Flower Emasculation as the Cause for Lack of Fruit Set in Japanese Plum Crosses

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ABSTRACT. Flower emasculation is widely used in breeding programs for hybridization of fruit trees. In Japanese plum (*Prunus salicina*), some genetic crosses made by emasculation have resulted in very low or lack of fruit set, but the causes leading to this situation are not clear. In this work, the influence of flower emasculation on fruit set was evaluated in four Japanese plum-type cultivars by comparing cross-pollinations performed with and without emasculation. Fruit set and fruit drop in the crosses were characterized until harvest. To ascertain which factors in the reproductive process could be related to the lack of fruit set, compatibility was determined for each cross by the observation of pollen tube growth under the microscope and by polymerase chain reaction. Likewise, the stage of ovule development was observed under the microscope in emasculated and non-emasculated flowers. An analysis of the different pollination treatments and the study of the compatibility relationships helped to dismiss factors that intervene in the reproductive process and to identify flower emasculation as the cause of premature degeneration of ovules and its implication in determining subsequent fruit set.

Flower emasculation is widely used in breeding programs for hybridization of deciduous fruit trees (Layne, 1983). This technique is also used in cytological and genetic studies (Okie and Hancock, 2008) and in different field experiments in which controlled pollination is required to avoid the interference of undesired pollen (Hedhly et al., 2009).

For fruit breeding purposes, flower emasculation is used to carry out controlled pollinations when the female parent is self-fruitful to avoid self-pollination and ensure crossing between cultivars. Emasculation consists of removing, with fingernails or other tools, the petals, sepals, and stamens before anther dehiscence (Layne, 1983). The technique makes flowers unattractive to pollinator insects (Free, 1964) and hand-pollinations can be done without the interference of undesired self- or cross-pollen. The late balloon stage 1 d before anthesis is considered most favorable for emasculation in stone fruit breeding (Bailey

and Hough, 1975). Other pollination techniques can be used to control crosses in *Prunus*. For example, trees used as male and female can be enclosed in portable screenhouses with honeybee (*Apis mellifera*) hives in them or single female trees can be enclosed with bouquets or potted trees with honeybee hives. However, a common problem for these techniques is synchronizing blooms of pollenizers with the female tree (Okie and Weinberger, 1996). Thus, flower emasculation is particularly used in *Prunus* breeding, including Japanese plum (Okie and Hancock, 2008; Okie and Weinberger, 1996; Weinberger, 1975).

Although many Japanese plum cultivars are either mutations or chance seedlings, others are the result of planned hybridizations (Boonprakob et al., 2001; Byrne, 1989; Okie and Ramming, 1999; Okie and Weinberger, 1996; Weinberger, 1975). However, many crosses made by emasculation have resulted in very low fruit set or not fruit set at all (Okie and Hancock, 2008; Okie and Weinberger, 1996). A negative effect of flower emasculation on fruit set has been reported in other *Prunus* species, including almond [*P. dulcis* (Kester et al., 1994)], sour cherry [*P. cerasus* (Brown et al., 1996)], and sweet cherry [*P. avium* (Hedhly et al., 2009)] and points to flower emasculation as one of the underlying causes.

Factors that have been suggested as the causes for low or lack of fruit set in emasculated flowers of *Prunus* species include adverse weather conditions during and after the blooming period (Okie and Weinberger, 1996), inadequate developmental stage

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of the bud when the emasculation is carried out (Hjeltne and Stanys, 1998), loss of the nutritive and protective functions of the perianth (Badr and Crane, 1965), possible damage to the pistil (Okie and Hancock, 2008; Okie and Weinberger, 1996), or acceleration of ovule degeneration (Hedhly et al., 2009). However, the causes leading to the lack of fruit set specifically in some Japanese plum crosses are not clear.

In this work, the influence of flower emasculation on fruit set was evaluated in four Japanese plum cultivars by comparing cross-pollinations performed with and without emasculation in orchard conditions. To ascertain which factors in the reproductive process could be related to the lack of fruit set, microscopic observations of pollen tubes and ovules were related with the behavior of flowers and developing fruit in the tree. The results revealed a high incidence of ovule degeneration caused by emasculation.

