Scientia Horticulturae 150 (2013) 283–289

Contents lists available at SciVerse ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Flower strategy and stigma performance in the apple inflorescence

Juan M. Losada [1], María Herrero *

Pomology Department, Aula Dei Experimental Station – CSIC, Spain

ARTICLE INFO

Article history:
Received 2 August 2012
Accepted 26 November 2012

Keywords:
Apple
Malus
Inflorescence
Stigma receptivity
Pollen germination

ABSTRACT

Flower gathering in inflorescences promote pollinator activity and assures seed and fruit set within the inflorescence. However, in this flower social behaviour, the possible contribution of each single flower gets diluted and has been overlooked. In this work we explore stigma receptivity in the different flower types of the apple corymb, an inflorescence with clear flower positions a central or king flower and four lateral flowers, where subsequent fruit set can be followed by the position along the flower axis. Flowers were receptive in turns, first in the king flower and thereafter in lateral flowers, prolonging in this way the whole inflorescence receptivity. But a closer look at pollen performance showed that king flowers had an intense but short stigmatic receptivity, whereas lateral flowers had a more discrete but much longer stigmatic receptivity. These divergences contribute to different strategies within a single inflorescence with different advantages under different scenarios. The king flower will have an advantage under good pollination conditions, whereas lateral flowers will have a better chance under poor pollination conditions. But in any circumstance these two stigma performances provide a strategy to deal with environmental uncertainty, ensuring a minimum of fruit production per inflorescence.

1. Introduction

Grouping flowers in inflorescences enhances evolutionary angiosperm fitness, favouring a higher floral display for pollinator attraction (Jordan and Otto, 2012), and the evolution of inflorescence architectures (Prusinkiewicz et al., 2007; Prenner et al., 2009) may have played a clear part as modifier of pollinator behaviour and hence pollen movement among flowers. In natural conditions, pollen limitation has been shown to regulate seed and fruit set (Ashman et al., 2004), and encourages female success of individual flowers within inflorescences (Zhang et al., 2012).

But all flowers of the inflorescence do not set a fruit, and some flowers have more reproductive success than others (Wyatt, 1982; Webberling, 1992). Indeed some flowers just behave as males (Diggle, 1995; Torices and Méndez, 2011) and the contribution of each flower inside the cluster to either male or female function depends on internal factors as architectural constrains and resources allocation between flowers (Diggle, 1995, 1997; Torices and Méndez, 2010; Cao et al., 2011; Zeng et al., 2009). All this converts in a flower social behaviour within the inflorescence, where each flower contributes to the whole inflorescence success. But the individual contribution of each flower has been overlooked. In this context differences in receptivity between flowers may play an important part.

While no much attention has been focused on the influence of flower longevity, it could be an important drive in mating system evolution (Weber and Goodwillie, 2012). Short receptive periods have been suggested under selection as a way to improve male genotype success (Castro et al., 2008). But also a delay in stigma receptivity will provide opportunities for gathering pollen landing and thus favouring pollen competition (Hormaza and Herrero, 1992, 1994; Herrero and Hormaza, 1996). This has been related to the female control of pollination (Lankinen and Kiboi, 2007; Lankinen and Madjidian, 2011), suggesting that stigma longevity ultimately determines pollination opportunities, and consequently the possibility of fertilisation.

Stigmatic receptivity duration varies from few hours to days, depending on the species (Heslop-Harrison, 2000), and has a crucial relevance in economical important crops such as fruit trees (Sanzol and Herrero, 2001) because it conditions the effective pollination period (Williams, 1966). Due to the implications on the subsequent fruit set, the duration of stigmatic receptivity has been evaluated in several fruit tree species such as kiwifruit (González et al., 1995a,b), apricot (Egea and Burgos, 1992), pear (Sanzol et al., 2003), or almond (Yi et al., 2006), showing big fluctuation in this trait. In fact the duration of stigma receptivity may vary from year to year, between cultivars of the same species (Ortega et al., 2004), or even within a same genotype (Sanzol et al., 2003; Castro et al., 2008). Indeed, variability exists between flowers of

* Corresponding author. Tel.: +34 976716125; fax: +34 976716145.
E-mail addresses: juanlosada@fas.harvard.edu (J.M. Losada), mherrero@eead.csic.es (M. Herrero).
[1] Present address: Arnold Arboretum of Harvard University, 1300 Centre Street, Boston, MA 02130, USA. Fax: +1 617 384 6531.

0304-4238/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scienta.2012.11.031

the same cultivar at constant temperatures in peach (Hedhly et al., 2005), suggesting that some flowers are more receptive than others. This variability also occurs between the different pistils of a flower in pear trees, and the stigmas become receptive and loose receptivity sequentially, extending the receptive period in a single flower (Sanzol et al., 2003). It has further been reported that environmental factors, as temperature and humidity (Hedhly et al., 2003, 2005, 2009; Lora et al., 2011), also affect the duration of stigmatic receptivity. In sum, the chance for receptivity appears to vary within flowers of a plant and is also modified by the environment.

