Flower bud differentiation and development in fruiting and non-fruiting shoots in relation to fruit set in apricot (*Prunus armeniaca* L.)

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Abstract Situations of high flower bud drop and low fruit set without apparent causes are common in fruit trees. The term *flower quality* has been coined to explain differences among flowers in their capacity to set fruit, but the causes underpinning these differences are largely unknown. This lack of knowledge is based on the fact that these differences are established a posteriori and there are no criteria to determine a priori what will make a flower to set a fruit or to drop. In this work, we profit of the empirical knowledge that there are fruiting and non-fruiting shoots to explore to which extent flower bud differentiation and bud development will affect the subsequent fruit set. For this purpose, the processes from flower bud differentiation to fruit set were sequentially analyzed in both types of shoots, over two years. More than half of buds from long shoots aborted development and dropped before flowering. At anthesis, most of the remaining flowers showed underdeveloped pistils that failed to sustain pollen germination or pollen tube growth along the pistil. This unsuccessful development resulted in clear differences in fruit set between both types of branches. These results highlight that flower bud differentiation and development play an important role for fruit set and that developmental timing appears critical to reach anthesis with a fully developed pistil.

Keywords *Bud drop · flower bud development · fruit set · pistil · pollen grains · pollen tubes*

Introduction

Apricot (*Prunus armeniaca* L.) is a species particularly prone to erratic fruit set. Low fruit set has been mainly related to external factors during the flowering period such as frosts (Gunes 2006), high pre-blossom temperatures (Rodrigo and Herrero 2002a) or pollination failure (Rodrigo and Herrero 1996). Likewise, internal causes in the flower have been also related to fruit set, such as male and female sterility (Lillecrapp et al. 1999), pollen-pistil incompatibility (Burgos et al. 1997), the length of the effective pollination period (EPP) (Egea and Burgos 1992), the nutritional status of the flower (Rodrigo and Herrero 1998, Rodrigo et al. 2000, 2009), and the stage of development of the ovule (Ruiz and Egea 2007) or the embryo-sac at anthesis (Egea and Burgos 1994).

Alterations during flower bud development may also cause lack of fruit set. Thus, flower bud drop in apricot has been repeatedly reported in different cultivars and situations (Legave et al. 1982; Alburquerque et al. 2004; Julian et al. 2007). A number of factors have been related to flower bud drop such as frosts before and during bud break (Julian et al. 2007), unsatisfied chilling requirements (Ruiz et al. 2007), water stress (Brown and Abi-Fadel 1953; Alburquerque et al. 2003), high bud density (Alburquerque et al. 2004), premature defoliation (Martinez-Gomez et al. 2002), or the nutritional status of shoots (Tabuenca 1969). Likewise, warm temperatures during dormancy have been related to flower bud drop in peach (Brown 1958; Weinberger 1967), and stresses during flower bud differentiation have been associated with the flower quality of the next season in almond (Lamp et al. 2001). However, these factors cannot entirely explain situations of high flower bud drop that are produced without apparent causes, and

the physiological mechanisms that induce flower bud drop remains largely unknown.

The term *flower quality* has been coined to explain differences among flowers in their capacity to set fruit (Williams 1965). But the causes underpinning these differences are largely unknown. This lack of knowledge is based on the fact that these differences are established a posteriori and there are no criteria to determine a priori what will make a flower to set a fruit or to drop. Differences in flower quality are empirically known in different fruit tree species among flowers located in different type of shoots and while there are fruiting branches, long shoots are largely unproductive and are commonly pruned (Alburquerque et al. 2003; Syvertsen et al. 2003; Volpe et al. 2008; Nortes et al. 2009). In this work, we profit of this empirical knowledge to explore to which extent flower bud differentiation and bud development will affect the subsequent fruit set. For this purpose the buds from two types of shoots, the productive short shoots and unproductive long shoots with different capacity of fruit set within the same tree, were analyzed.

Materials and methods

Plant material

Eight trees of apricot 'Moniqui' grafted on 'Montizo' plum rootstocks in 1992 and planted in an orchard distribution of 6 x 6 m were used from an experimental orchard at CITA, Montañana (Zaragoza, Spain), placed at 41º44'30"N latitude, 0º47'00"W longitude, and 220 m altitude. Two types of shoots of the current year were selected: twigs or short shoots, between 10 and 30 cm in length, and long shoots longer than 1 m and with a basal diameter higher than 1.5 cm.

The cultivar 'Moniqui' has been reported as having requirements of 1050-1150 chill units (CU) and 779-956 hours below 7ºC (Julian et al. 2007 and references therein). The time when the chilling requirements were covered were estimated for both years of experiments. Chilling requirements were fulfilled in both years in mid-January, when flower buds were still closed, some five weeks before bud break, and eight weeks before anthesis.

Flower bud differentiation and development

To characterize flower bud growth, 60 flower buds from short shoots and 130 flower buds from long shoots were randomly sampled weekly around the canopy from the beginning of bud differentiation in August-September until the end of dormancy in January, when chilling requirements where fulfilled. Flower buds were excised and weighed in a R200D Sartorius balance (Sartorius AG, Gottingen, Germany). This experiment was performed in two consecutive years, and in four trees per year.

