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A Comparison between Semi- and Fully Compatible Apple Pollinators Grown under Suboptimal Pollination Conditions

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***Abstract*. Apple (*Malus domestica*) has a gametophytic self-incompatibility (GSI) system. Consequently, fertilization is achieved by cross-pollination with a compatible pollinator. Compatibility is governed by a multiallelic *S* locus. Cultivars are fully compatible when both of their *S*-loci differ and are semi compatible when one locus is identical and the other differs. In a previous study we found that the fruit set and yield of the apple cultivar 'Topred' was reduced when it was pollinated by a semi compatible cultivar. To examine if this occurrence is a general feature in apples grown under suboptimal conditions, three additional cultivars, 'Golden Delicious', 'Granny Smith' and 'Royal Gala', were studied as pollen recipients of semi and fully compatible pollinators. Based on PCR analysis of the *S*-RNase allele, it was determined that the pollination rate of the semi compatible was significantly lower than that of the fully compatible pollinator in all cases. This was reflected by the lower fruit set and seed set of 'Golden Delicious' and 'Royal Gala', but not of 'Granny Smith'. In hand pollination experiments, where pollen was in excess, no difference was found between the semi and fully compatible pollinators in all three cases. These results indicate that the low yield, conferred by semi compatible pollinators, is due to insufficient cross-pollination (and not to cultivar characteristics). Thus, low yields due to semi compatibility may be avoided by appropriate honeybee management that will increase pollination. Still, under suboptimal conditions, for growth and pollination, full compatibility is preferable.**

Apples (*Malus domestica*) are self-incompatible, and therefore cross-pollination with a compatible cultivar is essential to achieve fruit set and yield (Dennis, 1979, 1986). In the gametophytic self-incompatibility (GSI) system, found in *Solanaceae*, *Scrophulariaceae* and *Rosaceae,* pollen growth inhibition is controlled by the *S* locus, which contains, among other genes, a multiallelic RNase gene (*S*-RNase) (Anderson et al., 1986). The S-RNases play a crucial role in conferring the incompatibility: they enter the pollen tubes during their growth in the style (Luu et al*.,* 2000), resulting in pollen growth arrest. Recent findings suggest that the non-self S-RNases are degraded by the ubiquitin/26S proteasome pathway, while the self S-RNase remains active (Qiao et al*.,* 2004).

Depending on their *S* loci, pairs of apple cultivars can be either fully compatible, when they differ in both *S* loci, semi compatible, when they carry one different and one identical *S* locus or incompatible, when both loci are identical. Use of the *S*-RNase alleles as markers provides an efficient tool for determining the level of compatibility. In addition, these markers also serve to identify the pollen source of a given fertilization event, and thus enable the measurement of the pollen flow in the orchard.

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Goldway et al. (1999) have found that the major reason for the low yields of 'Topred' apple (a sport of 'Red Delicious') in Israel was due to semi-compatibility with one of its pollinators ('Jonathan'). In the present study we questioned whether this observation was a common feature in apples cultivated in regions with suboptimal condition for growth and pollination.

Materials and Methods

Orchard design. All experiments were conducted in 10-ha commercial apple orchards in the north of Israel. Trees from all cultivars ('Golden Delicious', 'Red Delicious', 'Granny Smith', 'Royal Gala', and 'Jonathan') were of the same age (10 to 13 years), grafted on the rootstock Hashabi 13-4 and planted at a spacing of 2 × 4 m (1250 trees/ha). Row direction was north to south. The experimental plots consisted of a block of two rows of the fertilized cultivar and two adjacent rows of pollinator, semi or fully compatible, on each side.

Apple cultivars. Three combinations of semi versus fully compatible pollinators were examined. 'Granny Smith' [*S3, S23,* previously named *S10* (Broothaerts, 2003)] and 'Red Delicious' [*S9*, *S19,* previously named *S28* or *S30* (Broothaerts*,* 2003)] served as the semi and the fully compatible pollinators for 'Golden Delicious' (*S2*, *S3*), respectively; 'Golden Delicious' (*S2*, *S3*) and 'Granny Smith' (*S3*, *S23*) served as the semi and the fully compatible pollinators for 'Royal Gala' (*S2*, *S5*), respectively; and 'Golden Delicious' (*S2*, *S3*) and 'Red Delicious' (*S9*, *S19*) served as the semi and the fully compatible pollinators for 'Granny Smith' (*S3*, *S23*), respectively.

Seedling preparation and growth. Seeds were isolated from the fruits immediately after harvest, washed with running water, sown in boxes containing vermiculite medium treated with the fungicide, Marpan and stratified at 4 °C. Two months later the seeds were transferred to a nursery and left to germinate at 25 °C. Leaves were picked from one month-old seedlings, and stored at –70 °C until use.

