Flowering and Pollination Studies with European Plum (*Prunus domestica* L.) Cultivars

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Abstract

One of the most important factors affecting the financial outcome of commercial fruit growing is the success of pollination and fertilization, which in turn are dependent on weather conditions, activity of pollinators and the compatibility and overlap in flowering of the cultivars. Before introducing new cultivars, it is obviously important to know the compatibility and flowering characteristics of the genotypes. In order to find out these attributes of seven new and four established plum cultivars, pollen germination tests, hand pollinations and flowering phenology observations were carried out. 'Prosser 84' had 70% pollen germination. 'Opal' had just 3%, probably due to aged pollen. 'Anna Späth' flowered over a short period (13 days) and '1468' and 'DCA BO 46' over long (19 days) periods; the overlap of all cultivars was relatively good. With '1468', 'Victory', 'Anna Späth' and 'Prosser 84' flowering intensity varied between years, 'Jubileum', 'V70032', 'Tita' and 'DCA BO 46' flowered regularly, the others were intermediate. '1468' and 'WJ 65' flowered early, 'Prosser 84' and 'DCA BO 46' late and the rest fell in between. 'Victoria' proved to be self-fertile, 'Jubileum', 'V70032' and '1468' semi self-fertile. Fruit set percentages after June drop of all crosses were rather low; varying between 0 and 28%. With 'Tita', fruit drop occurred approximately two weeks later than with the other cultivars.

INTRODUCTION

In The Netherlands, European plums (*Prunus domestica* L.) are grown, with 'Victoria', 'Opal' and 'Monsieur Hâtif' as main cultivars and 'Jubileum' and 'Valor' as relatively new cultivars. Fruits are grown only for fresh consumption. Today, an increasing interest in new cultivars can be noticed. Before introducing new cultivars, it is very important to know the level of self-fertility, the bilateral compatibility and flowering characteristics, also in relation to the established cultivars. Because of the often relatively low temperatures during plum flowering in The Netherlands, cultivars with self-fertility are preferred. However, in order to prevent over-setting and the associated need for severe fruit thinning, semi self-fertile cultivars are considered the best for commercial fruit growing. Fully self-fertile cultivars, especially when planted in the presence of fully compatible pollinizers, easily tend towards over cropping and biennialism.

The profitability of fruit growing is strongly affected by fruit set percentage (Wertheim and Schmidt, 2005). In order to get sufficient yield, an adequate proportion of flowers must set fruit. With European plum, fertilization can take place after cross pollination and after self pollination with (semi) self-fertile cultivars only. Factors that have an effect on the fruit set percentage include temperature, air humidity, pollinator activity, availability of viable pollen, overlap of flowering periods, biennialism, and the compatibility of the pollinizer with the main cultivar.

Flowering starts in early spring as soon as the cultivar specific chilling requirement has been fulfilled and temperature has surpassed a threshold value. Temperature has a great effect on the duration of flowering. Cold weather prolongs the duration of flowering and warm weather shortens it. Consequently, the duration of flowering of a specific tree can vary from one week to several weeks (Wertheim and

Schmidt, 2005). Although the time of flowering may vary from year to year, the order of flowering of fruit-tree cultivars remains roughly about the same. However, even large fluctuations are possible, as the flowering time of fruit trees is determined by cultivar dependent fulfilment of the chilling requirement. Also, pre-chilling conditions, e.g. climatic conditions of the previous season, play a role. Keeping in mind the number of factors affecting the chilling requirement and the different responses of cultivars to these factors, it is not surprising that in some year's cultivars that normally have overlapping flowering periods, do not flower simultaneously (Tromp, 2005).

In addition to pollen of adequate quantity and quality, the success of fruit set is mainly determined by the overlap of spatially adjacent, compatible cultivars. The main cultivar and the pollinizer cultivar should flower at the same time and preferably the pollinizer should start flowering a few days before the main cultivar. Overlapping of the first and main part of flowering is important as the stigmas are receptive at or shortly after flower opening and moreover, the first flowers are inclined to give fruits of the best size and quality. Flowering overlap is defined as the difference in dates of full bloom. According to Kemp and Wertheim (1999) overlap is considered too short if the difference between the dates of full bloom is 6 days or more.

