International Journal of Horticultural Science 2007, 13 (3): 69–74
Agroinform Publishing House, Budapest, Printed in Hungary
ISSN 1585-0404

Severely pollen-limited fruit set in a pear (*Pyrus communis*) orchard revealed by yield assessments and DNA-based paternity assignment of seedlings

Mattison, H., Sehic J. & Nybom, H.

Balsgård–Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Fjälkestadsvägen 459, SE-29194 Kristianstad, Sweden; Author for correspondence: Nybom, H.

Summary: In commercial fruit tree orchards, consistently high yields are necessary for a durable economy. The Swedish pear cultivar 'Carola' has been noted for low setting in some orchards, possibly due to insufficient pollination. In this study, fruit set was evaluated in a research orchard where 'Carola' had been planted together with four potential pollinators. Total yield and number of fruits was noted during three and four years, respectively. In 2003, seeds were germinated from the harvested 'Carola' fruits, and the paternity of three seedlings from 50 trees was determined with RAPD analysis. 'Clapp's Favourite' had sired 39.6% of the seedlings, closely followed by 'Seigneur d'Espéren' (30.7%) and 'Clara Frijs' (26.7%) whereas 'Skånskt sockerpäron' only sired 1.1% of the seedlings. The remaining 2.3% appeared to have been derived by selfing. Pollen-limited seed set was indicated at surprisingly short distances; accumulated number of fruits on the 'Carola' trees was significantly higher when separated by only 2 m from one of the two most efficient pollinators, 'Clapp's Favourite' or 'Seigneur d'Espéren', compared to trees 4–10 m away in the same row. Number of viable seeds per fruit was also higher in fruits from trees immediately adjacent to the pollinators, suggesting an effect of improved pollination success. The importance of very short inter-cultivar distances for efficient pollen transfer became even more clear when comparisons involved the true pollination distances as determined by RAPD; the accumulated yields decreased linearly from 55 kg at a 2 m distance to only 17 kg at 13 m.

Key words: pear, pollination, RAPD, self-incompatibility, yield

Introduction

A high fruit set is a prerequisite of high yields and durable profitability in commercial fruit tree orchards. Some pear cultivars can set fruit without pollination (parthenocarpy) but very few cultivars achieve a commercially acceptable fruit set in this way (*Nyéki*, 1982). Therefore, fruit set usually requires efficient pollination. Pollination success is strongly affected by the self-incompatibility system. This system has evolved to prevent self-fertilization, and thereby inbreeding, in flowering plants (*de Nettancourt*, 2001; *Kao & McCubbin*, 1996). In pear and other fruit-tree species, self-incompatibility is usually controlled by a single locus, the S-locus, with allelic variants (S-alleles) (*Zuccherelli* et al., 2002). The S-alleles encode for the different S-RNases that determine the S-genotype for each cultivar. Two cultivars are normally incompatible if their S-alleles are shared, and when planted together only produce occasional fruits. Cultivars that share one S-allele are only semi-compatible and therefore cannot achieve their potential yield capacity in the field (*Schneider* et al., 2005; *Zisovich* et al., 2005). DNA-based identification of the S-allele composition has been undertaken for some European pear cultivars (*Zuccherelli* et al., 2002; *Zisovich* et al., 2004; *Sanzol* et al., 2006; *Takasaki* et al., 2006; *Moriya* et al., 2007) but is, as yet, not available for the majority of cultivars grown in the northern parts of Europe.

Because of the self-incompatibility system, specific pollinators must be introduced into the orchard or its immediate surroundings to ensure good fruit set. Pollination success however depends on many other factors besides S-allele constitution. The pollen donor must flower annually, and should produce ample, viable pollen at the most fertile stage for the recipient flowers (*Free*, 1993; *Granger*, 2004). Pear pollen grains are large and there is little transfer of pollen by wind. Therefore, the cultivars must be attractive to the pollinating insects that are responsible for pollen transfer. In Swedish pear orchards, honeybees together with wild bees of the genera *Andrena*, *Bombus* (bumblebees) and *Osmia* are usually the most important pollinators (*Pettersson* et al., 2004), and their flight patterns are strongly affected by inter-cultivar differences in flower colour, scent and the number of flowers per tree (*Free*, 1993).