To follow flower bud development, several branches completing over 460 flower buds per tree were monitored. To characterize the progression of flower bud stages, all the flower buds in each shoot were monitored. Thus, counts of

flower buds at each phenological stage were made every week from the end of dormancy until bud break and every two days from bud break to anthesis. Assessments were made using a previously adjusted scale (Austin et al. 1998), in which flower bud stage values are linearly related to apricot flower bud development: 1.6 separation of scales (bud break); 3: protrusion of sepals; 4.2: broadening of exposed sepals; 4.9: expansion and rounding of sepals; 5.5: initial profusion of petals; 5.9: expansion and rounding of petals; 6.1: the flower is fully open and functional (anthesis). Linear regressions were performed in both types of shoots to fit functions of chronological time through adjusted flower bud stage data. Slopes were compared and tested to determine if the rates of growth were significantly different (Rodrigo and Herrero 2002a). In order to characterize flower bud growth in relation to the stage of development, 15 flower buds at each phenological stage from both types of shoots were randomly sampled and individually weighed.

Pollination and fruit set

In order to characterize the size of the pistil at anthesis and to establish its influence on fruit set, between 90 and 250 flowers at balloon stage were randomly collected from each type of shoots. Pistils were individually weighed, and flowers were classified in three categories according to pistil size: well developed pistil with a swelled ovary, underdeveloped pistil and underswollen ovary with short style (Figure 1).

To follow pistil growth after anthesis, 270 flowers on short shoots and 120 flowers on long shoots were emasculated at balloon stage one day before anthesis and pollinated with the help of a brush the following day with compatible pollen of apricot ‘Canino’, since ‘Moniqui’ is self-incompatible (Rodrigo and Herrero 1996). Pollen was previously collected from flowers at balloon stage by removing

the anthers and placing them on paper at room temperature. Pollen was sieved 24-48 hours later with a 0.26 μm mesh and stored at 4 ºC until used. Flowers were randomly collected and weighed individually each three days from anthesis to 10 days afterwards. These experiments were performed in two consecutive years.

To characterize fruit set and flower drop and to determine the main drops of buds, flowers and developing fruits: between 1500 and 2000 flower buds were monitored for each type of shoot in two years. In the selected branches, weekly counts of all the flower buds were made from rest breaking, three weeks before anthesis, to harvest. In order to ensure the presence of compatible pollen in the stigma, a supplemental pollination was performed and these flowers were hand-pollinated at anthesis using a small brush with compatible pollen of apricot 'Canino' (Rodrigo and Herrero 1996). To determine the waves when drop was more pronounced, the relative fruit drop was assessed in each tree as the percentage of buds, flowers or developing fruits dropped each week in relation to the initial number of flower buds. Flower bud-, flower- and crop-density in both types of shoots were respectively assessed as the number of buds, flowers or fruits per square cm of basal branch section.

In vitro pollen germination

In order to determine pollen viability in each type of shoot, pollen from both populations of flowers was obtained following the same method described above. Pollen germination in vitro was carried out by scattering the pollen on a solidified germination medium consisting of 0.3 M sucrose, 1.6 mM boric acid and 0.6 mM calcium nitrate, and solidified with 0.8% (w/v) agar (Hormaza et al. 1996) in polystyrene Petri dishes (60 x 10 mm). Pollen was germinated for 24 h at 20 ºC and then frozen at - 18 ºC to arrest pollen germination. Preparations were defrozen during 24 h at 4 ºC and then observed under the microscope (Leitz Ortholux II,

Wetzlar, Germany). Pollen was considered as viable when the pollen tube was longer than the pollen grain diameter. For each treatment, viability was recorded in two Petri dishes by counting three fields per plate, each field containing between 100 and 200 pollen grains.

Controlled pollinations

Pollen tube growth in pistils from both types of shoots was monitored under the microscope. For this purpose, 10 flowers from each type of shoot and from each category of flowers (well developed pistil, underdeveloped pistil and underswollen ovary with short style, Figure 1) were randomly collected at balloon stage, emasculated, placed on water soaked florist foam at room temperature and hand-pollinated 24 h later with compatible pollen of apricot 'Canino'. After three days at room temperature, pistils were fixed in FAA [70% ethanol: glacial acetic acid: formaldehyde (18:1:1, v/v/v)]. Microscopic observations were made on squashed pistils previously washed in water three times, 1 h per wash, autoclaved for 10 minutes at 1 kg/cm^2 in 5% Na_2SO_3, and stained with 0.1% aniline blue in 0.1 N K_3PO_4 (Rodrigo and Herrero 2002b and references therein). Preparations were examined under an Olympus BH2 microscope (Olympus Optical Co, LTD, Japan) with ultraviolet epifluorescence, using a BP-405 exciter filter and a Y-455 barrier filter. The number of pollen grains onto the stigma and the presence of pollen tubes arriving at the base of the style were evaluated in individual pistils from each category of flowers and from each type of shoot.

