# Productive instability of sweet cherry orchards: microscopic methods for the study of its causes

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*Abstract*

**The cherry fruit yield in Chilean orchards is subject to variability year to year, which needs to be studied in order to stabilize and increase production and economic results. During flowering and fertilization, many flowers do not set, attributing generally climatic causes or floral overlap problems in auto-incompatible cultivars. The aim of this study was to assess microscopic methods to follow the flowering process and fruit set that allowed discriminating factors causing poor fertilization. For this, during the 2011 season in La Palma Experimental Station of Pontifical Catholic University of Valparaiso, we worked with 'Brooks' and 'Lapins'/MaxMa14 trees characterizing flower buds in 2-year-old branches, in different stages. Pollen was analyzed and monitored by fluorescence microscopy to the set process. In the dormant stage, pistils and stamens of both varieties were completely differentiated; the ovules were in early stages of differentiation, however, the pollen did not initiate this process yet. In both cultivars starch was not detected until starting green tip, where it was observed in anthers, style, ovules and ovaries, indicating that starch is detectable when the buds start scales opening and exposure of flower primordia. The pollen quality of all pollinator cultivars was good and for all observations it was possible to visualize its presence in the stigmatic surface. The migration of pollen tubes in the style and ovarian cavity, achieved a higher growth rate in 'Lapins', where it was possible to observe set from 72 h after the balloon stage, while in 'Brooks' it was scarce until 120 h.**

**Keywords:** *Prunus avium* L., flower buds, starch, pollen quality, pollination, pollen tube

## INTRODUCTION

Cherry orchards (*Prunus avium* L.) have variable production every year, which is due to abscission of flowers not pollinated, flowers that failed during fruit set and fruit not fully matured (Blanusa et al., 2005). These problems are often attributed to adverse climatic factors such as lack of winter chilling, spring frosts and rain during the ripening of fruit, and different flowering periods overlapping in the case of self-incompatible cultivars.

The plants of the genus *Prunus* require accumulated chilling during their endogenous latency to start flowering/budding. If not fulfilled, trees may experience problems such as starting the season with a late, uneven or weak flowering, (low floral quality). These effects have been observed in the Quillota valley, Valparaiso region, where the accumulation of winter chilling in recent years has caused problems in the releasing of dormancy (Table 1).

In order to increase and stabilize the production of this species, it was necessary to study the causes of the problem. Monitoring the process of floral differentiation until fruit set through microscopic methods in order to identify potential factors that explain instability of production was performed.

Table 1. Chilling accumulation and Ponderate Chilling Units (PCU) in the last 10 years in Quillota area, Valparaíso Región.

| Year | Chilling hours | PCU |
|---|---|---|
| 2002 | 645 | 727 |
| 2003 | 508 | 582 |
| 2004 | 438 | 768 |
| 2005 | 366 | 762 |
| 2006 | 285 | 607 |
| 2007 | 808 | 806 |
| 2008 | 726 | 923 |
| 2009 | 671 | 745 |
| 2010 | 888 | 871 |
| 2011 | 853 | 704 |
| 2012 | 717 | 712 |

**MATERIAL AND METHODS**

The study was carried out during the 2011 season at the Experimental Station La Palma, Pontificia Universidad Católica de Valparaíso (32°53'36"S; 71°12'09"W, 127 m), Quillota, Chile on 16 8-year-old cherry trees of 'Brooks' and 'Lapins' cultivars grafted on MAXMA 14 (*Prunus mahaleb* × *P. avium*). The trees have a Solaxe training system, with a plantation design of 5 m between rows and 2 m on the row, under a warm temperate climate with coastal influence, average temperature of 15°C and high relative humidity (Gastó et al., 1993).

Randomly in May 2011 two homogeneous branches (2 years old) per plant were marked, which were further divided into 3 sections (using for analysis only the middle third of these). To study the state of development of buds and the presence of starch in the different floral structures, buds were harvested from June to September (endodormancy and after anthesis, respectively), which were fixed in formalin solution, acetic acid and alcohol (FAA), later to be processed in the lab and get histological sections. Method PAS (periodic acid Schiff's reagent) described by Feder and O'Brien (1968) was used for starch determination. All samples were observed under a microscope Olympus CX31 model, allowing them to identify the states of differentiation of different floral structures (style, pistil, stigma, pollen grains and eggs) as the starch granules.