The pear cultivar 'Carola', developed at Balsgård from a cross between 'Johantorp' and 'Doyenné du Comice', was registered in 1983 and has now been marketed for almost 25 years. Initially, 'Carola' received considerable interest from pear growers due to very good fruit size and texture, high levels of tolerance against pear scab and good climate adaptability. Lately however, interest has decreased due to sparse fruiting in many orchards. It is generally believed among growers that this problem is due to insufficient pollination. One study was therefore recently performed in a commercial pear orchard in

North East Skåne (the southernmost province of Sweden) to determine the pollination success of 7 pear cultivars by DNA-based paternity assignment of seedlings from harvested 'Carola' fruit (*Nybom* et al., 2007). Due to the non-experimental layout of this orchard, pollination success could not be properly quantified, but some cultivars emerged as considerably more promising than others (especially 'Clara Frijs').

The present study was conducted at a research station in Kivik in South East Skåne. Various yield parameters (accumulated yield, number of fruits, average fruit weight and number of viable seeds per fruit) were analysed on 60 'Carola' trees to estimate pollination efficiency of four different cultivars planted as pollinators in the same field. In addition, seedlings obtained from fruit harvested on these 'Carola' trees were analysed using RAPD (Random Amplified Polymorphic DNA) to investigate pollen donors since the closest putative pollinator may not always be the one that has actually supplied the pollen.

Materials and Methods

Plant material

A pear trial was constructed in Kivik for research purposes in the autumn 1995 (*Figure 1*). Trees grafted on quince A were planted 2 m apart in three rows of 51–54 trees, with 4 m between rows in a north-south direction. Surrounding these rows were 'Carola' trees on the eastern side and an open grass area to the west. The trial consists mainly of 'Carola' with four different possible pollinators, 'Seigneur d'Espéren', 'Clapp's Favourite', 'Clara Frijs' and 'Skånskt sockerpäron', evenly distributed in the rows. Originally there were two trees of each pollinator cultivar but due to death and damage, only one 'Clara Frijs' and 'Skånskt sockerpäron' were present. The S-allele composition has been reported as SeSl for 'Seigneur d'Espéren' and SdSe for 'Clapp's Favourite' (*Takasaki* et al., 2006) but neither 'Carola' nor the other two putative pollinators, 'Clara Frijs' and 'Skånskt sockerpäron' (probably identical with 'Kleine Gelbe Frühbirne' and 'Jaune Précoce'; *Dahl*, 1929) have been investigated.

A total of 60 trees were selected for this study, five trees immediately south and five trees immediately north of each of the six pollinator trees. In October 1999, 2000 and 2002, all fruits were harvested, counted and weighed. In 2000, number of fully developed seeds according to ocular inspection was counted for 5 fruits per tree. Trunk circumference was measured in 2003 at a distance of 30 cm from the ground. In 2003, number of fruits on each tree was counted in August, and 10 fruits were harvested in October. The seeds were collected and all seeds from a tree were pooled, sown and subsequently stratified at 4C for 15 weeks. Young leaves from three seedlings per tree, if available, were collected 20 weeks after sowing, resulting in a total of 142 seedlings. DNA was extracted from the leaves immediately after collection. In addition, leaves from 'Carola' and the four pollinators were harvested in the pear trial for DNA extraction in 2003.