Statistical analyses

Statistical analyses were performed with SPSS 12.0 statistical software (SPSS Inc., Chicago, USA). Repeated measures ANOVA was used to analyze differences in bud weight in each stage of flower bud development from bud

break to anthesis among both types of shoots. Two-way repeated measures ANOVAs were also used to analyze flower bud growth and pistil growth over time in both types of shoots. Linear regressions of flower bud growth at seven phenological stages of development from bud break to anthesis were compared among shoots. Chi-square (χ^2) test for categorical variables was performed to analyze the percentage of flowers at anthesis with different pistil morphology. One-way ANOVAs were also performed to analyze pollen germination, in which germination percentage data were subjected to arcsine root square transformation, and number of pollen grains on the stigma. Multivariate analysis of variances (MANOVA) was used to ascertain possible differences in fruit set, bud drop, flower bud-, flower- and crop-density, and fruit weight among years and types of shoots. Finally, another MANOVA was performed to analyze the effect of type of shoot on the same variables, followed by one-way analyses of variances (ANOVAs) to ascertain the effect of type of shoot on each variable separately.

Results

Flower bud differentiation and development

Flower bud differentiation initiated in middle August and lasted between six and seven months until anthesis. The two-way repeated measure ANOVA (type of shoot-time) detected significant differences in bud growth between both types of shoots in this period. Thus, bud growth showed an increasing trend in both types of shoots from early differentiation until end of dormancy (Figure 2, $F_{(18,3438)} = 154.7$, $P < 0.001$). Flower buds from short shoots (Figure 2A) were also significantly heavier than buds on long shoots ($F_{(1,191)} = 534.2$, $P < 0.001$). In these long shoots, a population of flower buds remained small along the time (Figure 2B), with a significant interaction between time and type of shoot ($F_{(18,3438)} = 25.1$, $P < 0.001$). Differences among buds were also observed on their external appearance, since buds were lanceolate in short shoots and rounded in long shoots.

Bud development was also analyzed in both populations of buds from rest breaking to anthesis. Bud growth showed an increasing trend from bud break up to anthesis (Figure 3, repeated measures ANOVA, $F_{(7,105)} = 178.8$, $P < 0.001$). Flower buds from short shoots were also significantly heavier during bud break and the following stages of development before anthesis ($F_{(1,15)} = 7.9$, $P = 0.013$) with a significant interaction between type of shoot and time ($F_{(7,105)} = 2.3$, $P = 0.034$). Flowers from short shoots opened between two and three days earlier than flowers from long shoots. Thus, regression slopes, and therefore rates of growth, did not differ significantly between both types of shoots ($P > 0.01$, Figure 4). A delay in the development of buds from long shoots between two and five days was

also observed in all the previous stages of bud development from bud break (Figure 4).

Flowering and pollination

While in the short shoots most of the flowers at anthesis (73 %) had a morphologically well developed pistil, in the long shoots more than 90 % of the flowers at anthesis presented pistils not completely developed, with an underdeveloped pistil or an underswollen ovary (Figure 1). Thus, the percentage of flowers with underdeveloped pistils was significantly higher in long- than in short-shoots ($\chi^2 = 56.9$, d.f. = 2, $P < 0.001$, Figure 5).

Pollen viability evaluated through in vitro pollen germination did not differ significantly among both types of shoots (Table 1). However, in the flowers with underdeveloped pistils from both types of shoots, pollen grains did not germinated onto the stigma and therefore no pollen tubes were observed along the style. In the flowers with a well developed pistil, pollen tube performance was significantly different in each type of shoot. Thus, flowers from short shoots showed a higher number of pollen grains in the stigma (Figure 6A) than flowers from long shoots. While pollen tubes growing along the style (Figure 6B) were observed in both types of flowers, differences were detected in the number of pollen tubes reaching the base of the style (Figure 6C). While in all the flowers from long shoots pollen tube growth was arrested along the style, at least one pollen tube reached the base of the style in 59 % of the flowers from short shoots (Table 1).

Pistil growth showed an increasing trend in the 10 days following anthesis in both types of shoots (Figure 7, repeated measures ANOVA, $F_{(3,69)} = 18.5$, $P < 0.001$). The differences in pistil weight between both types of shoots observed at anthesis were maintained along this period, in which pistils from short shoots experimented a significantly larger growth than long shoot pistils ($F_{(1,23)} = 57.2$, P

< 0.001) with a significant interaction between type of shoot and time ($F_{(3,69)}$ = 7.9, $P <$ 0.001).