Materials and Methods

PLANT MATERIALS. Six Japanese plum-type cultivars, Ambra, Angeleno, Black Diamond, Early Queen, Fortune, and Golden Globe, located in different commercial orchards were used for pollination experiments to evaluate the effect of flower emasculation on final fruit set. Several pollination treatments were performed in which 'Black Diamond', 'Early Queen', 'Fortune', and 'Golden Globe' were used as maternal parents and 'Ambra', 'Angeleno', 'Black Diamond', and 'Fortune' were used as pollen donors (Table 1).

FIELD EXPERIMENTS. Cross-pollinations in both emasculated and non-emasculated flowers were performed with each female parent. Additionally, a population of open-pollinated flowers in the orchard was used as a control for each cultivar (Table 1). Pollen used in each pollination treatment was previously obtained from flowers collected at the balloon stage (Fig. 1A) by removing anthers and placing them on paper at room temperature for 24 h until anther dehiscence. Pollen was then sieved through a 0.26-mm mesh and stored at -20°C until required.

Cross-pollinations of non-emasculated flowers were performed by supplementary pollination (Williams, 1970a) in caged trees. To avoid the arrival of pollinating insects, two trees of each female parent were enclosed in a 0.8-mm mesh cage before bloom. In these caged trees, 120 to 1560 flowers per cultivar were cross-pollinated (Table 1) using a thin paintbrush every other day until all flowers had opened (Rodrigo and Herrero, 2002a). For cross-pollinations of emasculated flowers (Layne, 1983), old flowers and young buds were removed from a set of branches, leaving only flowers at the balloon stage (Fig. 1A). Between 200 and 560 of these flowers per cultivar and crossing

Table 1. Pollination treatment, number of treated flowers, number of fruit, percentage of fruit set, and percentage of pistils with both ovules degenerated in different Japanese plum-type cultivars and crosses performed in orchard conditions.

Cultivar/pollination treatment	Flowers (no.)	Fruit (no.)	Fruit Set (%)	Pistils with both ovules degenerated (%)
Black Diamond				
Open pollination	1622	20	1.2	0
Self-pollination	1763	0	0	0
× Fortune (S) [‡]	517	17	3.3 ^y	10 ^y
× Fortune (E) [‡]	531	0	0 ^y	78 ^y
Fortune				
Open pollination	1514	30	2.0	0
Self-pollination	1016	0	0	0
× Ambra (S)	396	13	3.3 ^y	0 ^y
× Ambra (E)	559	1	0.2 ^y	80 ^y
× Black Diamond (S)	121	5	4.1 ^y	0 ^y
× Black Diamond (E)	535	1	0.2 ^y	100 ^y
Golden Globe				
Open pollination	1611	58	3.6	0
Self-pollination	1222	4	0.3	0
× Angeleno (S)	1559	79	5.1 ^x	0 ^x
× Angeleno (E)	206	5	2.4 ^x	44 ^x
× Fortune (S)	1539	4	0.3 ^x	0 ^x
× Fortune (E)	211	0	0 ^x	22 ^x
Early Queen				
Open pollination	1831	54	2.9	0
Self-pollination	1562	2	0.1	0
× Ambra (S)	577	18	3.1 ^y	9 ^y
× Ambra (E)	450	48	10.7 ^y	70 ^y
× Angeleno (S)	540	28	5.2 ^y	0 ^x
× Angeleno (E)	527	65	12.3 ^y	30 ^x

[‡]E = emasculation; S = supplementary pollination.

^ySignificant at $P < 0.05$; association between flower emasculation and the proportion of fruit set as well as the proportion of flowers with both ovules degenerated analyzed for the same cross by two-way interaction contingency tables followed by χ^2 goodness-of-fit test with Yates' correction for continuity or the Fisher's exact test as appropriate.

^xNot significant at $P < 0.05$.

were emasculated and cross-pollinated the next day using a brush and previously collected pollen.