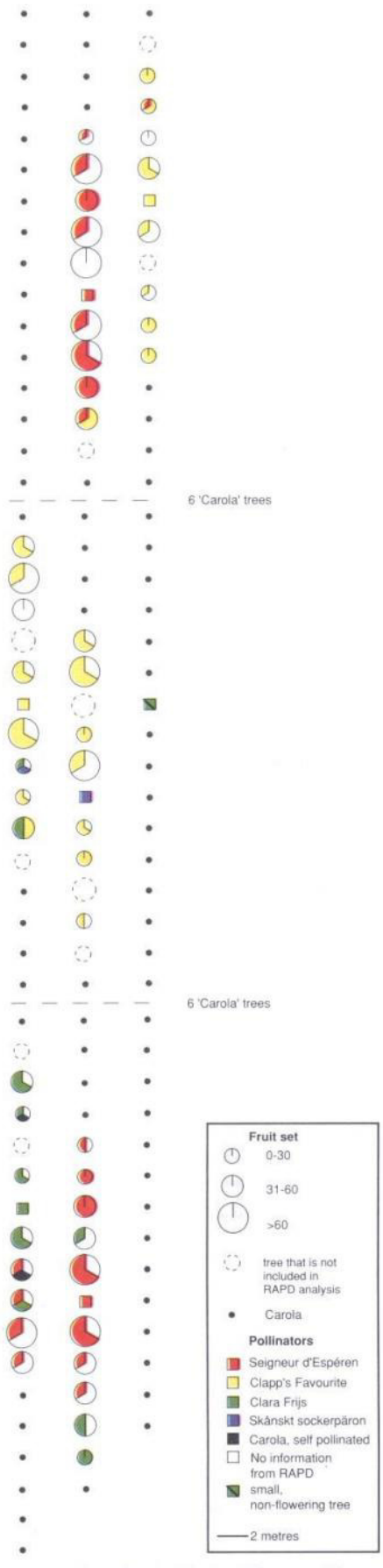

Figure 1 The research orchard with the different pollinators planted among 'Carola' trees. Accumulated number of fruits (1999, 2000, 2002 and 2003) and the actual pollinator, according to RAPD analysis of three seedlings per tree in 2003, is indicated for each of the 60 'Carola' trees included in the study

DNA analysis

Total genomic DNA was extracted using the Qiagen DNeasy™ Plant Mini Kit. The following changes were made to the manufacturer's protocol; 100 mg of leaf tissue was ground using a disposable plastic pestle in 200 µl of Buffer AP1 in 1.5 ml eppendorf tubes. An additional 200 µl of Buffer AP1 and 4 µl of RNase A (100 mg ml^{-1}) was added to the ground leaf tissue. In the last step the DNA was eluted two times with 50 µl Buffer AE.

A total of 43 primers (Operon Biotechnologies, Inc.) were screened for their ability to produce reproducible, polymorphic bands. Four of these primers (OPD09, OPD10, OPF13 and OPH02) were chosen since they could separate the pollinators from each other and from 'Carola' (*Figure 2*), and were subsequently used to analyse all 142 seedlings. The reactions contained 1 unit of *Taq* polymerase (ABgene), 2.5 µl of the supplied buffer, 3 mM $MgCl_2$, 0.2 mM dNTP, 0.6 µM primer and 5 ng DNA in a total volume of 25 µl. The amplifications were performed in a Px2 Thermal Cycler (Thermo Hybaid) programmed as follows: 1 cycle of 60 s at 96C; 35 cycles of 30 s at 94C, 30 s at 53C and 60 s at 72C; 1 cycle of 7 min at 72C. The PCR products were separated by agarose gel electrophoresis (1.8% agarose), and made visible by staining with ethidium bromide.

Figure 2 Schematic representation of RAPD band patterns obtained with four different primers. Cultivar-specific bands are marked with *.

Statistical analyses

Pearson correlation analyses were used for investigating associations between different yield parameters (number and weight of fruits, tree trunk circumference, number of seeds). T-tests were applied for analysis of how different factors (cultivar, distance between trees) affected the different yield parameters.

Results

In order to estimate overall pollination success, fruits were harvested, weighed and counted on 10 'Carola' trees surrounding each of the different pollinator trees in the same row. Calculated over all the 60 analysed trees, there was a significant correlation ($p < 0.001$, $N = 60$) between number of fruits for each year and the accumulated number of fruits for all years; Pearson correlation $r = 0.456$ (1999), 0.545 (2000), 0.701 (2002) and 0.738 (2003). Similarly, there was a significant correlation between the annual yield data and accumulated yield for all years ($r = 0.562$ (1999), 0.625 (2000) and 0.754 (2002) ($p < 0.001$, $N = 60$)). In the following analyses, we used the accumulated values since these should be more accurate than values obtained for single years.