Pollen germination is defined as an appearance of a pollen tube from one of the germination pores (Wertheim and Schmidt, 2005). Under favourable conditions pollen tube growth through the stigma and style are a fairly rapid processes (Sedgley and Griffin, 1989). However, both pollen germination and the rate of pollen tube growth are highly dependent on the prevailing temperature, and there are genotype-dependent differences between cultivars at divergent temperatures (Petropoulou and Alston, 1998; Vasilakakis and Porlingis, 1985). Furthermore, the optimal temperature for pollen germination is somewhat lower than that of pollen tube growth, as observed by Hedhly et al. (2004) and Vasilakakis and Porlingis (1985). It was also apparent that cultivars with pollen that germinates well at low temperatures also have fast pollen tube growth rates, which in turn affects the duration of the effective pollination period. Not only are the differences due to higher levels of pollen viability but also higher stylar receptivity. Maternal plants become more selective as the temperature decreases, which indicates that temperature acts as a selection pressure factor for genotypes that are better adapted for pollen tube growth in the style (Hedhly et al., 2004). This hypothesis is supported by the findings of Cerović and Ružić (1992), who discovered that the incompatibility reaction in sour cherries is most pronounced at the temperature of 5°C.

The aim of the current experiments was to determine the compatibility of 7 new plum cultivars by means of hand pollinations, pollen germination tests at 2 temperatures and flowering phenology observations. Although the pollinations should be repeated at least two years to increase reliability and to cover the year-to-year variability, the results of these one-season experiments can be viewed at least as trend setting.

MATERIALS AND METHODS

The Netherlands are situated between 50 and 53° (Randwijk at 52°) North latitude. In Randwijk, all trials are on plots with fertigation and the local soil is a river clay soil. The average rainfall is about 800 mm/year and the average temperature 9.8°C. The weather data (minimum, maximum and average temperature, relative humidity and rainfall) on the day of crossing and during the following five days were recorded (data not given). During the flowering period of 2005, trees of 'Jubileum', 'Opal', 'Victoria', 'Valor', '1468', 'DCA BO 46', 'Prosser 84', 'Tita', 'V70032', 'Victory', and 'WJ 65' were used for the experiments. Trees were between 4 and 10 years old, on St. Julien A or Ferlenain (with interstem 'Czar') rootstock and planted at a distance of 3.6 x 2.0 m.

Pollen Germination

Twigs were collected from the field before bud break, stored in a cool store for one month at +1°C, placed in water at room temperature and forced to flower. Pollen was acquired from the flowers as soon as the anthers dehisced. With '1468', one set of pollen

germination tests were placed in a cool store at 3°C (because of lack of time for counting). The pollen germination test for this cultivar was also repeated according to the normal practise (counting the results after 24 h). No differences were found between the two methods. The results presented for '1468' were obtained according to the method described above. For curiosity, the pollen viability for the supposedly male sterile cultivar 'Andreea' was also determined.

The germination tests were carried out in vitro. A drop of sucrose solution (10% in demineralised water, without boron) was placed on an objective slide and a little of pollen was mixed in the solution. The slide was placed in a Petri dish that contained a small amount of water to prevent the drop from drying out. Pollen was germinated in a climate chamber in the dark at a constant temperature of 12°C and in the lab room under natural light conditions (but no direct sunlight) at a room temperature of 20.5°C, in order to see the effect of temperature on the pollen germination. A treatment in a climate chamber in the dark at 20.5°C was carried out in order to determine the effect of light on the pollen germination rate. The progress of germination was checked under a microscope with 100 x enlargement after 24 h when the pollen germination percentage was counted for 100 pollen grains per replicate. There were three replicates per treatment. Pollen was considered germinated when the pollen tube was longer than the pollen diameter.