At anthesis flowers were collected at 59 of the BBCH state (Meier, 1997) scale to evaluate the quality of pollen over viability and germination tests. Viability analysis were performed according to the methods using triphenil tetrazolium chloride (TTC) and fluoresce indiacetato (FDA) described by Norton (1966) and Heslop-Harrison and Heslop-Harrison (1970), respectively. In the TTC method 40 mL of solution (2,3,5-TTC diluted to 1% in a solution of 5% sucrose) were added to the pollen grains and after 2 h incubation at 25°C were observed under the microscope; viable grains took a deep red color. For the FDA test, on a slide were placed 40 µL of FDA solution (2 mg FDA in 1 mL of acetone) and 40 µL of a solution of sucrose, to which were added the pollen grains. Samples were immediately observed in the microscope with WB filter where viable pollen presented a green color fluorescence.The germination of pollen grains was conducted according to Mortensen et al. (1964), which consisted of germinating pollen in a solution with 20% sucrose and boric acid (50 mg $L^{-1}$) for 2 h after incubation at 25°C to observe the extension of pollen tube.

Additionally flowers were labeled state BBCH 59, which were collected in steps up to 5 days after anthesis, to analyze the process of pollination and fertilization. The flowers were fixed in Carnoys solution then subjected to a process of softening and staining with blue aniline and viewed under fluorescence microscopy for the presence of pollen and pollen tubes in style and ovarian cavity (Sarker et al., 1993); besides observing the eggs viability.(Postweiler et al., 1985).

## RESULTS AND DISCUSSION

During endodormancy, pistils and stamens of both cultivars were completely differentiated (Figure 1), the eggs were starting their differentiation and pollen had not begun this process, indicating that the floral differentiation occurs gradually in the same bud. In the same stage no starch granules were detected in the flower structures, however, at the start of green tips was observed in anthers, styles, eggs and ovaries, which indicates that the starch is present in the buds during pre-anthesis (Figure 2).

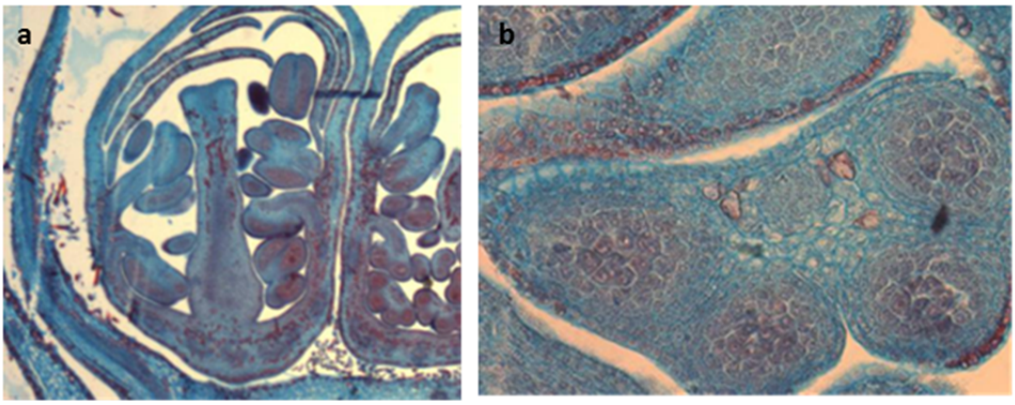


Figure 1. Differentiated pistil (a) and stamens (b) in 'Lapins' dormant.

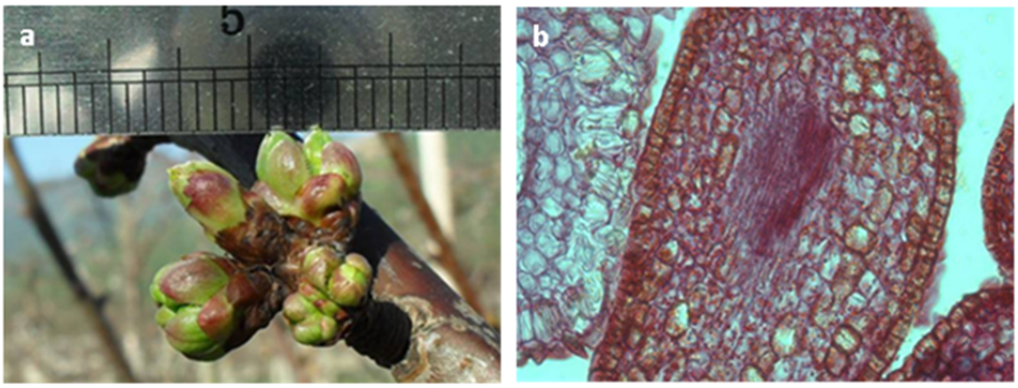


Figure 2. Reproductive buds harvested for starch analysis (a) and starch granules in anther filament detected in 'Brooks' (b).