DNA extraction. Extraction of DNA from the leaves was based on the method of Doyle and Doyle (1987). Briefly, 700 µL extraction buffer [2% hexadecyltrimethylammonium bromide (CTAB), 100 mM Tris-HCl pH 8, 20 mM ethylenediamine tetra acetic acid (EDTA) pH 8, 1.4 M NaCl, 1% polyvinylpyrolidone (PVP; MW 40000), 1% α-mercaptoethanol] were added to 100 to 200 mg of leaves and powdered in a pestle and mortar containing liquid nitrogen. This mixture was incubated for 30 min at 65 °C with occasional mixing. After cooling to room temperature, two extractions were performed with 24 chloroform : 1 octanol (v/v). DNA was pelleted with ethanol at room temperature and dissolved in double distilled water. The DNA extract was kept at –20 °C until use.

PCR analysis of S-RNase alleles. The analysis was performed on DNA extracted from the apple cultivars (as a control) and from the seeds (grown into seedlings). For the latter, one seedling per fruit was analyzed. PCR was carried out in a Minicycler (MJ Research). The primer pairs and PCR program for each of *S*-RNase alleles *S2*, *S3*, *S5*, *S7*, *S9, S19*, and *S23,* were described previously by Broothaerts et al. (1995), Janssens et al. (1995) and Schneider et al. (2001). In the PCR analysis of the *S*-RNase allele content in seeds of 'Golden Delicious', primers for all of the *S*-RNase alleles in the orchard were used, whereas in the analysis of 'Royal Gala' and 'Granny Smith' seeds, only the primers for the self and the adjacent pollinator *S*-RNase alleles were used.

Open-pollination experiment. Flowers of 'Golden Delicious', 'Royal Gala' or 'Granny Smith', in rows adjacent to the semi and fully compatible pollinators, were marked, 400 for each experiment (10 inflorescences on each of 4 branches of 10 trees). The bloom of the cultivars was simultaneous and to a comparable intensity. Fruitlets were counted a month later. Seed number/fruit were measured in 50 random fruit. Seeds from these fruit were isolated and the ones that germinated into seedlings served for DNA analysis (one from each fruit). 'Golden Delicious' open pollination experiments were conducted in 2001 and 2002, whereas 'Royal Gala' and 'Granny Smith' only in 2002.

Hand-pollination experiments. Hand pollination was carried out on 'Golden Delicious', 'Royal Gala' and 'Granny Smith'. Branches with eight to ten inflorescences were caged with screen nets (15 mesh, 30% shade) at the beginning of the balloon (mid-April). For each treatment 10 branches (one branch per tree)

Table 1. *S*-allele distribution within progeny after open pollination (2002).

Female parent	Adjacent pollinator	Fertilization by adjacent pollinator	Fertilization by nonadjacent pollinator	Self fertilization	Total seeds (no.)
Golden Delicious (*S2,S3*)					
	Red Delicious (*S9,S19*)*	29 (83%)	5 (14%)	1 (3%)	35
	Granny Smith (*S3,S23*)**	24 (60%)	13 (33%)	3 (7%)	40
Royal Gala (*S2*, *S5*)					
	Granny Smith (*S3,S23*)*	27 (90%)	3 (10%)	0	30
	Golden Delicious (*S2,S3*)**	16 (55%)	13 (45%)	0	29
Granny Smith (*S3,S23*)					
	'Red Delicious' (*S9,S19*)*	23 (88%)	3 (12%)	0	26
	Golden Delicious (*S2,S3*)**	17 (59%)	12 (41%)	0	29

*Fully compatible.
**Semi-compatible.

Table 2. PCR analysis of 'Golden Delicious' (*S2*, *S3*) progeny:

From row adjacent to Red Delicious (*S9*, *S19*)

	Fertilization with Red Delicious pollen				Fertilization with other cultivars' pollen			Self fertilization		
S3S-allele	*S2S9*[l]	*S2S19*	*S3S9*[z]	S3*S19*	*S2S23*	*S3S7*	*S3S23*	*S2S2*	*S3S3*	*S2S3*[y]
No. of seeds	10	7	6	6	3	1	1	0	0	1
Total	29 (83%)				5 (14%)			1 (3%)		

From row adjacent to Granny Smith (*S3*, *S23*)

	Fertilization with Granny Smith pollen		Fertilization with other cultivars' pollen					Self fertilization		
S-allele	*S2S23*	*S3S23*	*S2S7*	*S2S9*[z]	*S2S19*	*S3S9*[z]	*S3S19*	*S2S2*[x]	*S3S3*	*S2S3*
Number of seeds	14	10	2	4	2	2	3	2	0	1
Total	24 (60%)		13 (32.5%)					3 (7.5%)		

[z]Note that 'Red Delicious' and 'Jonathan' both carry *S9*.
[y]Could also occur by fertilization by the *S3* allele of 'Granny Smith'.
[x]Only *S2* was detected.

