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Compatibility and pollinator efficiency tests on *Pyrus communis* L. cv. 'Conference'

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SUMMARY
Effectiveness of pollination was predicted in a 'Conference' pear orchard by examining pollen tube growth. This histological technique allows a reliable and rapid estimation of pollination quality. Intra- and inter-cultivar compatibility was tested by hand pollinations with 'Conference' or 'Doyenné' pollen, respectively. In intra-cultivar pollinated flowers, limited pollen tube growth and large callose plugs were observed. This cultivar was thus considered self-incompatible. As insect pollinators deposit variable quantities and mixtures of intra-cultivar (incompatible) and inter-cultivar (compatible) pollen grains, their relative efficiency can differ greatly. The pollination efficiencies of honeybees (*Apis mellifera* L.) and of bumblebees (*Bombus terrestris* L.) were compared by examining pollen deposition and pollen tube growth following single floral visits. Bumblebees deposited higher quantities of more compatible pollen grains per stigma than honeybees; whereas no differences were detected in pollen tube growth. The implications for 'Conference' pear production are discussed.

Pear yield improvement remains an important issue as, for example, overall pear production in Europe (EU15) reached 2.5 million tonnes in 2004 (EUROFEL, 2005). The most widespread European pear cultivars are 'Conference', 'Williams' and 'Doyenné du Comice'. Fruit yield depends on successful achievement of a series of sequential processes, from floral induction to fruitlet retention. Each of these essential physiological processes is influenced by genetic, environmental, physiological and tree-management factors (Barbier, 1986; Webster, 2002). For the successful set of an optimum crop in most pear cultivars, the pollination and fertilisation of flowers must be effective. Most pear cultivars are self-incompatible or produce more and/or better fruit when cross-pollinated, which requires the transfer of pollen between different cultivars planted within the orchard (Webster, 2002; Stern *et al*., 2004).

Pollination is a critical factor in self-incompatible and early flowering species or cultivars. For this reason, rows of polliniser cultivars are usually planted between rows of the main cultivar in pear orchards, and 2.5 – 4.0 honeybee (*Apis mellifera* L.) hives ha^{-1} are introduced to ensure adequate pollen transfer (McGregor, 1976; Free, 1993; Monzon *et al*., 2004; Stern *et al*., 2004). Honeybees are considered to be the most important pollinators in pear. Nevertheless, limited pollen transfer has been cited as the main cause of poor fruit set in some cultivars (Webster, 2002). Pear flowers produce abundant pollen which attracts bees. Most of the cultivars studied produce little nectar (1.1–8.3 µl per flower) with low sugar concentrations [< 10% (w/v)] (McGregor, 1976; Benedek *et al*., 2000; Farkas *et al*., 2002a), which are highly variable and dependent on weather conditions (Benedek *et al*., 2000). *Apis mellifera* workers visit pear flowers mainly for pollen, and often switch to other fruit tree species or to other more attractive plants for nectar (Free, 1993; Benedek and Ruff, 1998; Farkas *et al*., 2002b; Monzon *et al*., 2004).

Pollination quality and subsequent yield are usually evaluated by fruit-set (before and after fruitlet drop). It would be useful to develop a more rapid estimation procedure in orchards. The presence of pollen tubes at the base of the style enables quantification of pollination efficiency after only a few hours, up to a few days, depending on the species (Davis, 1992). In pear, compatible and incompatible pollen tubes can easily be distinguished by fluorescence microscopy (Modlibowska, 1945; Lombard *et al*., 1973; Marcucci and Visser, 1987). In 'Conference' pear, pollen tubes reach the base of the styles after 4–5 d (Michotte-van der Aa and Jacquemart, 2003). The lengths of pollen tubes in the styles depend on the type of pollen (compatible *vs.* incompatible) placed on the stigma. Incompatible pollen tubes stop before reaching the basal third of the style. The presence of pollen tubes in the basal third of the styles in 'Conference' pear could thus be used as a reliable histological indicator of the effectiveness of pollination.

'Conference' pear flowers are visited infrequently by honeybees (Benedek and Ruff, 1998). The flowers offered a mean volume of nectar of 2.8 µl (SE = 1.8; n = 40). The nectar sugar concentration varies between 0.5–7.0% (w/v) when temperatures are lower than 14°C, and averages 9% (w/v) when temperatures are between

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15°–24°C (A.-L. Jacquemart, unpublished data). Fruit-set, following open pollination, exceeded 25% in Hungary (Nyéki and Soltész, 1998a, b) and averaged 60% in Belgium (Jacquemart and Michotte-van der Aa, 2003). No viable seeds were observed in selfed fruits, and open-pollinated fruits contained very little viable seed [0.1 ± 0.1 per fruit (mean ± SE); Jacquemart and Michotte-van der Aa, 2003]. This absence of viable seeds was attributed to a high tendency towards spontaneous or induced parthenocarpy (Nyéki *et al*., 1998; Deckers and Schoofs, 2002; Jacquemart and Michotte-van der Aa, 2003). Even if parthenocarpy enables the set of a crop, the shape and weight of economically valuable fruit depend on the presence of seeds, and thus on pollination events (Taseï, 1984; Wertheim, 1990).