At the pollen level, both varieties showed high viability and germination (Figure 3) and in all samples its presence was observed in the stigmatic surface (Figure 4), and was considered good pollen quality. However, in the case of 'Brooks' this was no guarantee of good pollination, because 'Lapins' did not behave as a good pollinator, because its flowers covered only part of flowering (Figure 5).

During pollination, pollen tube migration in the styles and the ovarian cavities (Figure 6a) achieved a higher growth rate in 'Lapins', where fertilization could be observed from 72 h after the state 59 scale BBCH while 'Brooks' was scarce until 120 h. This low rate of pollen tube growth could be one of the causes of low productivity of this cultivar, as the egg can degrade before the pollen tubes reaches the ovary (Cheung, 1996).

The aging of ovules can start immediately or a few days after anthesis (Postweiler et al., 1985; Cerović et al., 2000). In this study the ovules of both varieties began reducing their viability after 72 h (Figure 6b).

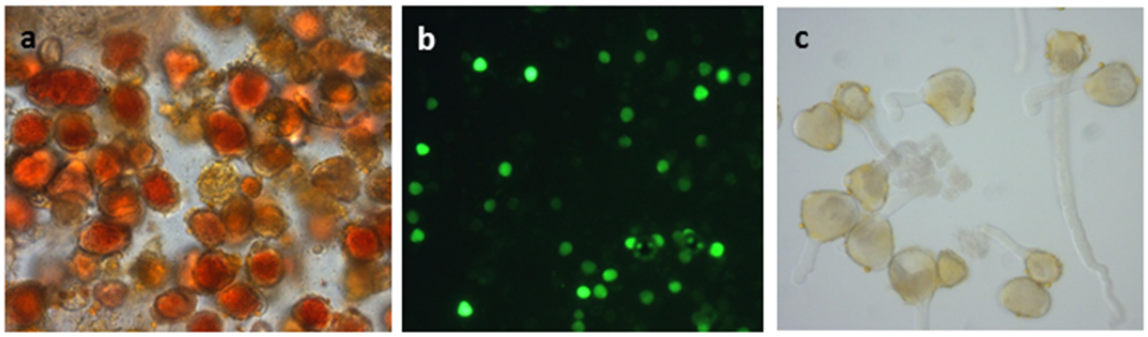


Figure 3. TTC viability, 40× (a) and FDA, 10× (b); and germination, 40× (c) of the pollen grains of 'Brooks'.

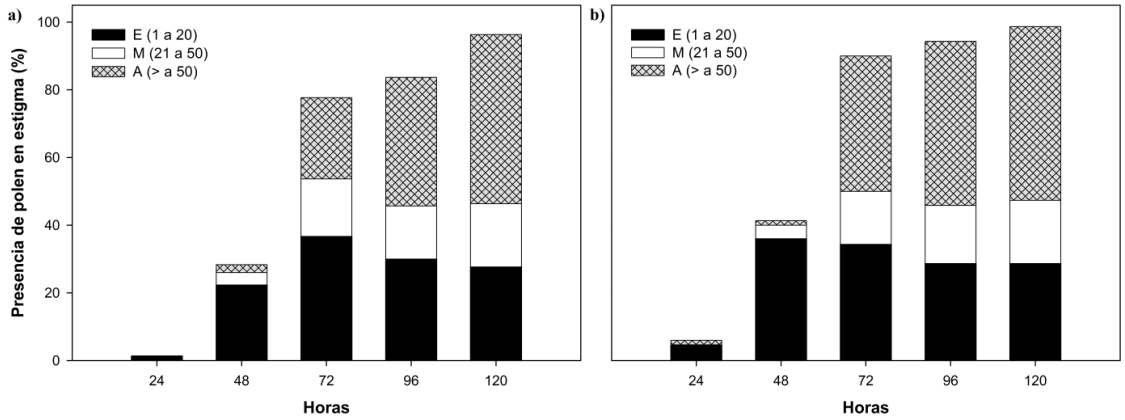


Figure 4. Presence of pollen on stigmatic surface of 'Brooks' (a) and 'Lapins' (b).

