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Effects of varying pollen load on fruit set, seed set and seedling performance in apple and pear

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Abstract. Two apple crosses and one pear cross were carried out using three different pollen densities. In one of the apple crosses, both fruit and seed set decreased when a mixture of pollen and powder (diluted pollen treatment) was used for pollination. Also, seedlings from the undiluted treatment were more vigorous than those originating from pollination with diluted pollen. In the other apple cross, however, no effect on fruit set, seed set or growth in the first year was found. Mean diameter after the second year differed among treatments, but did not seem to be related to pollen density. For pear, the number of good seeds decreased and the number of deaf, probably unfertilized, seeds increased with decreasing pollen density. However, there was no apparent significant effect of pollen density on seedling growth. With respect to scab resistance and mildew attack in both apple crosses, no differences among treatments were found. The results indicate that gametophytic competition may occur in apple crosses, but that it is probably weaker in pear.

Key words: Apple – Pear – Pollen – Gametophytic competition – Seedling vigor

Introduction

In higher plants, many genes are expressed in both the gametophyte and the sporophyte. This phenomenon is usually referred to as the "gametophytic-sporophytic genetic overlap" (Mulcahy 1979). Estimates of the extent of this overlap by comparing isozyme patterns in pollen and vegetative plant material indicated that in tomato 60% (Tanksley et al. 1981) and in maize 72% (Sari Gorla et al. 1986) of the sporophytically expressed genome is also expressed at the gametophytic level. For *Tradescantia* comparable results were found using hybridization between cDNA and mRNA from pollen and shoots (Willing and Mascarenhas 1984).

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The gametophytic-sporophytic genetic overlap can have several consequences. Mulcahy (1979) pointed out that due to the population size and the haploid status of pollen, gametophytic selection may have played an important role in the evolution of angiosperms. A practical consequence of selection on the pollen level is that, if competitive pollen tube growth and plant growth both depend on basic metabolic activities controlled by the same genes, the fitness of the fertilizing pollen grains actually influences the vigor of the sporophyte (Ottaviano and Mulcahy 1989). Thus, if pollen grains are allowed to compete with each other for the available ovules, the most vigorous ones will have highest chance to accomplish fertilization. If competition is low or absent, relatively more weak pollen grains will contribute to fertilization, and consequently the progeny will be less vigorous.

One of the techniques used to study this phenomenon is to apply limited amounts of pollen in crosses. Ter-Avanesian (1978) showed that pollination with low pollen loads increases variation in several growth parameters in progenies of cotton, *Vigna catjang* and spring wheat. Mulcahy et al. (1975, 1978) found that varying the quantity of pollen affected the sporophytic growth rate in *Petunia hybrida* and that this effect was consistent in the derived F_2 populations. For zucchini low pollen load also led to less vigorous seedlings (Stephenson et al. 1986).

Another interesting consequence of gametophytic-sporophytic genetic overlap is that efficient selection for specific traits may be possible by exerting a selective force to a gametophytic population. Successful examples of pollen selection have been found with respect to herbicide tolerance in maize (Sari Gorla et al. 1989), low temperature adaptation in tomato (Zamir and Gadish 1987) and heat tolerance in maize (Petolino et al. 1992), but negative results have been reported as well (see review of Ottaviano and Mulcahy 1989).

Apple and pear breeding programs are very time-consuming due to the long juvenile period, and therefore it is important to select vigorous seedlings at an early stage. These seedlings generally have a greater production capacity and a shorter juvenile period than seedlings with lower vigor (Visser et al. 1976). Also, they will be more precocious and productive when propagated on a rootstock (Visser and De Vries 1970).

Hand pollination is the usual procedure employed for making controlled crosses in fruit tree breeding programs. The number of pollen grains on the stigma will then far exceed the number of ovules available (for apple and pear usually one or two per style). However, no information is yet available on the effect of pollen load on the vigor of seedling progenies through gametophytic competition.

Visser and Verhaegh (1988) investigated the influence of pollen load on seed set and seedling vigor in apple and pear. For pear, no effect of pollen load on mean seedling length could be found, despite a clear influence on seed set. For apple, pollen diluted 1: 7 with dead pollen resulted in lower seedling length in one cross, but not in another. In both of the apple crosses there was no apparent effect on seed set.

These results needed reinvestigation. Visser and Verhaegh used dead (methanol-killed) pollen to prepare the dilutions. However, in some cases this may react as "mentor" pollen (Dayton 1974; Visser 1981): pollen acting as a stimulus to induce processes on the stigma and thus promoting other pollen grains to germinate and penetrate the stylar tissue. In this way, the dead pollen itself can affect fruit and seed set, leaving effects of pollen load per se undetected. Also, further dilutions than those used by Visser and Verhaegh (1988) may have a stronger effect, while measurements on older seedlings may provide more evidence.