Hand Pollinations

For reference, 'Jubileum' (semi self-fertile) and 'Victoria' (self-fertile) were selfed. 'Jubileum', 'Opal', 'Valor' and 'Victoria' were also used for a few cross pollination combinations. Per cross, 50 flowers were used, distributed over 2 or 3 trees. The hand pollinations were carried out according to the method described by Wertheim (1996). The selected flower clusters were thinned to two flowers, king and terminal flowers were removed. Pollinations were done on flowers in the late balloon stage, just before anthesis. Petals were not removed, nor were the flowers emasculated. Pollen of selected cultivars was applied by rubbing a flower of the selected pollinizer cultivar against the stigma of the mother parent. Stigmas were then covered with vaseline to prevent unwanted further pollination. The fruit set was observed before and after June drop (24–26 May and after 13 June). The fruit set percentage was expressed as the number of fruits per 100 flowers. Standards for evaluating fruit set were: 0–4% = poor, 5–9% = moderate, 10–24% = good and >25% = very good (Wertheim, 1996).

Flowering Phenology

The phenological stages of flower development were recorded from the B- or C-stage onwards according to the scale of Fleckinger (1955). The dates of four flowering stages were recorded: first open flower, 50% open flowers, 80% open flowers (full bloom) and 90% worn flowers. Flowering intensity and frost damage were recorded at the stage of full bloom. For flowering intensity a scale from 1 to 9 was used: 1 = no flowers and 9 = abundant flowering. For frost damage the scale ran likewise from 1 to 9: 1 = no damage and 9 = all flowers completely frozen (data not given). The flowering data from two years before − 2003 and 2004 – was taken into account and the average dates for the floral developmental stages were calculated.

Because of the nested design, a Generalized Linear Mixed Model was used. As the pollen germination results followed a binomial distribution, the results were analysed with the IRREML-procedure. 'Tita' was left out of the analysis, as it did not produce pollen. The results of the hand pollinations were analysed using Analysis of Variance (Genstat 8.0).

RESULTS AND DISCUSSION

Pollen Germination

Table 1 shows that there were significant differences in the pollen germination percentages between cultivars at both temperatures. Furthermore, there was an interaction

between the cultivar and the temperature. The standard deviations for the treatments were generally quite small, except for 'WJ 65' at 20.5°C. A further statistical analysis revealed an interaction between the cultivar and the temperature. Pollen germination − at 20.5°C − was not affected by the light conditions, as pollen germination was equal in light and dark; the F-probability for the factor "light" was 0.482. Surprisingly, the supposedly male sterile cultivar 'Andreea' did produce pollen, although the germination percentages for the cultivar were poor, ranging from 5 to 13.

The very low germination percentages of 'Opal' were probably due to aged pollen. However, according to Keulemans (1984, 1987), in previous experiments 'Opal' exhibits very divergent pollen germination percentages ranging from 3 to 64%, depending on the year. As the cultivar was not used as a pollen donor in the current experiment, it is unclear whether the low pollen germination percentages were due to aged pollen or to inviable pollen. 'Jubileum', 'Prosser 84', 'DCA BO 46' and 'WJ 65' had similar germination percentages at both temperatures. The germination percentage was better at 20.5°C for 'V70032' and 'Victoria'. This is in accordance with the results gained by Keulemans (1987) who reported that the germination percentage of the pollen of 'Victoria' increases by rising temperature. Cultivars having pollen that germinated better at lower temperature were 'Anna Späth', 'Valor', 'Victory' and '1468'. As plums flower early in the spring and the chances for low temperatures are high, it would be advantageous to have cultivars having pollen that germinates equally (or better) at lower temperatures.

Differences were also observed in pollen tube development (data not given); 'Jubileum' and 'Prosser 84' had especially well-formed pollen tubes at both temperatures with virtually no abnormalities whereas 15–40% of the pollen tubes of 'Victoria', 'DCA BO 46' and 'V70032' were distorted or had swollen ends.

Although a 10% sucrose solution with the addition of 15 mg L^{-1} application of boric acid is the mostly used and also recommended germination medium for fruit pollen, boron wasn't used, as boron application does not improve the germination of plum pollen according to Wertheim (1996).