Fruit set and fruit drop

Fruit drop followed the same pattern in both fruiting and non-fruiting shoots (Figure 8A), with three main waves from dormancy to harvesting (Figure 8B). The first drop took place before anthesis, the second in the two weeks after anthesis and the last drop between the fourth and fifth week after anthesis. However, the percentage of flower drop in each wave was different in each type of shoot. While in long shoots the main wave of drop took place during flower bud development before anthesis, resulting in the drop of most of the buds, in short shoots most flowers dropped after flowering (Figure 8B). Although bud-, flower- and fruit-drop showed a similar pattern in both types of shoots over the two years (Figure 8A), fruit set in relation both to the initial number of flowers and to the number of flower buds were higher in short shoots (22 and 18% in 2006 and 31 and 25% in 2007) than in long shoots (8 and 2% in 2006 and 11 and 2% in 2007). Flower bud-, flower- and crop-density, and fruit weight were also higher in short- than in long-shoots. To assess the significance of these differences among years and types of shoots, a MANOVA was performed. Both the independent variable year (Wilks value = 0.26, P = 0.34) and the interaction type of shoot-year (Wilks value = 0.22, P = 0.28) were non-significant, indicating that the behavior of each of the dependent variables tested was similar among the two years. Thus, data from both years were pooled in the subsequent analyses. However, significant differences depending on type of shoot were recorded on the same variables after MANOVA (Wilks value = 0.02, $P < 0.001$). The one-way ANOVAs performed to ascertain the effect of type of shoot on each variable showed significant differences between short- and long-shoots in fruit set, bud

drop, flower bud-, flower- and crop-density, but non-significant differences in fruit weight (Table 2).

Discussion

Flower buds in apricot grew and developed, from early differentiation up to dormancy, and resumed growth after dormancy following a pattern similar to other *Prunus* species (Luna et al. 1991). While in short shoots most buds followed a continuous development, in long shoots bud development was altered since more than half of buds did not further develop and subsequently dropped. Flower bud drop has been repeatedly reported in apricot (Legave et al. 1982; Alburquerque et al. 2003), and it has been considered of a physiological rather than an accidental nature (Legave et al. 1982), although in some situations external factors as frosts prior to bud break can cause drop of a high proportion of buds (Julian et al. 2007). The differences in flower bud drop reported herein among branches of the same tree clearly pointed to internal factors affecting bud development.

Differences in bud size among shoots were encompassed with differences in the timing of development. Thus, buds from short shoots were at an advanced external phenological stage resulting in an early flowering respect to those buds from long shoots. Differences in the phenology of short- and long-shoots have been reported in different tree species (Eysteinsson and Greenwood 1995, Miyazawa and Kikuzawa 2004). Likewise, variations in the timing of flowering (Stephenson 1981; Rodrigo and Herrero 2002a) and flower differentiation (Chandler and Tufts 1933; Brown and Abi-Fadel 1953; Diaz et al. 1981) have been also related to the subsequent ability of flowers to set fruits.

Variations in the size and timing of development of buds in both types of shoots reported herein resulted in differences in the pistil at anthesis. Long shoots showed a high number of flowers with underdeveloped pistils that had a reduced

capability to set fruit. Lack of fruit set related to abnormal flower buds have been reported in different apricot cultivars and seasons (Alburquerque et al. 2003; Ruiz and Egea 2008) and related to warm pre-blossom temperatures along bud break (Rodrigo and Herrero 2002a). While abnormally small pistils have been previously reported as a variable trait in different apricot cultivars (Rodrigo and Herrero 2002b) and have also been related to meteorological conditions (Rodrigo and Herrero 2002a), results herein showed that the presence of flowers with pistil alterations was also dependent of the type of wood in the same tree under the same meteorological conditions and therefore suggest that internal factors could influence these pistil abnormalities.

Differences in size among flowers with different capability to set fruit have been reported in apricot (Rodrigo and Herrero 2002a; Rodrigo et al. 2009) and other fruit tree species regardless of the different factors causing these differences, such as nitrogen summer applications (Williams 1965, Jordan et al. 2009), previous crop load (Buszard and Schwabe 1995), stresses during floral initiation (Lamp et al. 2001) or warm pre-blossom temperatures (Rodrigo and Herrero 2002a). Likewise, the age of the tree and wood (Robbie and Atkinson 1994) and the orientation of branches where flower buds are located (Robbie et al. 1993; Almeras et al. 2002) have been also related to a different reproductive success. Results herein showed that the buds and flowers from long shoots were more likely to have an underdeveloped pistil in which pollen grains did not germinate or pollen tubes did not reach the ovary and therefore subsequent fruit set was not produced.

The reasons behind this altered development have to be explored but, since long shoots carry on growing for a longer period of time than short shoots, competition for nutrients between buds and growing shoot could be behind these

alterations. This point needs experimental support and further work is needed to clarify the physiological mechanisms underlying the differences in bud drop and fruit set among buds of different type of shoots. Starch accumulated in the pistil plays a clear part in the support of pollen tube growth (Herrero and Dickinson, 1979), the onset of fruiting (Rodrigo et al. 2000; 2009) and ovule fate (Rodrigo and Herrero, 1998). An exam on the starch content of these underdeveloped pistils may throw light on the reasons behind a poor reproductive performance. While this point needs evaluation, the consistency of low fruit set related to small flowers, in different conditions, provides a good basis to explore this hypothesis. But what appears clear is that alterations along flower bud development reflected in clear differences in fruit set between both types of shoots. Fruit drop in both types of branches followed a pattern previously described in apricot (Rodrigo and Herrero 2002b; Rodrigo et al. 2009) and other *Prunus* species (Sedgley and Griffin 1989, Hedhly et al. 2007). However, most drops in short shoots occurred within the five weeks following anthesis, while the most important wave of drop in long shoots took place before anthesis. As a result of this high proportion of buds that prematurely drop, the percentage of fruit set in relation to the initial number of flower buds was considerably lower in long shoots. The fact that most of flower buds in these branches dropped before anthesis could explain previous reports in which not clear differences in fruit set were found between short- and long-shoots when the percentage of final fruit set was referred to the number of opened flowers instead to the number of buds (Alburquerque et al. 2003).