Material and methods

Two apple (*Malus pumila* Mill.) crosses were made: 'Golden Delicious' × 'Prima' and 'Cox's O.P' × 'Prima'. The male parent was chosen for its resistance to scab (*Venturia inaequalis* (Cke.) Wint.) and the presence of genes for partial resistance to mildew (*Podosphaera leucotricha* (Ell. & Ev.) Salm.). For pear (*Pyrus communis* L.) the cross 'Doyenné du Comice' × 'Bonne Louise d'Avranches' was made. Anthers from male parents were collected and kept at room temperature until pollen was shed. Pollen was kept in vials in a desiccator at 4°C until used.

When the female parent started flowering, petals from flowers that were still closed were removed and pollination was carried out using a fine brush. Three different pollination densities were used: (A) normal pollination with undiluted pollen; (B) pollen diluted 1: 7; (C) pollen diluted 1: 14. Inert Lycopodium powder (available at pharmacies) containing grains of a size equal to that of a pollen grain was used as the dilution powder. Also, pollination with powder only (D) was carried out for control purposes. For each flower, the brush was slightly dipped into the pollen or pollen/powder mixture until a small yellow dot was visible. Rough countings indicated that in this way each stigma received about 250 grains. Care was taken to distribute equally the different treatments over the clusters of the branches of three to four trees per cross. After pollination, the branches were bagged to prevent insect and wind pollination.

The number of fruits and seeds obtained was recorded for all treatments. Also, the number of normal ("good") and the number of "deaf" seeds (shrivelled and apparently not filled with a well-developed embryo) was recorded. All normal seeds from each treatment were weighed together. They were then sown and stratified for 8 weeks at 0°C before being brought to the greenhouse (18°C). From the first apple cross, 300 seeds of each treatment were sown separately. The young plants obtained were tested for scab resistance in the greenhouse after artificial inoculation with a spore suspension. All of the plants obtained from the remaining seeds were planted in the nursery at the end of May. The number of plants obtained varied from 92 to 191 for each treatment.

After the first growing season in the nursery the height was measured; after the second year both height and the diameter were determined. In the first season the apple seedlings were subjected to a normal spraying regime; in the second year no fungicides were applied in order to allow an assessment of scab and mildew susceptibility at the end of the second year. The pear seedlings were sprayed according to a normal scheme.

Results

Table 1 presents the results with respect to fruit and seed set. Fruit set was high in 'Golden Delicious' × 'Prima' but low in both of the other crosses. In the first cross, fruit set was significantly higher when the undiluted pollen load was used than for both diluted pollen loads. Also, the number of good seeds per fruit diminished with decreasing pollen density. The control treatment, pollination with Lycopodium powder only, unexpectedly yielded one seed, which was probably a result of slight contamination with pollen. For the second apple cross both fruit and seed set were similar for all treatments and relatively low. In both of the apple crosses, no differences

Table 1. Fruit set (number of flowers pollinated and resulting fruits), seed set (total number of good and deaf seeds and numbers per fruit) and mean seed weight of good seeds (in mg) for the different pollination treatments

Crosses[a]	Pollination treatment[b]	Number of flowers	Number of fruits	%[c]	Good seeds	Deaf seeds	Good seeds per fruit	Deaf seeds per fruit	Mean seed weight
GD × P	A	310	139	45 a	731	35	5.3	0.25	36.3
	B	516	182	35 b	857	44	4.7	0.24	37.4
	C	731	279	38 b	1131	37	4.1	0.13	36.5
	D	143	2	1.4 c	1	0	0.5	0	
C × P	A	534	41	7.7 a	99	130	2.4	3.2	41.2
	B	625	46	7.4 a	118	130	2.6	2.8	41.3
	C	750	80	11 a	198	226	2.5	2.8	40.9
	D	0	0						
DC × B	A	369	29	7.9 a	133	68	4.6	2.3	40.3
	B	504	46	9.1 a	162	190	3.5	4.1	42.8
	C	676	67	9.9 a	125	285	1.9	4.3	48.5
	D	0	0						

[a] GD × P, (apple cross: 'Golden Delicious' × 'Prima'); C × P, (apple cross: 'Cox's O.P.' × 'Prima'); DC × B, (pear cross: 'Doyenné du Comice' × 'Bonne Louise d'Avranches')
[b] A, Normal pollen load; B, pollen diluted 1:7 with powder; C, pollen diluted 1:14 with powder; D, powder only
[c] Figures followed by a different character (within a cross) differ significantly using a chi-square test at a 5% level of significance

Table 2. Mean length (in cm) after the first and the second growing season and mean diameter (in mm) after the second year in the seedling populations