Yield parameters

Calculated over all years with available data (1999, 2000, 2002 and 2003), the accumulated number of fruits per tree ranged from 3 to 102 with an average of 39.1 fruits per tree. Considerable variation in fruit number could be observed in connection with the different pollinators (*Figure 1*). The 20 trees surrounding 'Seigneur d'Espéren' (five trees immediately north and south, respectively, of the two pollinator trees) had a significantly higher number of fruits per tree than the trees surrounding 'Clapp's Favourite' (two-sample T-test $T = 2.93$, $p = 0.009$, $N_{Seigneur} = 20$, $N_{Clapp's} = 20$) and 'Skånskt sockerpäron' ($T = 2.19$, $p = 0.042$, $N_{Seigneur} = 20$, $N_{Skånskt} = 10$). All other pairwise comparisons between pollinators yielded non-significant results.

Accumulated number of fruits was relatively evenly distributed among trees north and south of the pollinators 'Clapp's Favourite' and 'Seigneur d'Espéren' (*Figure 1*, *Table 1*) suggesting that these had not been particularly affected by the pollinator in the neighbouring row. By contrast, trees north of 'Skånskt sockerpäron' and trees south of 'Clara Frijs' have a higher fruit number than their corresponding opposite positions. The trees with higher fruit nunbers were also in close proximity to pollinators in the neighbouring row.

Trees closest to a putative pollinator (i.e. in position 1 relative to the pollinator) sometimes differed from the other trees (in positions 2–5 relative to the pollinator). Thus, the accumulated number of fruits was 81.5 per tree for the 'Carola' trees closest to 'Seigneur d'Espéren', 34.5 for trees closest to 'Clara Frijs', 47.8 for trees closest to 'Clapp's Favourite' and 41.0 for trees closest to 'Skånskt sockerpäron'. Corresponding values for trees in positions 2–5 were 43.4 ('Seigneur d'Espéren'), 39.4 ('Clara Frijs'), 27.1 ('Clapp's Favourite') and 27.0 ('Skånskt sockerpäron'). Fruit number for trees in position 1 was significantly higher than for trees in positions 2–5 for 'Seigneur d'Espéren' ($T = 2.58$, $p = 0.019$, $N_{pos1} = 16$, $N_{pos2-5} = 64$) and 'Clapp's Favourite' ($T = 2.27$, $p = 0.033$, $N_{pos1} = 16$, $N_{pos2-5} = 64$) but not for the other two putative pollinators.

The accumulated yield calculated over the years 1999, 2000 and 2002 ranged from 0.28 to 18.04 kg per tree with an average of 7.24 kg. No significant differences could be found among the putative pollinators when the 10 trees surrounding each pollinator tree were compared for accumulated yield. Some differences in accumulated yield were however noted

Table 1 Yield parameters (no. of fruits per tree, average fruit weight per tree and number of normally developed seeds per fruit) given for trees of 'Carola' growing close (position 1) or further away (positions 2–5) on the northern and souther side, respectively, of 4 different pollinator cultivars.

	average number of fruits per tree[a]				average fruit weight per tree[b] (kg)				average number of viable seeds per fruit[c]			
	positon 1		positions 2–5		position 1		positions 2–5		position 1		positions 2–5	
pollinator	north	south	north	south	north	south	north	south	north	south	north	south
row 1												
Clara Frijs	39	30	57.5	21.3	9.25	3.94	12.11	5.37	4.2	4.0	3.3	3.0
Clapp's Favourite	76	35	24.3	53.0	12.82	7.70	4.95	11.25	4.0	3.0	3.4	4.1
row 2												
Seigneur d'Espéren	75	66	40.0	31.0	12.82	8.60	7.51	4.36	4.0	4.4	2.6	3.2
Skånskt sockerpäron	11	71	12.8	41.3	0.81	7.68	2.42	8.55	4.0	3.4	4.2	3.7
Seigneur d'Espéren	102	83	39.5	63.0	9.83	13.55	8.01	11.71	4.6	5.4	3.9	3.5
row 3												
Clapp's Favourite	35	45	18.0	15.0	7.22	8.56	2.57[d]	2.72	5.3	4.4	3.9	5.0