Because cross-pollinations in non-emasculated flowers were performed by supplementary pollination without removing the anthers of the flowers, self-compatibility of female cultivars was evaluated to determine the influence of self-pollen on the subsequent fruit set. For this purpose, self-pollinations were carried out in non-emasculated flowers by supplementary pollination of 1000 to 1760 flowers per cultivar (Table 1) in the same caged trees following the same protocol.

Additionally, a group of 1500 to 1830 non-emasculated flowers on each female cultivar was left for open-pollination in trees not covered and used as controls (Table 1). Weekly counts of flowers and developing fruit from anthesis to harvest (Williams, 1970b) were carried out to characterize fruit drop pattern and ascertain final fruit set in each pollination treatment.

POLLEN–PISTIL INCOMPATIBILITY. To establish the self-incompatibility of each female parent and the cross-compatibility with the parental cultivars, the same crosses carried out in the field were also performed in the laboratory and analyzed by the observation of pollen tube growth under the microscope. Flowers from each female cultivar were collected at the balloon stage, emasculated, and maintained on wet florist foam at room temperature (Rodrigo and Herrero, 1996). Thirty flowers per

treatment were hand-pollinated 24 h after emasculation. Seventy-two h later, when pollen tubes were expected to arrive at the base of the style (Guerra et al., 2009), the pistils were fixed in alcohol:acetic acid (3:1) (Williams et al., 1999). For microscope preparation, the fixed pistils were washed three times for 1 h with distilled water and left overnight in 5% sodium sulphite at 4 °C. On the next day, the pistils were autoclaved for 8 min at 1 kg·cm⁻² in 5% sodium sulphite to soften the tissues (Jefferies and Belcher, 1974). The pistils were stained with 0.1% (v/v) aniline blue in 0.1 N K₃PO₄ to stain callose (Linskens and Esser, 1957). Pollen tube growth was observed under a light microscope with ultraviolet epifluorescence using a BP 355/425 exciter filter and a LP 470 barrier filter (BH2; Olympus Optical, Tokyo, Japan). The number of pollen tubes at the base of the style was recorded in at least 10 pistils per cultivar and treatment.

The *S*-genotype of each cultivar used in the pollination experiments (Table 1) was also confirmed by *S*-*RNase* allele typing. Genomic DNA from each cultivar was isolated from young leaves following the protocol described by Hormaza (2002) and *S*-allele typing was carried out by *S*-*RNase* polymerase chain reaction (PCR) amplification using primers pairs Pru C2-PCER and Pru T2-PCER (Tao et al., 1999; Yamane et al., 2001) according to the protocol described by Guerra et al. (2009).

OVULE VIABILITY. From each cultivar and treatment made in the field, 40 to 50 flowers from selected branches were collected 4 d after pollination and fixed in alcohol:acetic acid (3:1) for examination of ovule development under the microscope. Ovules were scored for ovule viability through the presence of callose deposits in the chalaza of degenerating ovules (Arbeloa and Herrero, 1985; Hedhly et al., 2009; Morenol et al., 1992; Pimienta and Polito, 1982; Rodrigo and Herrero, 1998; Stosser and Anvari, 1982) in at least 10 pistils per cultivar and pollination treatment. Ovule development was monitored with the same staining procedure used to observe pollen tube growth. Preparations were observed under a light microscope equipped with ultraviolet epifluorescence with a BP340-390 exciter filter and a LP 425 barrier filter (DM2500; Leica Microsystems, Wetzlar, Germany).

STATISTICAL ANALYSES. Statistical analyses were performed with SPSS statistical software (Version 15.0; SPSS, Chicago, IL). Two-way interaction contingency tables followed by a chi-square goodness-of-fit test

with Yates' correction for continuity or the Fisher's exact test as appropriate were used to test for association between flower emasculation and the proportion of fruit set as well as the proportion of flowers with both ovules degenerated for each cross. Statistical significance was defined as $P < 0.05$.

Results

FRUIT SET AND FRUIT DROP. The percentage of fruit set in open-pollinated flowers ranged from 1.2% to 3.6%. Self-pollinations of the four cultivars used as female parents did not set fruit (Table 1); thus, they appeared to be self-incompatible. Although in 'Fortune' (Fig. 2A) and 'Golden Globe' (Fig. 2B) drop of all self-pollinated flowers was complete 6 weeks after

Fig. 1. Japanese plum flowers (A) at the balloon stage and (B) after emasculation.