Crosses[a]	Pollination treatment[a]	First year				Second year						
		Length				Length			Diameter			
		Mean	*n*	SD	[b]	Mean	*n*	SD	Mean	*n*	SD	[b]
GD × P	A	117.3	189	39.3	a	225.2	163	39.8 a	19.0	163	4.62	a
	B	106.9	181	39.5	b	216.4	149	43.4 ab	17.6	148	4.35	b
	C	108.6	180	35.9	b	214.2	150	44.5 b	18.4	146	4.73	ab
C × P	A	119.5	92	18.5	a	227.1	91	29.4 a	18.1	91	3.59	a
	B	117.8	111	18.7	a	216.1	109	37.2 b	16.2	109	3.70	b
	C	121.5	191	18.2	a	225.3	187	37.6 a	17.6	187	4.06	a
DC × B	A	65.6	113	21.3	a	173.8	106	35.0 a	16.8	106	4.01	a
	B	66.8	125	20.1	a	173.6	117	38.0 a	16.6	117	3.80	a
	C	64.2	100	22.0	a	165.3	98	36.4 a	16.1	98	4.10	a

[a] See Table 1 for abbreviations
[b] Figures followed by a different character (within each cross) differ significantly using a Students *t*-test at a 5% level of significance

Table 3. Number of scab susceptible (s) and resistant (r) apple seedlings in the progenies derived from different pollen loads after artificial inoculation in the greenhouse or after natural infection in the nursery

Crosses[a]	Pollination treatment[a]	s	r	Total	Not assessed[b]	% resistent[c]
Greenhouse						
GD × P	A	151	98	249	49	39.4 a
	B	138	104	242	68	43.0 a
	C	141	95	236	63	40.3 a
Nursery						
GD × P	A	115	65	180	39	36.1 a
	B	96	69	165	48	41.8 a
	C	107	58	165	46	35.2 a
C × P	A	39	53	92	0	57.6 b
	B	49	58	107	4	54.2 b
	C	66	122	188	3	64.9 b

[a] See Table 1 for abbreviations
[b] Not assessed = deformed or poorly growing seedlings
[c] Figures followed by a different character differ significantly using a chi-square test at a 5% level of significance

between treatments with respect to the mean weight of resulting good seeds were found. For pear, fruit set did not differ when varying pollen loads were used; however, the number of good seeds per fruit strongly decreased with reducing pollen load, while the number of deaf seeds simultaneously increased. Mean seed weight (of good seeds) increased with reducing pollen load.

Table 2 shows the results of growth measurements after the first and second year in the nursery. In the first cross, 'Golden Delicious' × 'Prima', seedlings resulting from pollination with undiluted pollen were significantly higher after 1 year than those derived from diluted pollen loads. After the second year, seedling growth can best be characterized by both height and diameter. Seedlings from undiluted pollen loads (treatment A) were significantly higher than those from treatment C. Seedlings from treatment A were also thicker than those from treatment B. Thus, in this cross, seedling vigor tended to decrease with reducing pollen load.

In the second apple cross, after the second growing season seedlings from treatment B were significantly smaller and thinner than those from treatments A and C. This tendency was already apparent after 1 year but was not yet at a significant level. For pear, no differences in seedling vigor between the populations were found.

The numbers of scab resistant and susceptible apple seedlings in both the greenhouse and nursery are shown in Table 3. The percentage of scab-resistant seedlings did not differ significantly between pollination treatments, neither in the greenhouse nor in the nursery.

For mildew, frequency distribution of attack in the nursery after natural infection did not differ between seedling populations derived from different pollen loads (Table 4).

Discussion

In the apple cross 'Golden Delicious' × 'Prima', the negative effect of reducing pollen load on fruit and seed set indicates that in the diluted treatments pollination density was suboptimal. Theoretically, even in treatments B and C there were sufficient viable pollen grains to fertilize the available ovules (usually one or two per style). However, as has been shown by Visser et al. (1988), comparative pollen-tube growth in the apical part of the style is higher when there are more germinated pollen grains present on the stigma. For apple and pear at least 40 germinating pollen grains per stigma are necessary to obtain optimal pollen-tube growth. A proportion of the pollen grains may be needed to initiate processes in the style apex, i.e., promoting tube growth of the pollen that subsequently germinate. This agreed with the findings of Visser and Marcucci (1983), who showed that in double pollinations the second pollen load (applied 1–2 days later) contributed more to fertilization than the first.