The use of empirical knowledge on the fruiting capacity of short- and long-shoots has proven to be a useful approach to understand how flower bud differentiation and bud development affect the subsequent fruit set. Alterations along flower bud development appeared to be behind a poor fruit set in long

shoots. Some of these buds fell close to flower opening and others gave rise to flowers with underdeveloped pistils that also failed to crop. It appears clear that flower bud development plays a clear part determining the success of that bud to become a flower and a fruit.

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Figure legends

Fig. 1 Flower buds at balloon stage (A, B, C) and flowers at anthesis (D, E, F) with different pistil morphology: Well developed pistil with a swelled ovary (A, D), underdeveloped pistil (B, E) and underswollen ovary with short style (C, F).

Fig. 2 Distribution of fresh weight of flower buds on short- (A) and long-shoots (B) in apricot 'Moniqui' from early differentiation to bud break.

Fig. 3 Fresh weight of flower buds on short- and long-shoots in apricot 'Moniqui' from bud break to anthesis. Flower bud stages according to Austin *et al.* (1998). Mean ± SE of the average values.

Fig. 4 Flower bud stages according to Austin *et al.* (1998) on short- and long-shoots in apricot 'Moniqui' from bud break to anthesis. Equations and determination coefficient (R^2) determined by linear regression.

Fig. 5 Percentage of flowers at anthesis with different pistil morphology in short- and long-shoots in apricot 'Moniqui'.

Fig. 6 Pollen performance in apricot 'Moniqui'. Germinated pollen grains in the stigma with pollen tubes growing through the style (A). Pollen tubes growing along the style (B). Pollen tubes reaching the base of the style (C). Bars = 30 µm.

Fig. 7 Fresh weight of pistil in flowers on short- and long-shoots in apricot 'Moniqui' from anthesis until 10 days after. Mean ± SE of the average values.

Fig. 8 Flower bud, fruit drop and fruit set, in relation to the initial number of flower buds on short- and long-shoots in apricot 'Moniqui' from rest break (3 weeks before anthesis) to harvest in 2006. Percentage of flower buds, flowers and developing fruits remaining in the tree (A). Percentage of buds, flowers and developing fruits dropped each week (B). Mean ± SE of the average values.

Table titles

Table 1 One-way analysis of variance (ANOVA) of percentage of in vitro pollen germination and amount of pollen grains on the stigma, and percentage of flowers with pollen tubes at the base of the style three days after pollination in flowers with well developed pistil on short- and long-shoots in apricot ‘Moniqui’.

Table 2 One-way analysis of variance (ANOVA) of percentage of fruit set, in relation both to the initial number of flowers and to the initial number of flower buds, percentage of bud drop, flower bud-, flower- and crop-density per square cm of basal branch section and fruit weight (g) on short- and long-shoots in apricot ‘Moniqui’.

Table 1 One-way analysis of variance (ANOVA) of percentage of in vitro pollen germination and amount of pollen grains on the stigma, and percentage of flowers with pollen tubes at the base of the style three days after pollination in flowers with well developed pistil on short- and long-shoots in apricot 'Moniqui'.

	Short shoots			Long shoots				
Trait	Mean	SE	df	Mean	SE	df	*F*	*P*
In vitro pollen germination (%)	25.6	2.4	5	20.8	2.6	5	3.85	0.067 ^{NS}
Number of pollen grains in the stigma	66.8	8.6	16	13.5	5.1	7	16.5	< 0.001***
Flowers with pollen tubes (%)	58.8			0				

SE: standard error, df: degree of freedom; *F*, F statistic.
***Significant at $P<0.001$; NS, not significant.

Table 2 One-way analysis of variance (ANOVA) of percentage of fruit set, in relation both to the initial number of flowers and to the initial number of flower buds, percentage of bud drop, flower bud-, flower- and crop-density per square cm of basal branch section and fruit weight (g) on short- and long-shoots in apricot 'Moniqui'.

