

## Short communication. Influence of storage temperature on the viability of sweet cherry pollen

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### Abstract

The conditions to store sweet cherry pollen of six cultivars ('Brooks', 'Cristobalina', 'Marvin', 'New Star', 'Ruby' and 'Somerset') for a long period of time were studied. Pollen samples were stored at 4°C or -20°C and were taken at 7, 15, 30, 60, 90, 180, 270, 365 or 540 days of storage for all cultivars (with the exception of 'Somerset' at 30 days). 'Cristobalina' showed the highest pollen germination (over 60%). For the rest of cultivars, maximum pollen germination ranged from 36% to 44%. Pollen viability was completely lost for most cultivars after only 60 days of storage at 4°C. However, percentages of germinated pollen in all cultivars were not different from the control after one year of storage at -20°C, with the exception of 'New Star' and 'Marvin' that showed a reduction in pollen germination. Storage for periods longer than one year at -20°C produced a decrease in pollen viability with the exception of 'Cristobalina' and 'Somerset' pollen that maintained similar viability at all times. Results indicate that pollen viability could be maintained at reasonably high percentages after storage at -20°C during one year for all cultivars studied.

**Additional key words:** *in vitro* pollen germination, low temperature storage, *Prunus avium* L.

### Resumen

#### Nota corta. Influencia de la temperatura de conservación sobre la viabilidad del polen de cerezo

Se han estudiado las condiciones de conservación del polen de seis variedades de cerezo ('Brooks', 'Cristobalina', 'Marvin', 'New Star', 'Ruby' y 'Somerset') durante un largo período de almacenamiento. El polen se conservó a 4°C y -20°C y se tomaron muestras a los 7, 15, 30, 60, 90, 180, 270, 365 y 540 días de almacenamiento de todas las variedades, con la excepción de 'Somerset' a los 30 días. 'Cristobalina' mostró los porcentajes de germinación mayores (por encima de 60%). Los valores alcanzados por el resto de las variedades oscilaron entre el 36% y el 44%. La mayoría de las variedades perdieron completamente la viabilidad del polen tan solo a los 60 días de conservación a 4°C. Sin embargo, los porcentajes de germinación del polen conservado a -20°C no variaron después de un año, excepto en las variedades 'New Star' y 'Marvin', que mostraron una reducción en su germinación. La conservación del polen a -20°C durante un periodo superior al año disminuyó la viabilidad del polen, excepto en el caso de 'Cristobalina' y 'Somerset', donde la viabilidad fue similar durante todo el tiempo. De los resultados de este trabajo se puede concluir que la viabilidad del polen de cerezo se puede mantener en porcentajes razonablemente elevados durante un año a -20°C en todas las variedades estudiadas.

**Palabras clave adicionales:** conservación a bajas temperaturas, germinación del polen *in vitro*, *Prunus avium* L.

Sweet cherry (*Prunus avium* L.) fruit tree can be found in many different countries with a temperate climate, between 35° latitude North and 55° latitude South (Lichou *et al.*, 1990). Fruits are very appreciated for their flavour and beneficial characteristics for health (Serrano *et al.*, 2005).

Pollen viability may decrease quickly depending upon the storage conditions. Mayer *et al.* (1988) found that pollen viability decreased to half after 4 hours at 24°C or 1 hour at 27.7°C.

Controlled cross-pollinations require using selected pollen from elite cherry cultivars, since most of them are self-incompatible and bloom times often do not overlap between cultivars (from 3 to 4 weeks differences between late and early flowering). Due to these differences usually pollen is collected and dried before hand

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pollination. Also, exchange of pollen between breeders is a common practice that simplifies most quarantine requirements. Pollen needs adequate storage conditions to avoid losing viability. Viability of *Prunus* pollen, including old sweet cherry cultivars, after storage for long periods of time were carried out and published many years ago (Griggs *et al.*, 1953). However, cultivars studied here are relatively new selections of great interest obtained from different breeding programmes or local Spanish cultivars and, as far as we know, their pollen viability has not been tested before.

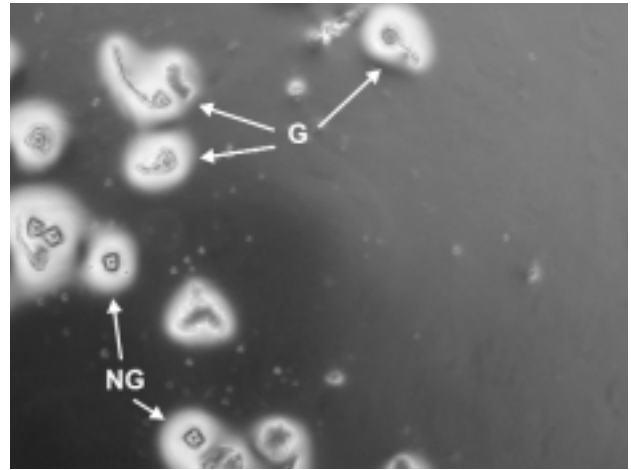
In the last years, many new cultivars have been released from different breeding programmes all over the world. Some of them are of low-chilling requirements which would be imperative in most Mediterranean areas. Many of them are being introduced and tested in different conditions, including Mediterranean climate. Here, the conditions to store sweet cherry pollen for a long period of time are studied. Such methodology is useful to efficiently plan hybridizations between cultivars flowering very separately in time.