Fig. 2. Fruit drop in Japanese plum-type cultivars as affected by different pollination treatments: open-, self-, and cross-pollination in emasculated and non-emasculated flowers. Percentage of fruit from the original number of flowers remaining in the tree during the 13 weeks after anthesis in (A) 'Fortune', (B) 'Golden Globe', (C) 'Black Diamond', and (D) 'Early Queen'.

anthesis, in ‘Black Diamond’ (Fig. 2C) and ‘Early Queen’ (Fig. 2D), a group of self-pollinated flowers remained on the trees until 8 to 9 weeks after anthesis.

Fruit set in cross-pollinated flowers performed by supplementary pollination of non-emasculated flowers ranged from 0.1% to 5.1% and was higher than the fruit set obtained in open-pollinated flowers for the same female cultivar, except in the case of ‘Golden Globe’ × ‘Fortune’ in which no fruit were obtained (Table 1). Cross-pollinated flowers stopped dropping at the time when drop of self-pollinated flowers was completed in each cultivar. However, emasculated flowers dropped between 1 and 2 weeks earlier than flowers crossed by supplemental pollination (Fig. 2).

Flower emasculation had a variable effect on fruit set when compared with non-emasculated flowers in the different compatible crosses (Table 1). Thus, chi-square analysis revealed significant association between emasculation and lack of fruit set in ‘Black Diamond’ × ‘Fortune’ (N = 1048, Yates’ $\chi^2 = 15.748$, df = 1, $P < 0.001$), ‘Fortune’ × ‘Ambra’ (N = 955, Yates’ $\chi^2 = 13.386$, df = 1, $P < 0.001$), and ‘Fortune’ × ‘Black Diamond’ (N = 656, Yates’ $\chi^2 = 12.875$, df = 1, $P < 0.001$). However, no significant association between flower emasculation and fruit set reduction was reported in ‘Golden Globe’ × ‘Angeleno’ (N = 1765, Yates’ $\chi^2 = 2.246$, df = 1, $P = 0.134$) and ‘Golden Globe’ × ‘Fortune’ (N = 1750, Yates’ $\chi^2 < 0.001$, df = 1, $P = 1$). The cross ‘Golden Globe’ × ‘Fortune’ did not produce fruit either in emasculated or in non-emasculated flowers indicating the possible cross-incompatibility of the crossing. On the other hand, fruit set in emasculated flowers was significantly higher than fruit set in flowers crossed by supplemental pollination in the crosses in which ‘Early Queen’ was the female parent: ‘Early Queen’ × ‘Ambra’ (N = 1027, Yates’ $\chi^2 = 22.708$, df = 1, $P < 0.001$) and ‘Early Queen’ × ‘Angeleno’ (N = 1067, Yates’ $\chi^2 = 16.245$, df = 1, $P < 0.001$).

SELF- AND CROSS-INCOMPATIBILITY. In the four self-pollinated cultivars, Black Diamond, Early Queen, Fortune, and Golden Globe, pollen tube growth was arrested in the style (Fig. 3A) and no pollen tubes reached the base of the style (Table 2).

Cross-pollinations displayed variable percentages of pistils with pollen tubes growing through the pistil (Fig. 3B) and reaching the base of the style (Table 2). Although some pistils of ‘Black Diamond’ × ‘Fortune’, ‘Fortune’ × ‘Black Diamond’, ‘Early Queen’ × ‘Ambra’, ‘Early Queen’ × ‘Angeleno’, and ‘Golden Globe’ × ‘Angeleno’ crosses had pollen tubes at the base of the style, pollen tubes did not reach the base of the style in the pistils analyzed from the cross ‘Golden

Fig. 3. Pollen tube growth along the style and ovule development in pistils of Japanese plum flowers (A) pollen tube (arrow) arrested in the style in an incompatible cross (bar = 50 μm), (B) pollen tubes (arrows) advancing to the base of the style in a compatible cross (bar = 50 μm), (C) both ovules degenerated with callose accumulation at the chalazal end (*) (bar = 100 μm), and (D) well-developed ovule (bar = 100 μm).