Variable	Source	SS	df	MS	*F*	*P*
Fruit set (flowers)	Between Groups	1037	1	1037	18.5	< 0.001***
	Within Groups	674	12	56.2		
	Total	1711	13			
Fruit set (flower buds)	Between Groups	1570	1	1571	69.7	< 0.001***
	Within Groups	270	12	22.5		
	Total	1840	13			
Bud drop	Between Groups	13779	1	13779	220	< 0.001***
	Within Groups	748	12	62.4		
	Total	14527	13			
Flower bud density	Between Groups	2517	1	2517	15.3	0.002**
	Within Groups	1979	12	165		
	Total	4496	13			
Flower density	Between Groups	9391	1	9391	107	< 0.001***
	Within Groups	1049	12	87.4		
	Total	10440	13			
Crop density	Between Groups	837	1	837	165	< 0.001***
	Within Groups	61	12	5.06		
	Total	898	13			
Fruit Weight	Between Groups	145	1	145	3.09	0.104 ^{NS}
	Within Groups	563	12	46.9		
	Total	708	13			

SS, sum of squares; df, degrees of freedom; MS, mean of squares; *F*, F statistic.
**Significant at $P < 0.01$; ***Significant at $P<0.001$; NS, not significant.

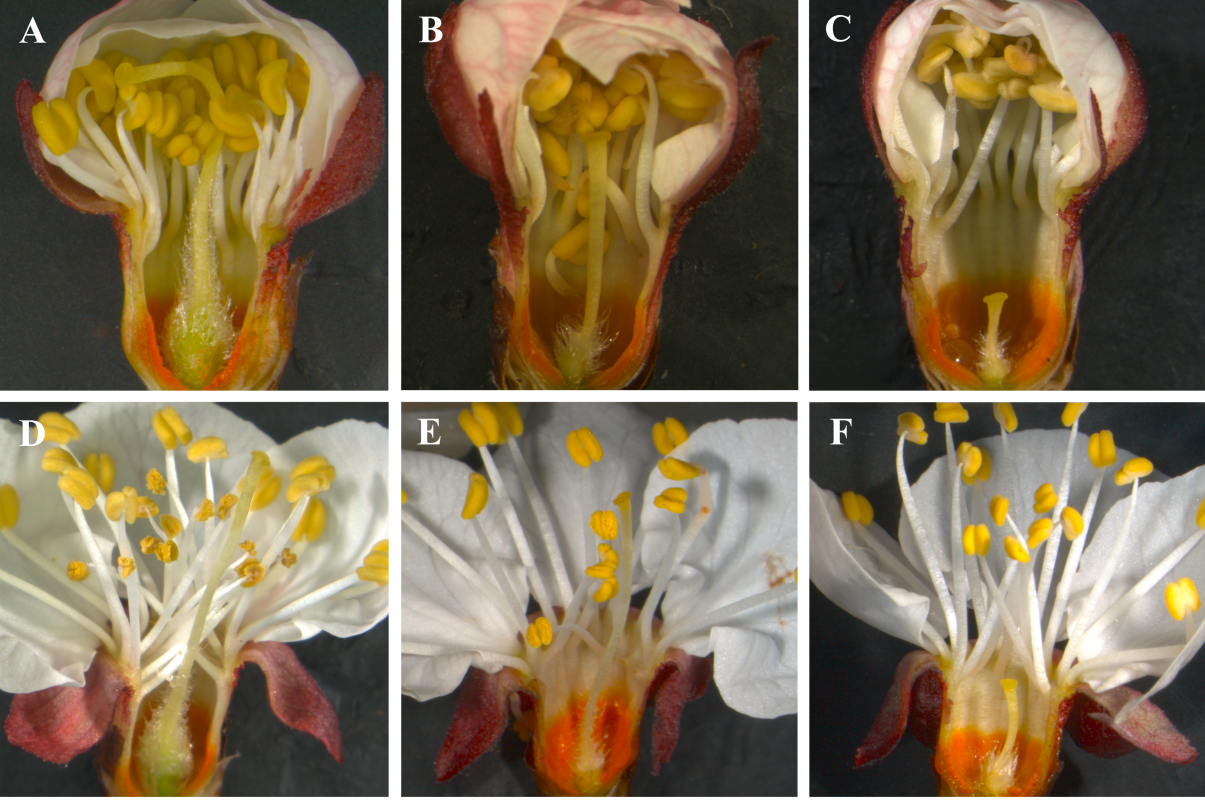

A
B
C
D
E
F

FIG. 2

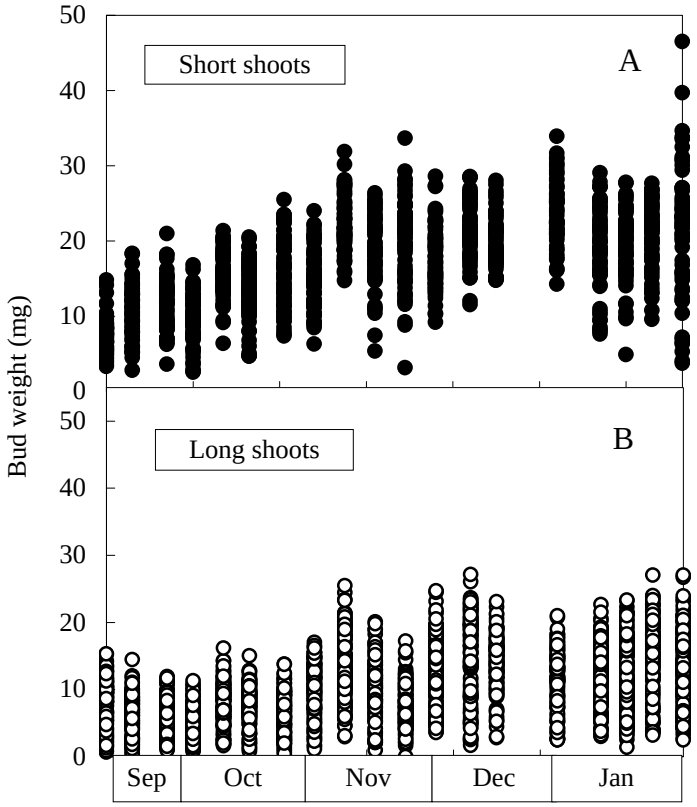

Short shoots
A
Long shoots
B
Bud weight (mg)
50
40
30
20
10
0
Sep
Oct
Nov
Dec
Jan

FIG. 3

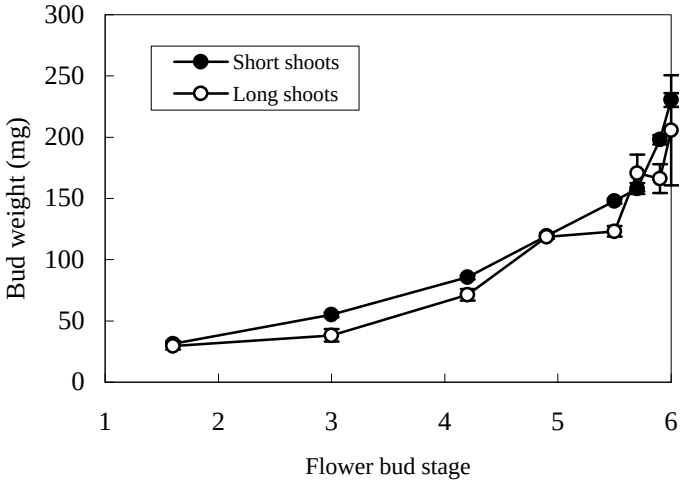

300
250
200
150
100
50
0
Bud weight (mg)
Short shoots
Long shoots
1
2
3
4
5
6
Flower bud stage

FIG. 4

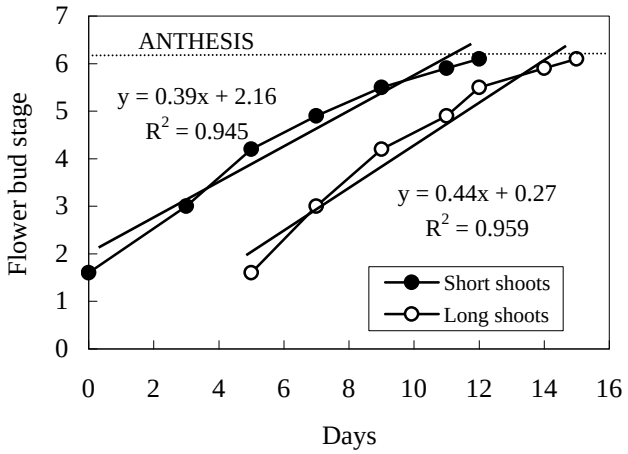
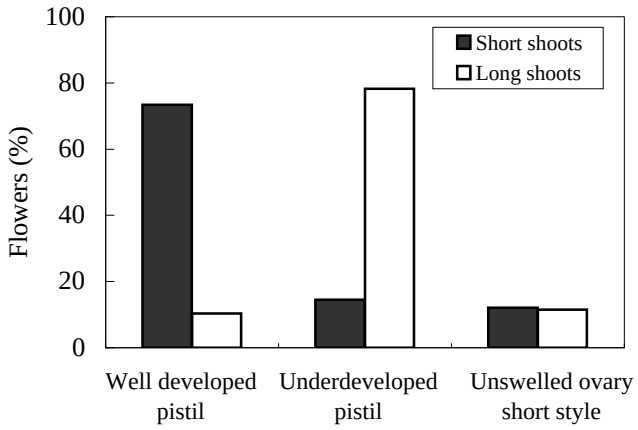

ANTHESIS
y = 0.39x + 2.16
R2 = 0.945
y = 0.44x + 0.27
R2 = 0.959
Short shoots
Long shoots
Flower bud stage
Days
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16

FIG. 5


100
80
60
40
20
0
Flowers (%)
Short shoots
Long shoots
Well developed pistil
Underdeveloped pistil
Unswelled ovary short style