Table 3. PCR analysis of 'Granny Smith' (*S*3, *S23*) progeny:

From row adjacent to 'Red Delicious' (*S9*, *S19*)

	Fertilization with Red Delicious pollen				Fertilization with other cultivars pollen	
S-allele	*S3S9*	*S3S19*	*S23S9*	*S23S19*	*S3Sx*[z]	*S23Sx*
Number of seeds	7	7	7	2	2	1
Total	23 (88%)				3 (12%)	

From row adjacent to 'Golden Delicious' (*S2*, *S3*)

	Fertilization with Golden Delicious' pollen		Fertilization with other cultivars pollen	
S-allele	*S3S2*	*S23S2*	*S3Sx*	*S23Sx*
Number of seeds	10	7	5	7
Total	17 (59%)		12 (41%)	

[z]*Sx* = the second allele was not determined.

Table 4. PCR analysis of 'Royal Gala' (*S2*, *S5*) progeny:

From row adjacent to 'Granny Smith' (*S3*, *S23*)

	Fertilization with Granny Smith' pollen				Fertilization with other cultivars pollen	
S-allele	*S2S3*[z]	*S2S23*	*S5S3*	*S5S23*	*S2Sx*	*S5Sx*
Number of seeds	8	8	6	5	1	2
Total	27 (90%)				3 (10%)	

From row adjacent to 'Golden Delicious' (*S2*, *S3*)

	Fertilization with Golden Delicious' pollen		Fertilization with other cultivars pollen	
S-allele	*S2S3*[z]	*S5S3*	*S2Sx*	*S5Sx*
No. of seeds	7	9	5	8
Total	16 (55%)		13 (45%)	

[z]*S3* could be of the adjacent 'Granny Smith' or of the remote 'Golden Delicious'.

served as a replication. Flowers were pollinated by gently rubbing a pollen donor flower on a pollen recipient flower. All nonpollinated flowers were removed and the branches were closed again with the screen nets. Fruitlets were counted 1 month after pollination.

Pollen vitality was determined by germination in a solution containing 10% sucrose, 2 mM H_3BO_3 and 3 μM $Ca(NO_3)_2$. In all treatments the pollen applied had a vitality >50%.

Statistical analysis. Percentage data were subjected to arcsin transformation before analysis to provide a normal distribution. Data were analyzed for statistical significance by the general linear model (GLM) procedure of SAS (SAS, 1990). Duncan's multiple range test was applied to compare treatments when ANOVA showed significant differences among the means.

Results and Discussion

Fertilization rates of fully versus semi-compatible pollinators in open pollination. Three cultivars served as pollen recipients, 'Golden Delicious', 'Royal Gala', and 'Granny Smith'. Fertilization rates of the semi and fully compatible pollinator were determined by analysis of the *S*-RNase allele content of the seeds, isolated from the apple fruits of the recipient cultivars from rows adjacent to each of the pollinators. As can be seen in Table 1, the fertilization rate of the semi was lower than that of the fully compatible pollinator. The progeny of 'Golden Delicious', as the female parent, were analyzed for all the S-RNase alleles in the orchard (Table 2). Thus, apart from identifying the pollination efficiency of the adjacent row, fertilization by pollen of remote cultivars could also be observed. In the 'Golden Delicious' row adjacent to the fully compatible pollinator 'Red Delicious', 11% of the progeny carried *S23*-RNase alleles from 'Granny Smith', which is located two rows away from the 'Golden Delicious' row, and 3% (see comment for Table 2) carried the *S7*-RNase allele from 'Jonathan', which is located in a distant block, 12 rows away from

Table 5. Fruit set and number of seeds per fruit after open pollination.

Female parent	Adjacent pollinator	Fruit set (%) 2001	Fruit set (%) 2002	Seeds/fruit (no.) 2002
Golden Delicious (*S2,S3*)	Red Delicious (*S9, S19*)*	48 A[z]	51 A	7.6 A
	Granny Smith (*S3,S23*)**	34 B	43 B	6.4 B
Royal Gala (*S2,S5*)	Granny Smith (*S3,S23*)*	ND[y]	23 A	6.2 A
	Golden Delicious (*S2,S3*)**	ND	16 B	5.1 B
Granny Smith (*S3,S23*)	Red Delicious (*S9, S19*)*	ND	19 A	7.2 A
	Golden Delicious (*S2,S3*)**	ND	17 A	7.0 A

*Fully compatible.
**Semicompatible.
[z]Results with in a column referring to the same female parent followed by different letters differ significantly by Duncan's multiple range test, $p < 0.05$.
[y]ND = no data.

