
	$F_{7,343}$=17.00 P<0.0001	$F_{7,36}$=27.48 P<0.0001	$F_{7,32.49}$=17.39 P<0.0007	$F_{5,12}$=2.67 P=0.075

[1]ND: no data.

To assess the pollen composition, we quantified the polypeptides and amino acid content since their composition was reported as a useful indicator of diet performance in bees (Vanderplanck et al., 2014). Polypeptide concentration ranged from 31 µg mg^{-1} for 'Joséphine de Malines' to 107 µg mg^{-1} for 'Sweet Sensation' while the total amino acid concentration did not differ among cultivars (Table 3). The percentage of essential amino acid reached 42-44% and all cultivars contained the full spectrum of amino acids. Asparagine and glutamine were the dominant amino acids whatever the cultivar and accounted each for 13% of total amino acid content in pollen. Although they are not essential amino acids, asparagine and glutamine are important as energy and nitrogen sources for bees (Vanderplanck et al., 2014). Amino acid composition varied among cultivars ($F_{5,12}$=13.14, P=0.002) (Figure 2) mainly regarding 'Doyenné du Comice'

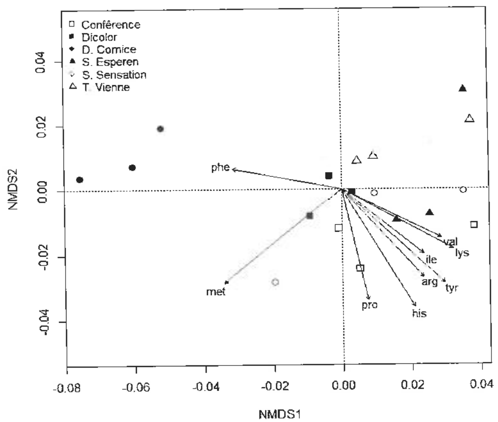

Figure 2. Pollen amino acid composition in the eight studied pear cultivars. NMDS ordination plot based on Bray-Curtis distances calculated on amino acid (µg mg^{-1}) concentrations. Only significant amino acids are shown (P<0.05): arg, arginine; his, histidine; ile, isoleucine; lys, lysine; met, methionine; phe, phenylalanine; pro, proline; thr, threonine; tyr, tyrosine; val, valine.

Despite some differences among cultivars, pear pollen was rich in polypeptides and amino acids and their concentrations were similar to pollen considered as good quality pollen for the development of bumblebee colonies (Vanderplanck et al., 2014). The high quality of pear pollen compared to nectar may explain why pollinating insects mainly visit pear for pollen resource (Díaz et al., 2013).

CONCLUSIONS

We tested seven pear cultivars as pollinizers for 'Conférence' and all could be valuable pollinizers for 'Conférence' except 'Dicolor'. The highest fruit size resulted from the crossings of 'Conférence' with 'Triomphe de Vienne' and 'Doyenné du Comice', suggesting that these cultivars are the best pollinizers for 'Conférence' in our growing conditions.

Although floral resource production varied among cultivars, all studied cultivars produced high quality pollen (rich in polypeptides and amino acids) and nectar. Flowers of 'Doyenné du Comice' contained more sugar in their nectar and more pollen grains anther^{-1} than flowers of 'Triomphe de Vienne'. In consequence, this cultivar could be more attractive to pollinators. Precise observations of insect pollination under orchard conditions would confirm pollinator attractiveness and pollination efficiency of these cultivars.

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