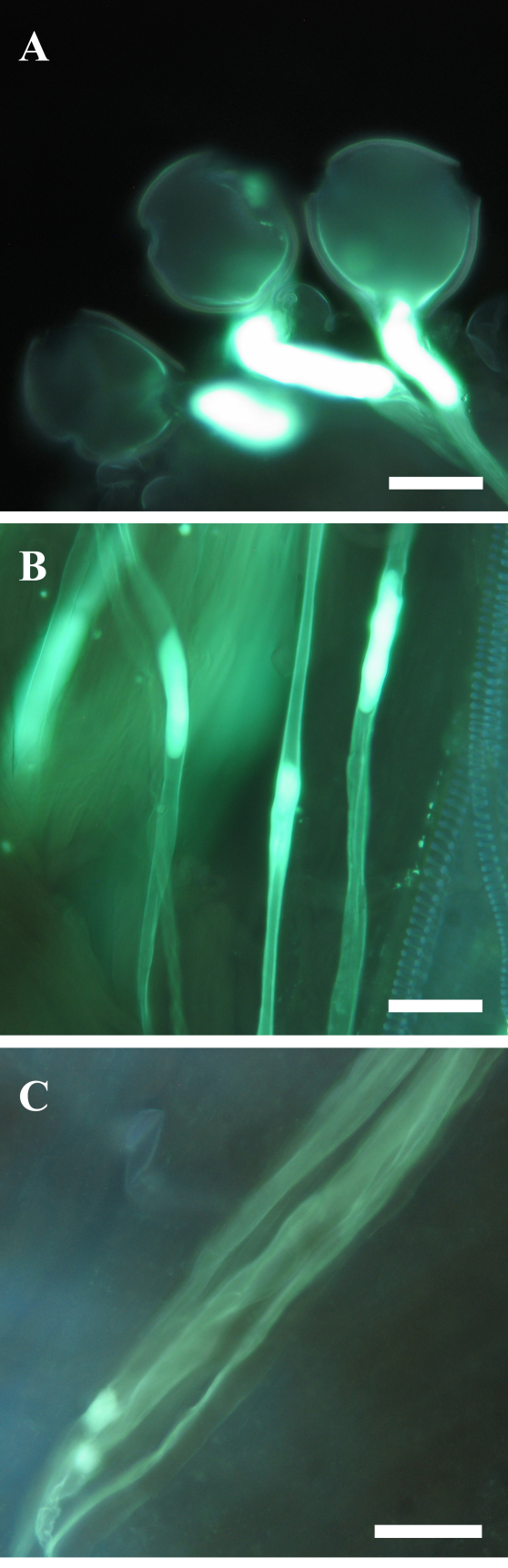

A
B
C

FIG. 7

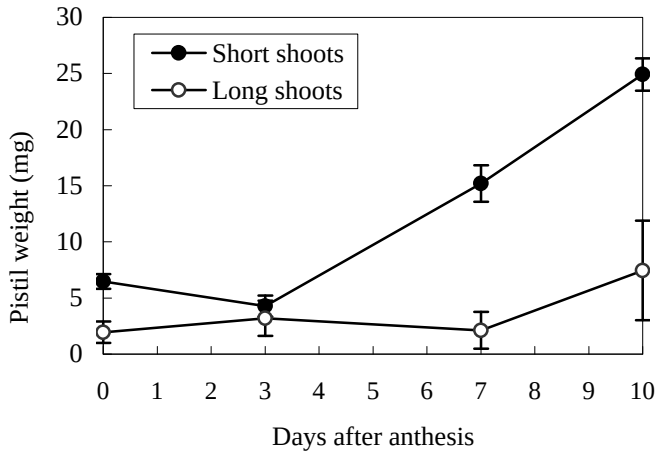
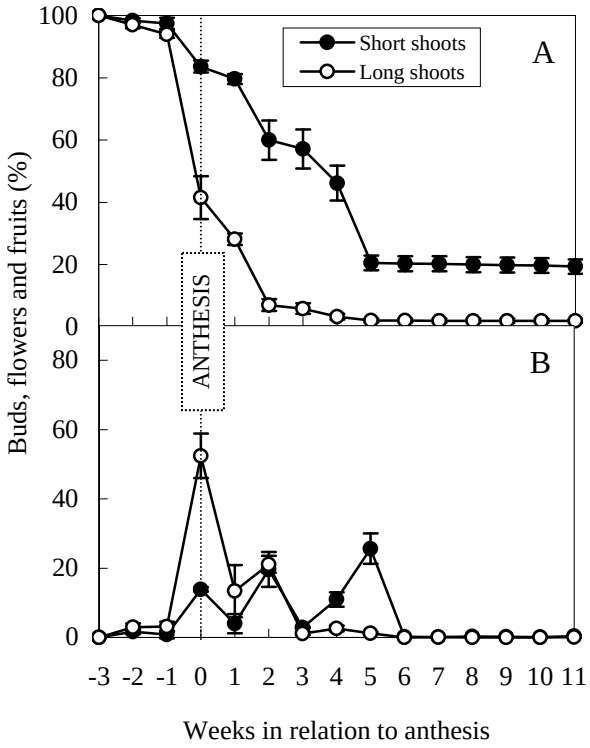

Short shoots
Long shoots
Pistil weight (mg)
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10
Days after anthesis

FIG. 8


Short shoots
Long shoots
A
B
ANTHESIS
Buds, flowers and fruits (%)
100
80
60
40
20
0
-3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11
Weeks in relation to anthesis