Hand Pollinations

Fruit set percentages following self-pollinations were generally low. There was a marked fruit drop occurring between the two counts, especially in cultivars where the initial fruit set had been high. With 'Tita', a very late fruit drop occurred approximately two weeks later than for the other cultivars. The final fruit set percentages for 'Tita' are presented in Table 2 in italics. All the fallen fruits had flat, inviable seeds. After the fruit drop, the average fruit set percentage for 'Tita' was 7.2, and only the pollination with 'Valor' pollen induced a good fruit set (20%).

Only 'Victoria' produced a good fruit set upon selfing, 'Jubileum', 'V70032' and '1468' had a moderate fruit set and the rest produced a poor fruit set. However, within the self-pollinations, significant differences in final fruit set could not be found. In spite of this, it is still obvious that certain cultivars are indeed self-incompatible, while others show different degrees of self-compatibility. Self-pollinations showed that 'V70032' and '1468' are partially self-compatible, despite the low fruit set percentages, as also the known partially self-compatible 'Jubileum' had relatively low fruit set. According to Kemp (1996), in a previous trial 'Victoria' showed a fruit set of 23%. The reason for low fruit set of 'Jubileum' and '1468' might be the weather conditions during and after the pollinations; the mean temperature during the five days after the pollinations was merely 6.7°C and there was some rain after the pollinations were made. The rather low mean temperature (avg. 10.2°C) and some rain during the five days following the selfing of 'V70032' may be the cause for the low fruit set percentage of this cultivar as well. Furthermore, pollen germination of 'V70032' was decreased notably by the decreasing temperature. On the other hand, on the same day pollination was made using 'V70032' pollen on 'Tita', resulting in a fruit set percentage of 12. It seems that 'V70032' is only partially self-compatible. 'DCA BO 46' did not produce any fruits as a result of selfing, which may be due to self-incompatibility or detrimental weather conditions; on the day of

the pollination there was a heavy rain and a mild frost three days after the selfing of the cultivar. Before June drop, 'Victory' and 'WJ 65' had fruit set percentages of 10 and 2, respectively, but after the drop the percentages were zero for both cultivars. It seems that the self-pollen was able to fertilize the egg-cell, but the development of the embryo stopped 3–4 weeks after pollinations, resulting in embryo abortion. According to Tromp and Wertheim (2005), embryo abortion may be caused by external competition for nutrients between the developing embryo and the growing shoots or neighbouring fruits or internal competition between the embryo and the endosperm. As none of the trees of the cultivars in the experimental field were bearing many fruits, it is probable that embryo abortion was due to competition caused by either growing shoots or the endosperm.

The only pollinated cultivar that had generally a poor fruit set percentage was '1468'. All the other cultivars included in the experiment showed compatibility of different degrees with the tested pollen donors. When '1468' was used as a maternal cultivar in the one-way crosses, the fruit set percentages were low. This might further suggest that the *S*-allele activity in the pistil of '1468' is strong. On the other hand, the cultivar managed to set fruit when self-pollinated, which should not happen if the *S*-RNase concentration is high in the pistil. In contrast to '1468', the *S*-RNase concentration in the pistil of 'Tita' may be lower than in other cultivars, as the cultivar had good fruit set percentages independent of the pollinizer cultivar.

In the crosses 'Tita' × '1468', 'Tita' × 'WJ 65' and 'Tita' × 'V70032' some of the branches used as replicates were infected with bacterial canker (*Pseudomonas syringae*). At the time of pollinations there were no symptoms of the disease to be seen. Thus, the fruit set percentages for these crosses were not as high as they would have been without the effect of the disease. Well-setting cultivars were 'Jubileum', 'V70032', 'Opal' and 'Victoria', which had average fruit sets of 16.3, 12.7, 11.5 and 10.0%, respectively. Furthermore, the maternal parents had a significant effect on the fruit set percentage. Although the experimental design did not enable reliable statistical analysis in this respect, it is safe to assume that the mentioned cultivars have a tendency to set well. Paternal parents had differences in their fruit-setting capability, although not statistically significant. On average, 'Valor', 'WJ 65', 'Prosser 84', '1468' and 'V70032' were able to induce fruit set percentages greater than 10%.