Table 2. Pollination treatment, percentage of pistils with pollen tubes at the base of the style, and *S*-genotype of different Japanese plum-type cultivars and crosses performed in laboratory conditions.

Cultivar/cross	Pistils with pollen tubes at the base of the style (%)	<i>S</i> genotype
Black Diamond		<i>SeSh</i>
Self-pollination	0	
× Fortune	59	<i>SeSh</i> × <i>SbSc</i>
Fortune		<i>SbSc</i>
Self-pollination	0	
× Ambra	—	<i>SbSc</i> × <i>SbSo</i>
× Black Diamond	50	<i>SbSc</i> × <i>SeSh</i>
Golden Globe		<i>SbSc</i>
Self-pollination	0	
× Angeleno	91	<i>SbSc</i> × <i>ScSh</i>
× Fortune	0	<i>SbSc</i> × <i>SbSc</i>
Early Queen		<i>SeSh</i>
Self-pollination	0	
× Ambra	36	<i>SeSh</i> × <i>SbSo</i>
× Angeleno	27	<i>SeSh</i> × <i>ScSh</i>

Globe' × 'Fortune'. Thus, the four self-pollinated cultivars appeared self-incompatible, and all the crosses except 'Golden Globe' × 'Fortune' were cross-compatible.

S-RNase typing by PCR was used to identify the *S*-genotype of the different cultivars used in the pollination experiments (Table 2; Fig. 4). All the cultivars analyzed had the expected *S*-RNase alleles (Guerra et al., 2009; Sapir et al., 2004) except 'Golden Globe' that had *S*-alleles different from those previously reported (Guerra et al., 2009). The PCR analysis confirmed the cross-compatibility of all the crosses except for 'Golden Globe' × 'Fortune' that was cross-incompatible because both cultivars had the same *S*-genotype.

OVULE VIABILITY. To determine the causes resulting in the different behavior of emasculated and non-emasculated flowers of the same crosses, ovule development was analyzed under the microscope in pistils from all the pollination treatments performed in the field. Both ovules were degenerated (Fig. 3C) in a variable percentage (22% to 100%) of the pistils analyzed from crosses with emasculated flowers (Table 2). However, most supplemental pollinated flowers from the same crosses (90% to 100%) and all the self- and open-pollinated flowers had at least one well-developed ovule (Fig. 3D; Table 1). Thus, the number of flowers with both ovules degenerated were significantly higher in emasculated than in non-emasculated flowers in 'Black Diamond' × 'Fortune' ($N = 19$, Yates' $\chi^2 = 6.363$, $df = 1$, $P = 0.005$), 'Fortune' × 'Ambra' ($N = 18$, Yates' $\chi^2 = 8.508$, $df = 1$, $P = 0.001$), 'Fortune' × 'Black Diamond' ($N = 15$, Yates' $\chi^2 = 11.251$, $df = 1$, $P < 0.001$), and 'Early Queen' × 'Ambra' ($N = 21$, Yates' $\chi^2 = 4.033$, $df = 1$, $P = 0.024$). However, no significant association between flower emasculatation and ovule degeneration were reported in 'Golden Globe' × 'Angeleno' ($N = 17$, Yates' $\chi^2 = 2.508$, $df = 1$, $P = 0.082$), 'Golden Globe' × 'Fortune' ($N = 19$, Yates' $\chi^2 = 0.685$, $df = 1$, $P = 0.211$), and 'Early Queen' × 'Angeleno' ($N = 21$, Yates' $\chi^2 = 1.859$, $df = 1$, $P = 0.104$).

Discussion

Flower emasculatation caused premature ovule degeneration and affected fruit set in the japanese plum-type cultivars analyzed. The analysis of the different pollination treatments performed in orchard conditions and the study of the compatibility relationships among cultivars allowed dismissal of other factors that could intervene in the reproductive process. Premature degeneration of both ovules was identified as the cause of female

sterility and their implications for subsequent fruit set in emasculated flowers.