The relevance of stigmatic receptivity on the subsequent fruit set sometimes is not easy to follow since evaluation of stigmatic receptivity implies a destructive method. Still the apple corymb is an excellent model system to evaluate this performance, since it has just five flowers – a number that can be easily followed – and the position of the flower and the subsequent fruit in the short inflorescence axis can be tracked. The apical flower – king flower – opens first, while lateral flowers open almost synchronically one to three days after the king flower (Pratt, 1988; Hancock et al., 2008). However, only a small proportion of flowers within the corymb set a fruit (Williams, 1966), suggesting distinct individual contributions of flowers during the reproductive phase in this species. With this perspective, studies on apple fruit abscission elucidated an apical dominance controlled by hormones (Dal Cin et al., 2005, 2009) as well as the genetic control of abscission (Bottom et al., 2010). But, before fruit set, the reproductive implication of the different flowers in the corymb to the reproductive outcome has been overlooked.

The aim of this work is to evaluate stigma performance in both king and lateral flowers within the apple corymb, and the subsequent implications in fruit set, to elucidate the possible contribution of each kind of flower to the general inflorescence strategy.

2. Materials and methods

2.1. Plant material

Apple trees (*Malus* × *domestica*, Borkh) cv Golden Delcious Spur were grown in an orchard located in the Aragón region on the North-East of Spain. The compatible cv Royal Gala was used as the pollen source. Before flower opening, at advanced balloon stage, 42 king and 42 lateral flowers were depetaled and emasculated leaving a 5 mm length pedicel. The flowers were placed in humid florist foam at room temperature of about 20 °C, and left for 24 h.

In the field, fifty king and fifty lateral flowers were selected at balloon stage to observe their development. Each day, five king and five lateral flowers were weighed for six days after anthesis. Field photographs were taken with an Olympus μ760 camera.

2.2. Pollination procedures

Since the cv Golden Delicious is self incompatible, pollen was obtained from flowers from the compatible cv Royal Gala. Flower buds were picked at balloon stage, just prior to flower opening. The anthers were removed and left on paper at room temperature of 22 °C for 24–48 h until dehisced. Then pollen was sieved using a 0.26 μm diameter mesh and conserved at −20 °C until used.

Batches of six different Golden flowers – 30 stigmas – were hand pollinated with a paint brush each day. One day after pollination, each batch of pistils was fixed in FAA – formalin: acetic acid: 70% ethanol – (1:1:18) (Johansen, 1940) for at least 24 h, and then transferred to 70% ethanol.

2.3. Microscopic preparations

Stigmatic receptivity was evaluated through the ability of pollen grains to adhere, and germinate on the stigma surface. With this aim, gynoecia were washed three times in distilled water, for one hour each time, and then they were left in 5% sodium sulphite overnight. The next day gynoecia were autoclaved for 10 min at 1 kg cm^{-2} in 5% sodium sulphite (Jefferies and Belcher, 1974), and finally individual styles were dissected and squashed onto glass slides with 0.1% aniline blue in 0.1 N K_3PO_4 (Currier, 1957; Linskens and Esser, 1957) to visualise callose and pollen tubes. Slides were observed under an epifluorescent LEICA DM2500 microscope with a filter 340/425 nm. Fluorescence photographs were taken with a CANON Power Shot S50 camera linked to the CANON-Remote Capture software.

Stigmatic area of 30 styles from each flower type at anthesis was measured with the Leica Application Suite software.

2.4. Fruit set measurements

To evaluate the final fruit set of king or lateral flowers in field conditions, 100 corymbs were selected after June drop in branches oriented to all directions, and then the position of the fruit in the corymb was recorded.

2.5. Statistical analysis

Statistical analyses were performed with the SPSS 17.0 software (SPSS Inc., Chicago, USA). General ability of stigmas to adhere and germinate pollen grains was assessed by comparison of mean percentages between flower types each day-after-pollination with one way ANOVA at a P value ≤ 0.05. Same proof was used to evaluate mean number of adhered and germinated pollen grains on stigmas among pollination days in each flower type, and seeking for differences between number of adhered/germinated pollen grains between flower types each pollination day. Finally, pollen germination percentage on both flower types in regard of day of pollination was evaluated by same ANOVA mean comparison test after a data transformation into the $(\arcsin\sqrt{\%\text{germination}})^{-1}$. When possible, significant independent groups were separated by Duncan multiple range test at the 95% confidence level.

Flower weights were correlated with pollination day with a T pair comparison proof, and thereafter, mean weights between flower types were compared by one way ANOVA each pollination day. Finally, ANOVA test served to compare fruit set percentages between fruit types at a P value ≤ 0.05.

3. Results

3.1. Stigmatic receptivity

Monitoring flower development in field conditions showed that king flowers lasted for four days, when petal wilting occurred concomitantly to stigma browning (Fig. 1). Lateral flowers had a slower developmental pace and lasted for five days. King flowers opened ahead of lateral flowers (Fig. 2A and B), but hand pollinating both kinds of flowers at anthesis, showed a surprising different pollen performance. Pollen grains abundantly germinated on stigmas of king flowers (Fig. 2C), contrasting to lower levels of pollen germination on stigmas of lateral flowers (Fig. 2D). However, when pollination was performed on flowers that had been opened for three days after anthesis, king flowers had a very poor pollen germination (Fig. 2E), while lateral flowers showed a high pollen germination (Fig. 2F).

Quantifying the proportion of flowers with at least one pollen grain adhered or germinated confirmed microscopy observations.