Besides honeybee hives, bumblebees and other wild bees are increasingly used to pollinate crops (Corbet, 1993; Macfarlane *et al*., 1994; Williams, 1996; Walther-Hellwig and Frankl, 2000; Monzon *et al*., 2004). Bumblebees are considered to be efficient pollinators due to their morphology, speed and high activity rate, even in adverse weather (Lundberg, 1980; Westerkamp, 1991; Fussell, 1992; Kwon and Saeed, 2003). Differences in pollination efficiency have been observed among different species of *Apidae* foraging on other plant species (Dag and Kammer, 2001; Javorek *et al*., 2002; Fuchs and Muller, 2004), but we wanted to compare the pollination efficiency of honeybees and bumblebees in 'Conference' pear.

The study reported here predicted the effectiveness of pollination in a 'Conference' pear orchard in central Belgium by assessing self-compatiblity (intra-cultivar) and comparing bumblebee and honeybee efficiency, by measuring pollen tube growth. The objectives of this study were: (1) to verify whether *Pyrus communis* cv. 'Conference' is intra-cultivar compatible; and (2) to compare the pollinating efficiency of two groups of potentially effective pollinators, honeybees (*A. mellifera* L.) and bumblebees (*Bombus terrestris* L.).

MATERIALS AND METHODS

Pollination experiments

This study was carried out on 'Conference' pear trees in a commercial orchard in Nodebais, central Belgium (50°44' N; 4°44' E) in April 1999. Four pollinator-exclusion tents, made of a metallic frame covered by a white windbreak net, were positioned before flower-set, with two trees per tent. The first two tents were devoted to intra-cultivar hand-pollinations. The other two tents were devoted to inter-cultivar pollinations, one tent with honeybees, the other with bumblebees. Fifteen flower clusters were selected per tree and covered with exclusion bags to prevent visits by pollinators. For each tree, ten flower clusters were used for honeybee and bumblebee comparisons and the remaining five for hand-pollination. A minimum of ten branches of the cultivar 'Doyenné' were placed in bottles of water and hung in the trees for insect pollination. Insects could transfer intra- or inter-cultivar pollen, and pollination was thus considered to be mixed. Hand-pollinations were performed on freshly open flowers using a thin paint brush.

Honeybee colonies consisted of approx. 30,000 bees (CARI, Louvain-la-Neuve, Belgium), and hives of bumblebees (Biobest® Biological System, Westerloo, Belgium) consisted of approx. 50 workers of *B. terrestris*. The difference in insect numbers did not influence the results as we measured pollination efficiency after the first insect visit on newly opened flowers. The exclusion bag was removed from a flower cluster and the flowers were observed until a bee visited. Open flowers (first day of anthesis) were watched continuously until either an *Apis* or *Bombus* worker visited them. The visited flowers were noted and, after the bee had left, all unvisited flowers were removed from the flower cluster and the branch was rebagged.

Four d after pollination, two flowers per cluster and per treatment were collected. These flowers were conserved in 70% (v/v) ethanol and used to count pollen grains and to observe pollen tube growth in the styles.

Pollination efficiency

Pollen grains and pollen tubes were examined by fluorescence microscopy (Kearns and Inouye, 1993). The callosic contents of the pollen wall and pollen tube plugs fluoresce heavily under UV-excitation (λ = 365 nm). Styles were placed in 1.0 M NaOH for 1 h to soften them. The styles were then rinsed in distilled water and crushed in 0.1% (v/v) aniline blue solution in 1.0 M KH_2PO_4 pH 9.0. As high numbers (> 100) can not be counted exactly, the number of pollen grains on the stigmas of each flower was estimated and classified into six categories of abundance: category 1, 0–19 pollen grains; category 2, 20–39 pollen grains; category 3, 40–59 pollen grains; category 4, 60–79 pollen grains; category 5, 80–99 pollen grains; and category 6, 100+ pollen grains. We observed five styles per flower and distinguished compatible pollen tubes, incompatible pollen tubes, or a mixture of both types. Compatible pollen tubes contained many small and regularly distributed callosic plugs in the first half of the style. Incompatible tubes showed much longer and frequent callosic plugs, and became swollen at their tip, with the occurrence of loops and branching. The number of styles with pollen tubes growing through the different parts of each style (upper, middle and lower parts, each of equal length) was quantified. It was not feasible to quantify the number of pollen tubes in each part of the style because of their high numbers.

Categories of pollen number, pollen compatibility types, and numbers of pollen tubes in the third part of the styles were compared using pooled t-tests with SAS Software (SAS Institute, Cary, NC, USA).