Table 4. Level of mildew attack in the nursery in the progenies of the different pollination treatments for apple

Crosses[a]	Pollination treatment[a]	Mildew attack score[b]						Total
		0	1	2	3	4	5	
GD × P	A	0(0%)	0(0%)	8(4.4%)	46(25.4%)	68(37.6%)	59(32.6%)	181
	B	0(0%)	0(0%)	9(5.4%)	38(22.6%)	61(36.3%)	60(35.7%	168
	C	0(0%)	0(0%)	4(2.4%)	40(24.0%)	61(36.5%)	62(37.1%)	167
C × P	A	0(0%)	0(0%)	0(0%)	19(20.6%)	38(41.3%)	35(38.0%)	92
	B	0(0%)	0(0%)	0(0%)	19(17.1%)	47(42.3%)	45(40.5%)	111
	C	0(0%)	0(0%)	0(0%)	34(17.8%)	80(41.9%)	77(40.3%)	191

[a] See Table 1 for abbreviations
[b] Number and percentage (%) of seedlings with 0 = no, 1 = very little, 2 = little, 3 = moderate, 4 = rather severe, 5 = severe attack

In the experiments described here, each stigma received about 250 pollen grains when an undiluted pollen load was used. Thus, in treatment A pollen competition will be strong. When diluted 1: 7, the amount of pollen may be limiting for optimal tube growth, and therefore the competitive ability is probably reduced. With a 1: 14 dilution of pollen, pollen-tube growth may be less than optimal, and the level of competition will be strongly reduced. With lower competition, relatively more weak pollen grains will contribute to fertilization. In the concept of genetic overlap this would give a weaker progeny.

The decrease in fruit and seed set when using diluted pollen in 'Golden Delicious' × 'Prima' indicates that suboptimal pollen concentrations have been used. The simultaneous decrease in seedling vigor (Table 2) is evidence for the occurrence of gametophytic competition. These results also provide additional evidence for gametophytic-sporophytic overlap of fitness-determining genes.

In the apple cross 'Cox's O.P.' × 'Prima', however, our results were less clear. The treatments did not affect fruit or seed set, which were low in all cases. Also, the number of deaf seeds was high. In apple, deaf seeds usually contain a degenerated embryo and/or endosperm (Kobel 1954). Pollinations for this cross were carried out at a different location and time than for the first apple and the pear cross. Weather conditions were bad at that time. These conditions, as well as unfavorable conditions in the following period, may be responsible for the low fruit and seed set. The results of growth measurements in the progenies (Table 2) are not unequivocal. Treatment B gave less vigorous seedlings than A, as expected when gametophytic competition plays a role. The vigor of seedlings from treatment C, however, was the same as for those from the undiluted treatment. It may be possible that the bad weather conditions during pollination also influenced the results of the treatments in this cross.

For pear, the number of good seeds per fruit decreased dramatically when the diluted pollen loads were used. Simultaneously, the number of deaf seeds increased. These seeds were thin, flat and, in contrast with the deaf seeds obtained in the 'Cox's O.P.' × 'Prima' apple cross, did not contain any visible embryonic structure or endosperm. It is known that stimulative parthenocarpy is quite common in pear (Gorter and Visser 1958). Deaf seeds as described here regularly occur in fruits that are not fertilized. They consist of the integuments of the ovule that have developed in the absence of zygote development (Karnatz 1963; Kobel 1954).

The results presented here agree with the findings of Wertheim (1984) who varied the number of pollinator trees in plots of 'Doyenné du Comice'. With increasing pollinator densities in the orchard, fruit set increased. Simultaneously, more good and fewer deaf seeds were formed, but the total number of seeds was not affected.

The drastic decrease in good seed set and the increase in the number of unfertilized deaf seeds in the experiments described here indicate that the pollen loads in treatments B and C were far from optimal. Thus, gametophytic competition would be expected to be shown. However, no differences between treatments with respect to seedling vigor were found (Table 2). Similar results were found by Visser and Verhaegh (1988). In this respect, it should be considered that in the pear cross the mean weight of good seeds increased with decreasing pollen load. This is probably the result of the lower seed set in these treatments resulting in a better supply of nutrients for the remaining seeds. Heavier seeds may initially result in more vigorous seedlings. In this way the possible effects of gametophytic competition could be nullified. However, it does not seem likely that effects of seed weight are still strong after two growing seasons. It would be expected that genetic effects, such as those of gametophytic competition, would gradually exceed these physiological effects. This was found for *Petunia hybrida* in progenies from different pollination densities (Mulcahy et al. 1975). This leads to the conclusion that gametophytic competition is probably weaker in pear than in apple.

With respect to scab and mildew resistance in both apple crosses, the results indicate that these resistance genes do not seem to be linked to genes determining fitness. The percentage of scab- or mildew-resistant seedlings can thus not be manipulated by changing the pollen densities.

Our results indicate that apple breeders should be aware of the fact that the vigor of progenies may be affected when low densities of pollen are used in hand pollinations. This is especially important while less vigorous seedlings may be expected to have a longer juvenile period, a smaller production capacity and require a longer time till fruiting on a rootstock. These effects will, however, only be shown on a long-term basis. It should be emphasized that similar effects may occur when large amounts of pollen with low viability are used.

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