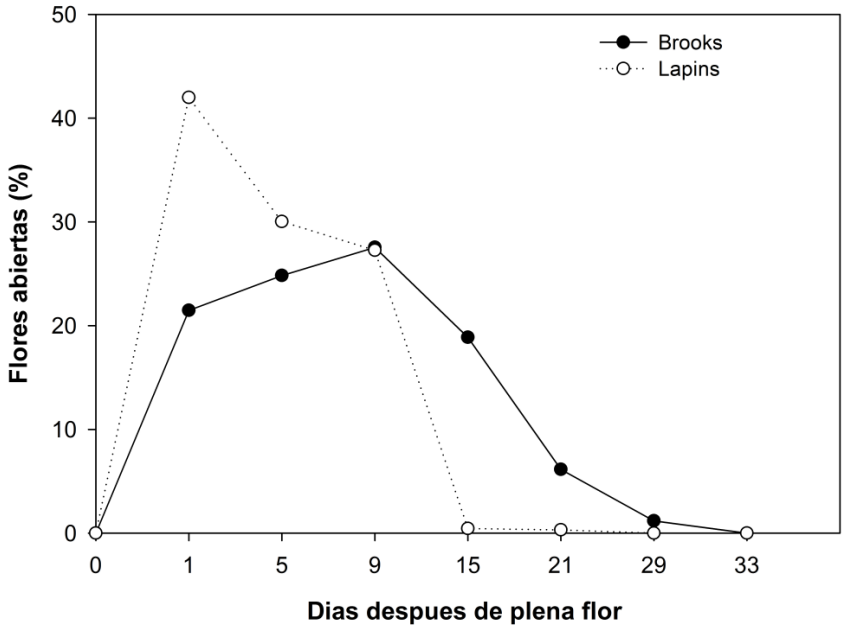


Figure 5. 'Lapins' and 'Brooks' blooming curves recorded during the 2011-12 season Experimental La Palma, Quillota, Chile Station.

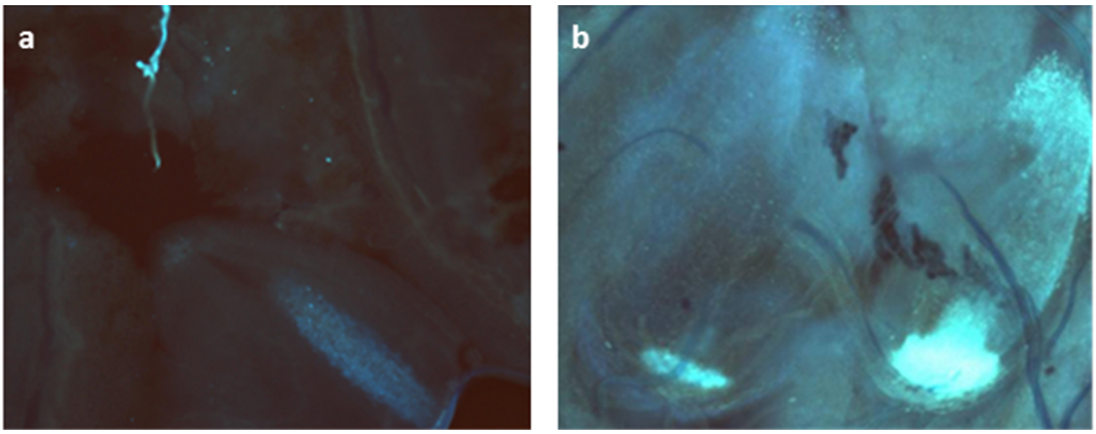


Figure 6. Pollen tube reaching ovarian cavity (a) and (b) ovules in degenerating process.

## CONCLUSIONS

The proposed methodology effectively allows establishment of factors influencing productivity. In 'Brooks' it was determined phenologically that: a) there was an early ending of flowering in the pollinator used; b) there is a slow movement toward pollen tubes in the ovarian cavity; c) an anticipated degeneration of the ovules, which started before the arrival of pollen tubes. However, pollen quality was not a limiting factor for productivity. On the other side, it was not possible establish causal factors in 'Lapins'.

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