Two of the four reciprocal crosses showed full compatibility in both ways, although the fruit set percentages differed slightly depending on the direction of the crossing (Table 3). 'Jubileum' and '1468' were compatible only when 'Jubileum' was used as a mother cultivar, the reciprocal cross being incompatible. The zero fruit set percentage induced by 'Jubileum' pollen on '1468' is neither caused by detrimental weather conditions as the reciprocal pollination had lower temperatures during pollination and five days after, nor pollen of poor quality, since 'Jubileum' had good pollen germination percentages at both temperatures.

Similarly, 'WJ 65' and 'V70032' had significantly different fruit set percentages depending on the direction of the crossing. The difference can't be explained by divergent weather conditions, nor by differences in the pollen viability of the father cultivar. According to Wertheim and Schmidt (2005), unilateral incompatibility doesn't occur often in fruit species and has been so far recorded in apples, pear and sweet cherries. The mechanism behind unilateral incompatibility is not well-known, but it is thought to be the result of varying levels of stylar proteins, caused by the combined effect of all of the *S*-alleles present in the pistil. A similar combined effect of the stylar *S*-alleles has been reported for Japanese pear (Wertheim and Schmidt, 2005).

Flowering Phenology

In the light of three year's flowering data (Table 4), the cultivars seem to have nearly the same flowering order every year (data not given). '1468', 'Victory', 'Anna Späth' and 'Prosser 84' had rather big differences in terms of flowering intensity between years, indicating a biennial bearing habit. On the contrary, 'Jubileum', 'V70032', 'Tita' and 'DCA BO 46' had a regular and good flowering habit. '1468' and 'WJ 65' flowered

early, ‘Prosser 84’ and ‘DCA BO 46’ late and the rest of the cultivars fell in between. It can be debated whether the average flowering dates based on only three years can give accurate estimations on the flowering development. When comparing the three years’ averages of ‘Jubileum’, ‘Opal’ and ‘Anna Späth’ to twenty years’ averages (Kemp and Wertheim, 1999), there indeed are differences. However, the differences are not big enough to change the flowering order between groups early-medium-late. According to Wertheim (1996), accurate flowering time estimations require at least 10 years of observations.

CONCLUSIONS

In the light of the current experiments, ‘V70032’ and ‘Tita’ would be interesting choices as new good and productive cultivars. As ‘Tita’ is male sterile, it requires a good pollinizer cultivar. Taking into account the unexpected and ample late fruit drop of ‘Tita’, it is clear the pollinations should be repeated, and pollinations with other commercial cultivars should be included. ‘V70032’ is interesting not only because of its partial self-fertility, but also as it was found to be a good maternal parent as well as pollen donor. Furthermore, the cultivar had very good pollen germination rates at 20.5°C, and good germination at 12°C. These cultivars have quite well-overlapping flowering periods with ‘Jubileum’ and ‘Opal’. Both ‘Tita’ and ‘V70032’ also have reliable flowering every year. Also, ‘WJ 65’ produced a good fruit set on average.

The self-incompatible ‘1468’ showed very unreliable fruit set and flowering characteristics, and poor pollen germination at 20.5°C. ‘Victory’, ‘DCA BO 46’ and ‘Prosser 84’ were not used as crossing parents very extensively and unfortunately it is not possible to say anything with certainty on the compatibility of these cultivars. ‘Victory’ and ‘Prosser 84’ showed signs of irregular flowering. However, ‘DCA BO 46’ induced good fruit set percentages on ‘Jubileum’, ‘Opal’ and ‘Victoria’. Keeping in mind that reciprocal crosses usually are equally compatible, this could suggest that the mentioned commercial cultivars could induce an acceptable fruit yield on ‘DCA BO 46’. Furthermore, the flowering times of ‘Opal’ and ‘Victoria’ overlap well with ‘DCA BO 46’.