[a] data from 4 years; [b] data from 3 years; [c] data from 1 year; [d] no data from position 3 year 2000, position excluded

between trees growing either north or south of the putative pollinators, especially for 'Clara Frijs' and 'Skånskt sockerpäron' (*Table 1*). No significant differences were found between trees in position 1 and positions 2–5 when calculated separately for each putative pollinator.

As expected, accumulated number of fruits and accumulated yield were strongly correlated when calculated across all 60 trees ($r = 0.953$, $p < 0.001$, $N = 60$). However, tree size (measured as trunk circumference) was correlated with both the accumulated number of fruits ($r = 0.316$, $p = 0.014$, $N = 60$) and the accumulated yield ($r = 0.322$, $p = 0.012$, $N = 60$) suggesting that tree size and thus also number of available flowers may have been a major factor in determining fruit set.

Mean fruit weight differed considerably among trees and ranged from 55 to 400 g, with an average of 248 g. No correlation was detected between accumulated yield and mean fruit weight ($r = 0.229$, $p = 0.081$, $N = 60$). In addition, the overall very low yields in our trial suggest that most trees achieved a fruit set far below their carrying capacity. Possibly, fruit set has therefore been affected mainly by the amount of pollinated flowers (tree size and pollination success) instead of by resource availability.

Number of seeds

Number of fully developed seeds per fruit was counted only in 2000, and ranged from 1.0 to 5.7 with an average of 3.7. No significant differences could be found among the putative pollinators when the 10 trees surrounding each pollinator tree were compared for number of seeds. In contrast, tree position again seems to have played a role (*Table 1*). Fruits of the two 'Carola' trees immediately adjacent to each pollinator had a higher percentage viable seeds compared to fruits in trees that were further away from the pollinators ($T = 2.26$, $p = 0.026$, $N_{pos1} = 58$, $N_{pos2-5} = 168$) (*Table 1*) when analysed for all 6 pollinator trees together. Comparisons among trees in the other positions did not show any significant differences in seed number. Since the closest trees were also noted for higher yield, the increased seed number in fruits from these trees is most likely an effect of improved pollination success. By contrast, fruit size is obviously not affected by seed number in this trial since there was no correlation between the mean fruit weight and the average number of seeds per fruit when calculated across all 60 trees ($r = -0.050$, $p = 0.727$, $N = 60$).

Pollinator success

Clearly scorable and completely reproducible pollinator-specific bands were few (*Figure 1*). We succeeded in finding only one or two such bands for each pollinator, and some of these bands appear to be heterozygous and therefore not transmitted to all offspring. Using the pollinator-specific bands, paternity could be determined for 86 of the analysed seedlings; 'Clapp's Favourite' had sired 40, 'Seigneur d'Espéren' 31, 'Clara Frijs' 14 and 'Skånskt sockerpäron' one of these seedlings. In addition, there were two seedlings that apparently originate from selfing since they have only 'Carola' bands. Both 'Clapp's Favourite' and 'Seigneur d'Espéren' occurred twice in the orchard, whereas 'Clara Frijs' and 'Skånskt sockerpäron' occurred only once. Correcting for the different number of pollinator trees (dividing the no. of seedlings sired by 'Clapp's Favourite' and 'Seigneur d'Espéren' by 2), the most successful pollinator was 'Clapp's Favourite' which had sired 39.6% of the seedlings, closely followed by 'Seigneur d'Espéren' with 30.7% and 'Clara Frijs' with 26.7% whereas 'Skånskt sockerpäron' had sired only 1.1% and 'Carola' itself 2.3%.