The percentage of fruit set in open-pollinated flowers was representative of fruit set of japanese plum-type cultivars in orchard conditions (M.E. Guerra, A. Wunsch, M. López-Corrales, and J. Rodrigo, unpublished data). Supplementary pollination increased fruit set in most crosses, and only self-pollinations and one cross-incompatible crossing did not produce fruit. On the other hand, flower emasculatation had a variable effect on fruit set in the crosses depending on the female parental cultivar. Emasculatation of flowers has been reported previously as the cause of an increase in the percentage of fruit set in different *Prunus* species such as peach (*P. persica*) (Arbeloa and Herrero, 1991), sweet cherry (Theiler-Hedtrich, 1994), apricot (*P. armeniaca*) (Rodrigo and Herrero, 2002b; Rodrigo et al., 2009), almond (Socias i Company et al., 2005), or interspecific crosses (Arbeloa et al., 2006). This increase in fruit set in emasculated flowers can be explained by the fact that flowers with anomalies or underdeveloped pistils are usually taken away when older and younger flowers are removed from the branches selected in the experiments. Likewise, hand-pollination of the emasculated flowers can improve the chances of flowers to set fruit, because this ensures the arriving of compatible pollen to the stigma of emasculated flowers. However, the emasculatation of flowers has been also related to a significant reduction of fruit set in almond (Kester et al., 1994), sweet cherry (Hedhly et al., 2009), and japanese plum-type cultivars (Okie and Hancock, 2008; Okie and Weinberger, 1996).

The results of this work showed that emasculatation of flowers in japanese plum influenced fruit set, causing an increase or a reduction, even a lack, of fruit set depending on the cultivar. Although flower emasculatation resulted in an increase of fruit set in 'Early Queen', in the other cultivars, the fruit set was drastically reduced ('Golden Globe') or even no fruit set was achieved ('Black Diamond' and 'Fortune'). Furthermore, the pattern of fruit drop was also different in 'Early Queen' in relation to the other cultivars analyzed. Thus, the drop of emasculated flowers in 'Early Queen' followed the same pattern as supplemental pollinated flowers, and fruit drop in both populations was not completed until 9 weeks after pollination. However, in the other cultivars, the drop of emasculated flowers was complete in the 5 weeks after pollination, several weeks before the establishment of the fruit set in the population of supplemental pollinated flowers.

The different behavior observed among the japanese plum-type cultivars regarding both the effect of flower emasculatation and the pattern of drop could be related to the different origin of each cultivar. Nowadays the term "japanese plum" does not correspond to a pure species, but it comprises a heterogeneous group of diploid plums that were derived from the interspecific hybridization of the original species, *P. salicina*, with other diploid plums ($2n = 16$) such as *P. americana*, *P. hortulana*, *P. munsoniana*, *P. simonii*, *P. nigra*, *P. besseyi*, *P. angustifolia*, and *P. cerasifera* (Byrne, 1989; Faust and Suranyi, 1999; Okie, 2006).

Observations of pollen tube growth confirmed that 'Black Diamond', 'Early Queen', 'Fortune', and 'Golden Globe' are self-incompatible, because no pollen tubes were observed at the base of the style in self-pollinated flowers and no fruit were obtained from self-pollinations in the field. All the crosses analyzed were compatible except for 'Golden Globe' × 'Fortune' in which no pollen tubes were observed at the base of the style. The percentages of pistils with pollen tubes at the base of

Fig. 4. Polymerase chain reaction amplification of *S*-RNase alleles of japanese plum-type cultivars Black Diamond (B), Fortune (F), Early Queen (E), Ambra (A), Angeleno (An), and Golden Globe (G) from the different orchards using primers Pru C2-PCR. SS = size standard.

the style in compatible crosses were lower than in other *Prunus* but representative of Japanese plum-type cultivars in previous studies (Guerra et al., 2009).