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Tables

Table 1. Pollen germination percentages of 11 plum cultivars at 2 constant temperatures.

Cultivar	12°C		20.5°C	
	Germination %	Standard deviation	Germination %	Standard deviation
Opal	2.7 a	2.1	3.3 a	1.6
WJ 65	29.7 b	1.5	29.0 c	17.0
V70032	32.7 bc	0.6	61.3 ef	6.1
Victoria	39.3 bcd	4.9	51.0 de	7.0
Anna Späth	42.7 cde	4.2	32.3 c	4.0
Valor	44.7 de	6.7	31.3 c	3.5
Victory	48.0 de	2.0	16.0 b	1.0
DCA BO 46	48.0 de	1.0	48.7 d	0.6
Jubileum	48.3 de	5.9	43.0 d	2.6
1468	52.0 f	2.6	13.7 b	7.1
Prosser 84	69.7 f	1.5	64.7 f	3.5

Values in 1 column followed by the same letter(s) do not differ significantly.

Table 2. Fruit set percentages of one-way crosses and selfings.

Crossings	Fruit set percentage before June drop	Fruit set percentage after June drop
1468 × DCA BO 46	2	2 ef
1468 × Valor	8	2 ef
1468 × 1468	18	4 ef
DCA BO 46 × DCA BO 46	0	0 f
Jubileum × Jubileum	56	6 def
Jubileum × DCA BO 46	30	14 bcdef
Jubileum × Valor	50	28 ab
Opal × Victory	14	2 ef
Opal × Prosser 84	26	14 bcdef
Opal × 1468	34	14 bcdef
Opal × DCA BO 46	30	16 bcde
Tita × 1468	22	14 bcdef (*2*)
Tita × Prosser 84	30	20 bcd (*4*)
Tita × WJ 65	32	16 bcde (*4*)
Tita × V70032	28	12 cdef (*6*)
Tita × Valor	42	36 a (*20*)
V70032 × V70032	12	6 def
V70032 × Valor	14	8 def
V70032 × Prosser 84	18	10 cdef
V70032 × 1468	22	14 bcdef
Victoria × 1468	18	8 def
Victoria × Victoria	24	10 cdef
Victoria × DCA BO 46	40	12 cdef
Victory × Victory	10	0 f
Victory × DCA BO 46	12	0 f
WJ 65 × WJ 65	2	0 f
WJ 65 × Valor	18	10 cdef
WJ 65 × DCA BO 46	12	12 cdef

Values followed by the same letter(s) do not differ significantly (P=0.05). The LSD-value used was 15.0.

Table 3. Fruit set percentages of reciprocal crosses.

Crossing	Fruit set percentage before June drop	Fruit set percentage after June drop
Jubileum × 1468	46	14 bcdef
1468 × Jubileum	0	0 f
Jubileum × WJ 65	56	20 bcd
WJ 65 × Jubileum	22	18 bcd
Jubileum × V70032	26	16 bcde
V70032 × Jubileum	16	14 bcde
V70032 × WJ 65	26	24 abc
WJ 65 × V70032	10	8 def

Values followed by the same letters do not differ significantly (P=0.05). The LSD-value used was 15.0.

Table 4. Flowering overlap of 11 plum cultivars (Randwijk, average of 2003, 2004 and 2005).

1 April	10 April	20 April	30 April	Cultivar	Flowering intensity
A----------B------C----------D				1468	5
A----------B------C----------D				WJ 65	6
A----------B------C----------D				Jubileum	6
A----------B-C----------D				V70032	7
A----------B-----C----------D				Tita	6
A----------B------C----------D				Victoria	6
A----------B-C----------D				Opal	6
A----------B---C----------D				Victory	5
A----------B-C----------D				Anna Späth	5
A----------B-C----------D				Prosser 84	4
A----------B-------C----------D				DCA BO 46	6

A=first open; B=50% open flowers; C=80% open; D=90% worn flowers.