By comparing the band pattern of each seedling with its known parent, i.e. 'Carola', and its putative parent, i.e. the set of four possible pollinators, we were able to exclude both 'Clara Frijs' and 'Skånskt sockerpäron' for the 54 remaining seedlings, which leaves us with either 'Seigneur d'Espéren' or 'Clapp's Favourite' as possible fathers. Therefore, their success as pollinators relative to 'Clara Frijs', 'Skånskt sockerpäron' and 'Carola' was apparently even higher than the above given percentages.

Information about true paternity was inserted into *Figure 1*, and demonstrated that pollen from 'Seigneur d'Espéren' and 'Clapp's Favourite' in a neighbouring row had boosted fruit set in trees surrounding the obviously less efficient pollinators 'Clara Frijs' and 'Skånskt sockerpäron'. The latter actually proved to be almost totally inefficient as a pollinator, having sired only one seedling.

Distance between the two trees intended to cross-pollinate each other is crucial for seed set. As shown in *Figure 3*, fruit set decreases rapidly with increasing distances, from 55 kg at a 2 m distance to only 17 kg at 13 m. A linear relationship is obtained when the distances between 'Carola' trees and the actual pollen donor (according to DNA-based paternity assignments) are used as the independent variable.

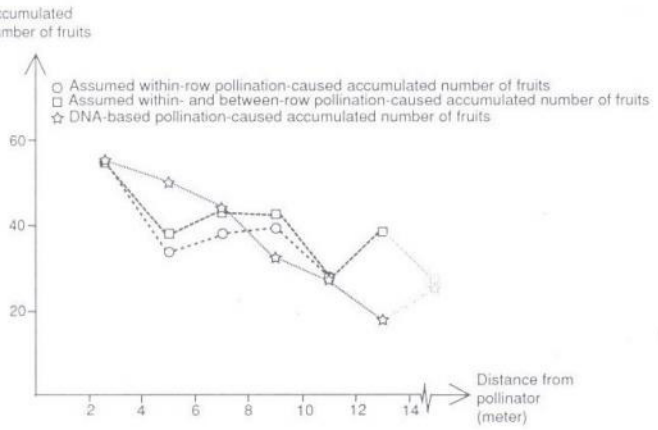

Figure 3 Accumulated number of fruits in 'Carola' trees at different distances from the pollinators, with distances based on (1) assumed within-row pollination for the 10 trees surrounding a certain pollinator, (2) assumed within- and between-row pollination for the 20 trees surrounding two adjacent pollinators, and (3) information from DNA-based paternity assignment.

Discussion

Fruit set in commercial orchards is dependent on the availability of compatible cultivars. The S-alleles of 'Doyenne de Comice', one of the parents of 'Carola', have been identified as SaSb (*Zuccherelli* et al., 2002). Neither 'Seigneur d'Espéren' nor 'Clapp's Favourite' share these S-alleles as they have SeSl and SdSe, respectively (*Takasaki* et al., 2006) and therefore it is not surprising that they can act as pollinators to 'Carola'. Unfortunately there is no information published for the other cultivars in this study. 'Carola' and 'Skånskt sockerpäron' are very dissimilar in appearance and ripening time, but a close relationship and sharing of one or two S-alleles cannot be refuted without evidence.

Cross-compatibility is, however, only one of the prerequisites for successful pollination. Many other, insufficiently known factors like flower shape, amount of pollen and nectar composition may affect the flight patterns of pollinating insects. Inter-cultivar differences in flower shape can thus reduce cross-pollination, at least in apples (*Schneider* et al., 2005). We are not aware of any major flower-shape differences among the cultivars used in our study, and they also overlap in flowering time. However, even small differences in peak flowering time can affect the pollination patterns considerably (*Visser & Marcucci*, 1983).