Pollen of six sweet cherry cultivars, 'Brooks', 'Cristobalina', 'Marvin', 'New Star', 'Ruby' and 'Somerset' were used in this study. Flowers at E stage of Baggiolini (1952), showing the stamens, were collected from the field in Murcia and carried to the laboratory. Anthers were removed from flowers and immediately dehydrated in a chamber under controlled conditions (22°C and 20% RH) during 24–28 hours. After desiccation, 15 mg pollen samples were placed in 1.5 ml eppendorf tubes and stored at 4°C or –20°C.

Pollen samples were taken at 7, 15, 30, 60, 90, 180, 270, 365 or 540 days of storage for all cultivars (with the exception of 'Somerset' at 30 days). Pollen was dusted onto Petri dishes with 25 ml of a medium containing 15% sucrose and 1.2% bactoagar (Remy, 1953; Parfitt and Almehdi, 1984). Since the appropriate temperature for sweet cherry pollen germination was found between 22°C and 25°C (Bargioni, 1980), dishes were incubated for 20 hours at 23°C.

To evaluate pollen germination, an optical microscope with a 40x ocular was used and pollen grains were considered as germinated when the length of the pollen tube exceeded its diameter (Fig. 1).

For each treatment combination (pollen genotype, temperature and storage time), germination was recorded by counting ten different ocular fields with a similar number of pollen grains (35–50 each one), to avoid a possible effect of high pollen density on germination (Kwack, 1965; Giulivo and Ramina, 1974). Each count



**Figure 1.** Sweet cherry pollen grains germinated (G) showing the pollen tube and no germinated, without pollen tube (NG).

was considered as a replicate. Germination percentages were transformed by arcsine root square and ANOVA analysis was carried out. Means were compared with the first record after 7 days storage, as the control, using a Dunnett test. Statistical analyses were performed with SAS.

There were significant differences among pollen genotypes, temperatures, time of storage ( $P < 0.001$ ) and all possible interactions were also significant (Table 1). Therefore, the effect of storage time on pollen viability was analysed separately for each cultivar and storage temperature.

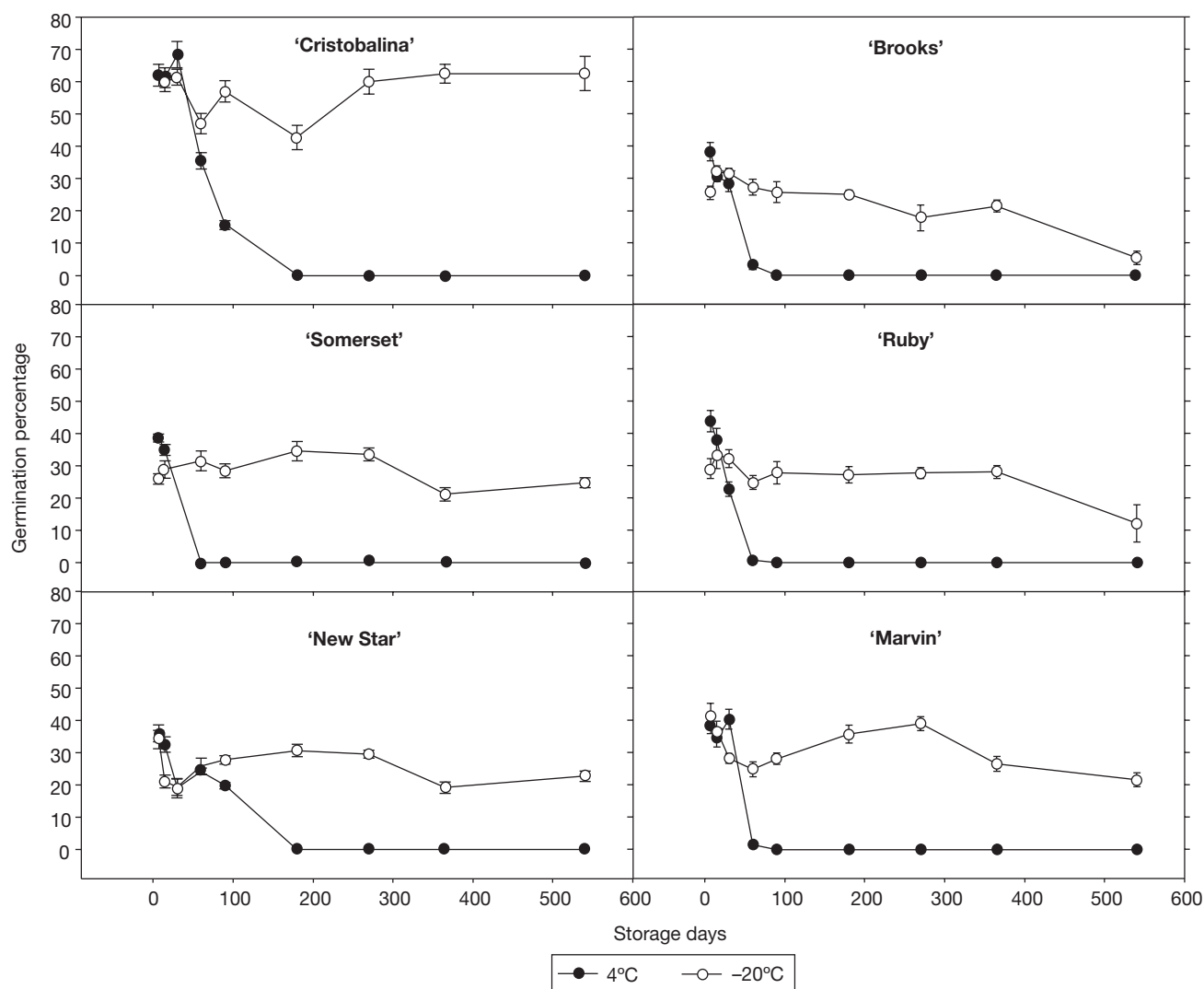
Pollen viability decreased after 15 or 30 days of storage at 4°C (Fig. 2). However, pollen remained viable in most cultivars up to one year of storage at –20°C.