The identification of the *S*-alleles of the six cultivars analyzed complemented the results obtained by pollination treatments and pollen tube growth. Cross-compatibility results correlated well with the percentages of fruit set obtained in each cross performed in non-emasculated flowers and allowed dismissing cross-incompatibility as the cause of the lack of fruit set in the crosses performed on emasculated flowers. Results confirmed the *S-RNase* genotype of 'Ambra', 'Angeleno', 'Black Diamond', 'Early Queen', and 'Fortune' (Guerra et al., 2009; Sapir et al., 2004). However, the *S-RNase* genotype of 'Golden Globe' reported here (*SbSc*) differed from that previously reported (*SbSf*) (Guerra et al., 2009). Because this cultivar descends from Laroda (*SbSc*) and Queen Ann (*SbSh*) (Ramming, 1994), it seems plausible that the *S*-genotype of 'Golden Globe' is *SbSc*. The existence of homonyms in this and other Japanese plum-type cultivars could explain situations of lack of fruit set in orchards in which the apparent compatible pollenizers are improperly named.

Results from both field and laboratory experiments indicated that the four female cultivars were self-incompatible. However, 'Black Diamond' and 'Early Queen' have the *Se*-haplotype that has been correlated with self-compatibility in some Japanese plum cultivars (Beppu et al., 2005; Guerra et al., 2009). Further fruit set and pollen tube growth experiments as well as molecular analysis are needed to confirm that *Se*-allele is correlated with self-compatibility in other cultivars.

The high proportion of emasculated flowers showing callose in both ovules in which fertilization could not take place correlated with the high reduction of fruit set in the affected cultivars. Flowers of *Prunus* contain two ovules within a single carpel, and fertilization of at least one ovule is required for fruit set. Thus, one of the two ovules, the primary ovule, can be fertilized and become a seed; the other, the secondary ovule, usually aborts (Bradbury, 1929). The two ovules within each flower are similar in size at anthesis. Although the primary ovule continues growing in the days after anthesis, the secondary ovule arrests its growth and degenerates. Degeneration of the secondary ovule has been linked to the appearance of callose that starts to accumulate at the chalazal end of the nucellus and then spreads into the whole ovule, which then shows clear symptoms of degeneration with a shrunken nucellus (Arbeloa and Herrero, 1991; Cerovic and Micic, 1999; Cerovic et al., 2000; Pimienta and Polito, 1982; Rodrigo and Herrero, 1998; Stosser and Anvari, 1982). Although the premature degeneration of one of the two ovules is part of the normal developmental process of *Prunus* flowers, the degeneration of both ovules in flowers has been associated with female sterility in different *Prunus* species and cultivars (Lillecrapp et al., 1999; Mert and Soyulu, 2007). Likewise, ovule degeneration has been reported as the cause of reduction of fruit set in emasculated flowers of some cherry cultivars (Hedhly et al., 2009). Our results show that ovule degeneration caused by emasculation can also reduce and even prevent fruit set in some Japanese plum cultivars.

Flower emasculation as the cause of ovule degeneration and lack of fruit set was clear in three cultivars analyzed in this work. This may well be a general problem that could explain situations of lack of offspring in some crosses performed for breeding purposes between different Japanese plum-type cultivars with no apparent reasons for the lack of fruit set (Okie and

Hancock, 2008; Okie and Weinberger, 1996). Alternatively, crosses in those cultivars in which flower emasculation prevents fruit set could be performed by supplemental pollination of non-emasculated flowers with pollen of the desired male parent in caged branches or trees. For self-incompatible cultivars used as female parents, all the offspring obtained would be expected to descend from the male parent cultivar used as the pollen source. However, for self-compatible cultivars, the offspring would include individuals whose male parent could be the cultivar used as the female parent or the cultivar used as the supplemental pollenizer. In this case, the use of the self-incompatibility locus (*S*-genotype) as a genetic marker could be useful to identify those offspring of the desired cross, because it has been successfully used in *Prunus* to differentiate among different pollen sources (Sebolt and Iezzoni, 2009).

This study revealed a strong effect of emasculation on final fruit set that was related to degeneration of both ovules, because the crosses carried out by this technique can increase, reduce, and even prevent fruit set depending on cultivars. This information may be valuable to identify the causes for lack of offspring in some crosses performed for breeding purposes. Once known, this situation could be solved through the use of alternative methods to flower emasculation for crosses made on sensitive female cultivars.

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