Paternity assignment

Ideally, accurate estimation of pollination success should involve both the checking of paternity of seedlings from fruits of the target cultivar, and the resulting crop load. Paternity of apple and pear seedlings has been ascertained using allozymes (*Kron* et al., 2001a, b; *Routley* et al., 2004), RFLP with minisatellite DNA probes (*Nybom & Schaal*, 1990) and S-alleles (*Schneider* et al., 2001a, b; 2005; *Zisovich* et al., 2005). Amount of selfing (usually very low) versus cross-pollination has thus been estimated, and pollen transfer within and between rows has been monitored. In the previous studies, usually only one to three cultivars had to be discriminated and then allozymes as well as S-alleles may be sufficient. When more cultivars are involved, multi-locus methods like RAPD or multiple single-locus markers like SSR (Simple Sequence Repeats) are usually the markers of choice (*Weising* et al., 2005).

Unfortunately we were not able to determine the father of all seedlings, but the patterns obtained (*Figure 1*) suggest that 'Seigneur d'Espéren' and 'Clapp's Favourite' sired most of the seedlings obtained from the same-row trees surrounding these two pollinators. 'Clara Frijs' was slightly less efficient, and 'Skånskt sockerpäron' was almost completely ineffective as a pollinator. Interestingly, pollination success of these latter two cultivars would probably have been estimated as quite similar if based only on fruit yield parameters. Although some pear cultivars are partially self-compatible (*Moriya* et al., 2005), selfing in 'Carola' was suggested only in approximately 2% of the seedlings in our study, and in 6% of the seedlings in a previous study (*Nybom* et al., 2007).

Orchard design

To achieve an optimal orchard design, it is important not only to find the most compatible cultivars, but also to maximize pollen transfer within the orchard (*Kron* et al., 2001b). Honeybee pollination occurs mainly in rows or between adjacent rows, resulting in an average pollen dispersal distance of only 5.8 m in an apple orchard (*Kron* et al., 2001b). Occasional pollen transports can be much longer; in a previous 'Carola' study, we found that some pollen must have travelled more than 40 m (*Nybom* et al., 2007). In our present study, we could clearly see that pollen transfer had taken place mostly within rows for the two most efficient pollinators, 'Seigneur d'Espéren' and 'Clapp's Favourite'. Transfer between rows was quite limited and was evident mainly when the in-row pollinators were less effective.

The significantly higher fruit set in trees immediately adjacent to the pollinators 'Seigneur d'Espéren' or 'Clapp's Favourite' suggests that all 'Carola' trees that are planted more than one tree away from these pollinators apparently suffer from pollen-limited fruit set. We did not find a corresponding pattern for 'Clara Frijs' and 'Skånskt

sockerpäron' only because these cultivars were not sufficiently efficient as pollinators, and therefore the surrounding trees were pollinated by 'Seigneur d'Espéren' or 'Clapp's Favourite' instead. Quantitative analysis of the effects of tree distance from pollinator (*Figure 3*) shows that fruit set is effectively linearly dependent on the distance from the pollen source. Harvests thus decrease to only 50% when the distance increases from 2 m to approximately 10 m between 'Carola' and its pollinator. The insufficient yields noted in many 'Carola' orchards probably derive from the common practice to plant three to four rows together before switching to a different cultivar. Since interplanting two cultivars within a row is usually avoided, the solution could be to plant alternating rows with 'Carola' and the pollinator.

Acknowledgements

We are very grateful to the late Nils-Arthur Ericson who was the director of Kivik Research Station and who designed and initiated the pear trial, and to Paul Ilg for managing the trial and collecting yield data in 1991–2001. The study was supported by a grant to Hilde Nybom from FORMAS.

References

Dahl, C.G. (1929): Pomologi. Albert Bonniers, Stockholm.

De Nettancourt, D. (2001): Incompatibility and incongruity in wild and cultivated plants. Springer, Berlin.

Free, J.B. (1993): Insect pollination of crops. 2nd ed., Academic Press, London.

Granger, A.R. (2004): Gene flow in cherry orchards. Theor. Appl. Genet. 108: 497–500.

Kao, T.H. & McCubbin, A.G. (1996): How flowering plants discriminate between self and non-self pollen to prevent inbreeding. Proc. Natl. Acad. Sci. USA 93: 12059–12065.

Kron, P., Husband, B.C. & Kevan, P.G. (2001): Across- and along-row pollen dispersal in high-density apple orchards: Insights from allozyme markers. J. Horticult. Sci. Biotechnol. 76: 286–294.