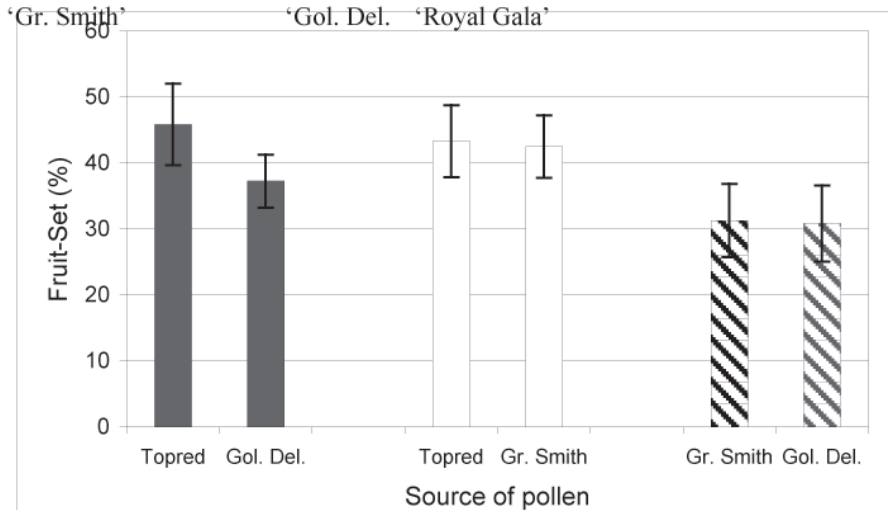

Fig. 1. Fruit set after hand pollination (2004).

'Golden Delicious'. In the 'Golden Delicious' row adjacent to the semi compatible pollinator 'Granny Smith', 13% carried the *S19*-RNase allele from 'Red Delicious', which is located two rows away, and 5% carried the *S7*-RNase allele from 'Jonathan', which is located 10 rows away. An additional 15% of the progeny were either from 'Red Delicious' or 'Jonathan' (since the *S9*-RNase allele is carried by both of these cultivars, see Table 2). Thus, as expected, pollination efficiency was reduced as the distance from the pollinator increased.

Table 1 also provides data on the maternal *S*-RNase alleles among the progeny. Summing up the presence of the maternal *S*-RNase allele for each of the three cultivars, that served as female parents, revealed that the distribution was more or less equal, as expected: 'Golden Delicious', *S2* = 50% and *S3* = 50% , 'Royal Gala' *S2* = 49% and *S5* = 51%, and 'Granny Smith' *S3* = 56% and *S23* = 44%.

Fruit set and seed set in open pollination. Analysis of the *S*-RNase allele content of the seeds indicated, that in the rows adjacent to the semi compatible cultivar, much of the fertilization was performed by remote cultivars. To determine whether the lower fertilization rate of the semi compatible pollinator was compensated by fertilization by remote pollinators, fruit set and seed set were measured. As shown in Table 5, the fruit set of 'Golden Delicious' and of 'Royal Gala' in the rows adjacent to the semi compatible pollinator was significantly lower than that of the row adjacent to the fully compatible pollinator. In accordance, significantly lower seed set was found in 'Golden Delicious' and 'Royal Gala' rows adjacent to the semi compatible pollinators. Yet, fruit set and seed set of 'Granny Smith' demonstrated no significant difference between the rows adjacent to the semi or to the fully compatible pollinators. Based on the results of the progeny PCR-*S*-RNase analysis, it was concluded that the low pollination rate of this cultivar, by the semi-compatible pollinator, was compensated by nonadjacent cultivars, whereas this was not the occurrence in the other two cases. We assume this could be explained by the longer period full blooming of 'Granny Smith'. 'Granny Smith' fully bloomed for about 10 d, whereas 'Golden Delicious' and 'Royal Gala' fully bloomed for 4 d. Thus there was a higher probability that cross-pollination by remote trees would occur in 'Granny Smith'.

Fruit set after hand pollination. To see if the reduced fruit set and seed set conferred by the semi compatible pollinator could be compensated by the addition of pollen, hand pollination experiments with pollen in excess, were carried out. As can be seen in Fig. 1, there was no significant difference between semi and fully compatible pollinators for all three pollen recipient cultivars. The results imply that pollen in excess could compensate for the lower fruit set, conferred by semi compatible pollinators. Hence, by adequate honeybee management, low fruit set, conferred by semi compatibility could be overcome. However, it seems that under suboptimal conditions for apple pollination it is preferable to design orchards with full compatibility between adjacent cultivars.

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