TABLE I

Comparison of intra- and inter-cultivar compatibility in Pyrus communis *cv. 'Conference' following hand-pollinations under experimental tents*

Pollen source	n	Compatible pollen tubes	Pollen tubes in lower third part
Intra-cultivar	83	5.0 ± 3.6	8.7 ± 3.2
Inter-cultivar	123	96.9 ± 1.5	89.1 ± 2.3
t-test		24.27	18.63
P		< 0.0001	< 0.0001

Inter-cultivar pollination was performed with 'Doyenné' pollen.
Percentages of styles showing compatible (pure and mixed) pollen tubes, and percentages of styles showing pollen tubes in their lower third part were compared with pooled t-tests.
n = number of observed styles. All values are means ± standard error.

TABLE II
Comparison of honeybee and bumblebee pollination efficiency in Pyrus communis *cv. 'Conference' under experimental tents in central Belgium*

Pollinator	n	Pollen grain number (category)[†]	Compatible pollen tubes	Pollen tubes in lower third part
Honeybees	97	4.0 ± 0.1	22.3 ± 4.3	17.2 ± 3.9
Bumblebees	99	4.5 ± 0.1	39.4 ± 4.9	18.2 ± 3.9
t-test		2.19	2.59	0.18
P		< 0.0001	0.0104	0.8602

[†]See Materials and Methods for definition of pollen grain number categories 1–6.
Mixed-pollination was performed with 'Doyenné' and 'Conference' insect pollen deposition on virgin flowers.
Percentages of styles showing compatible (pure and mixed) pollen tubes, and percentages of styles showing pollen tubes in their lower third were compared by pooled t-tests.
n = number of observed styles. All values are means ± standard error.

RESULTS AND DISCUSSION

Self-compatibility: intra- and inter-cultivar hand-pollinations

Pollen source (intra- *vs.* inter-cultivar) had a highly significant effect on the percentage of styles showing the different types of pollen tubes (Table I). A high percentage of styles showed compatible (pure and mixed) pollen tubes (96.9%) following inter-cultivar pollination. The majority of these styles (89.1%) showed pollen tubes in their (lower) third part. On the other hand, intra-cultivar pollination resulted in many incompatible pollen tubes that stopped growing at different levels in the styles, with only a few styles (7.1%) showing some pollen tubes in their lower part (Table I). Thus 'Conference' pear can be considered to be self-incompatible, as are many other *Pyrus* species or cultivars (Taseï, 1984; Wertheim, 1990; Zhang and Hiratsuka, 1999; Hiratsuka *et al*., 2001; Sanzol *et al*., 2003; Zisovich *et al*., 2004; 2005).

Polliniser cultivars such as 'Packham's Triumph' or 'Devoe' in Hungary (Nyéki and Soltész, 1998a) and 'Comice' or 'Triomphe de Vienne' in Belgium (Warnier, 2000) are thus recommended in 'Conference' pear orchards to achieve good yields. In growth-chamber pollination experiments, 'Doyenné' was also considered to be a good polliniser with 80% of styles showing pollen tubes reaching the lower third part (Michotte-van der Aa and Jacquemart, 2003). It would be interesting to compare all available polliniser cultivars for 'Conference' under field conditions. Moreover, as full compatibility occurs only when the cultivars carry no *S*-allele (incompatibility allele) in common, it would be useful to identify the *S*-alleles from cultivated polliniser cultivars in European orchards (Ishimizu *et al*., 1996; Zisovich *et al*., 2004; 2005)

Relative efficiency of honeybees and bumblebees

In mixed-cultivar pollinations, bumblebees deposited more pollen grains onto stigmas (mean category 4.5, corresponding to more than 90 pollen grains per stigma) than honeybees (Table II). Some stigmas (4%) received no pollen grains after a single honeybee visit. In our experiments, more compatible (pure and mixed) pollen tubes were observed following bumblebee visits than following honeybee visits (39.4% *vs.* 22.3%, respectively). However, no significant difference was observed between honeybee and bumblebee pollinations in terms of the proportion of styles showing pollen tubes in their lower third part (< 20%; Table II).

The better pollen carrying-capacity of bumblebees, and even of solitary bees, has been reported for other species (Walther-Hellwig and Frankl, 2000; Javorek *et al*., 2002; Fuchs and Muller, 2004). For example, *Osmia cornuta* (*Megachilidae*) was more efficient than honeybees in 'Comice' pear pollination (Monzon *et al*., 2004). Recent studies have discussed the possibility that a diverse range of pollinators contribute to overall pollination in more ways than just by increasing pollinator abundance (Stern *et al*., 2004). Different pollinator species might complement each other in terms of their spatial or temporal distribution. Several species can be used to improve pear pollination, and their numbers would have to be studied and adjusted (Monzon *et al*., 2004; Stern *et al*., 2004).

More data under field conditions are needed to estimate the relative efficiency and importance of all potential pollinators in 'Conference' pear. It could be interesting to test the efficiency of bumblebee pollination under open orchard conditions. Honeybee pollination efficiency on 'Conference' pear needs to be reassessed as it appeared insufficient under our conditions.

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