Kron, P., Husband, B.C., Kevan, P.G. & Belaoussoff, S. (2001b): Factors affecting pollen dispersal in high-density apple orchards. HortScience 36: 1039–1046.

Moriya, Y., Yamamoto, K., Okada, K., Iwanami, H., Bessho, H., Nakanishi, T. & Takasaki, T. (2007): Development of a CAPS marker system for genotyping European pear cultivars harboring 17 S alleles. Plant Cell Rep. 26: 345–354.

Nybom, H. & Schaal, B.A. (1990): DNA „fingerprints" applied to paternity analysis in apples *(Malus* x *domestica)*. Theor. Appl. Genet. 79: 763–768.

Nybom, H., Sehic, J. & Mattisson, H. (2007): DNA-based determination of suitable pollinating cultivars for the pear cultivar 'Carola' (*Pyrus communis*). Int. J. Horticult. Sci. 13: 15–19.

Nyéki, J. (1982): Pear varieties in Hungary. In: The pear. Cultivars to marketing. (van der Zwet, T. & Childers, N. F., eds.). Horticultural Publications, Gainesville, Florida, 115–120.

Pettersson, M.W., Cederberg, B. & Nilsson, L.A. (2004): Grödor och vildbin i Sverige. Report, Artdatabanken, SLU and Uppsala University.

Routley, M.B., Kron, P. & Husband, B.C. (2004): The consequences of clone size for paternal and maternal success in domestic apple (*Malus* x *domestica*). Amer. J. Bot. 91: 1326–1332.

Sanzol, J., Sutherland, B.G. & Robbins, T.P. (2006): Identification and characterization of genomic DNA sequences of the S-ribonuclease gene with self-incompatibility alleles S1 to S5 in European pear. Plant Breeding 125: 513–518.

Schneider, D., Stern, R.A., Eisikowitch, D. & Goldway, M. (2001a): Analysis of S-alleles by PCR for determination of compatibility in the 'Red Delicious' apple orchard. J. Horticult. Sci. Biotechnol. 76: 596–600.

Schneider, D., Stern, R.A., Eisikowitch, D. & Goldway, M. (2001b): Determination of the self-fertilization potency of 'Golden Delicious' apple. J. Horticult. Sci. Biotechnol. 76: 259–263.

Schneider, D., Stern, R.A. & Goldway, M. (2005): A comparison between semi- and fully compatible apple pollinators grown under suboptimal pollination conditions. HortScience 40: 1280–1282.

Takasaki, T., Moriya, Y., Okada, K., Yamamoto, K., Iwanami, H., Bessho, H. & Nakanishi, T. (2006): cDNA cloning of nine S alleles and establishment of a PCR-RFLP system for genotyping European pear cultivars. Theor. Appl. Genet. 112: 1543–1552.

Visser, T. & Marcucci, M.C. (1983): Pollen and pollination experiments. IX. The pioneer pollen effect in apple and pear related to the interval between pollinations and the temperature. Euphytica 32: 703–709.

Weising, K., Nybom, H., Wolff, K. & Kahl. G. (2005): DNA fingerprinting in plants: principles, methods and applications. Taylor & Francis/CRC press, Boca Raton USA.

Zisovich, A.H., Stern, R.A., Shafir, S. & Goldway, M. (2004): Identification of seven S-alleles from the European pear (*Pyrus communis*) and the determination of compatibility among cultivars. J. Horticult. Sci. Biotechnol. 79: 101–106.

Zisovich, A.H., Stern, R.A., Shafir, S. & Goldway, M. (2005): Fertilization efficiency of semi- and fully-compatible European pear (*Pyrus communis*) cultivars. J. Horticult. Sci. Biotechnol. 80: 143–146.

Zuccherelli, S., Tassinari, P., Broothaerts, W., Tartarini, S., Dondini, L. & Sansavini, S. (2002): S-allele characterization of self-incompatible pear (*Pyrus communis* L.). Sexual Plant Reprod. 15: 153–158.