When cultivars were studied separately, 'Cristobalina' showed the highest pollen germination (over 60%). For

**Table 1.** Analysis of variance using GLM procedure for pollen germination *in vitro* of six cherry genotypes

| Source                                       | d.f. | F value  | Pr > F   |
|--|------|----------|----------|
| Pollen genotype                              | 5    | 310.63   | < 0.0001 |
| Temperature                                  | 1    | 1,212.95 | < 0.0001 |
| Storage time                                 | 8    | 215.51   | < 0.0001 |
| Pollen genotype × temperature                | 5    | 23.24    | < 0.0001 |
| Pollen genotype × storage time               | 39   | 10.72    | < 0.0001 |
| Temperature × storage time                   | 8    | 152.98   | < 0.0001 |
| Pollen genotype × temperature × storage time | 36   | 9.22     | < 0.0001 |
| Error  | 927  |          |          |

d.f.: degrees of freedom.



**Figure 2.** *In vitro* germination of pollen grains of six sweet cherry genotypes after storage at 4°C or -20°C during different periods of time. Vertical bars represent the standard error and the absence of bars indicates that the standard error was zero.

the rest of cultivars, maximum pollen germination ranged from 35.98% to 43.76%. The relatively low germination percentages found with these cultivars contrast with those reported by Bargioni and Cossio (1980) who found germination values between 70% and 80% for the cherry cultivars. However, results from this work are in agreement with those found by Hedhly *et al.* (2005), who studied pollen germination of nine sweet cherry cultivars testing *in vitro* pollen performance under two temperatures regimes (15°C and 30°C). They found a highly significant effect of pollen genotype and temperature. Higher temperature reduced pollen germination, which maximum values were between approximately 40% in 'Talaguera Brillante' and 'Ambrunés' cultivars and 70% in 'Van' or 'Bing'

cultivars. Also differences in pollen performance have been found in different genotypes of sweet cherry by Hormaza and Herrero (1999) or in other *Prunus* species such as apricot (Egea *et al.*, 1992) and almond (Martínez-Gómez *et al.*, 2002).

In this study, for most cultivars pollen completely lost viability after only 60 days of storage at 4°C. Remarkably, 'Cristobalina' and 'New Star' maintained viable pollen in relatively high percentages up to one more month at this temperature.

Pollen viability in all cultivars was not different from the control after one year of storage at -20°C, with the exception of 'New Star' and 'Marvin' pollen which viability was significantly lower after 365 days from the first recorded germination percentage measured

after 7 days. However, results at 365 days were not significantly different from those obtained at some of the previous sampling days (i.e. 30, 60 or 90 days). Pollen from 'Marvin' and 'New Star' stored at  $-20^{\circ}\text{C}$  showed a lower viability after 30 days than after 180 and 270 days. A high density of pollen grains in the culture medium has been described to have a positive influence on pollen germination (Kwack, 1965; Giulivo and Ramina, 1974). Although care was taken to uniformly distribute pollen grains and to choose ocular field where a similar number of pollen grains was present, small differences could explain the unexpected results observed.

Fogle (1975) and Brown *et al.* (1996) indicated that freezing or freeze-drying of cherry pollen, in general, delays loss of viability when the pollen is stored for many years. In this study pollen germination remained similar in the case of 'Cristobalina' and 'Somerset' after storage for periods longer than one year at  $-20^{\circ}\text{C}$ . However, pollen germination was reduced in 'Brooks', 'Marvin', 'New Star' and 'Ruby' after 540 days of storage at  $-20^{\circ}\text{C}$ , which was approximately half of that recorded in the control. Griggs *et al.* (1953) found a slight decrease of germination percentages in pollen of 'Black Tartarian' and 'Napoleon' cultivars stored more than 400 days at  $-18^{\circ}\text{C}$ . All these results seem to indicate that pollen viability can be affected by long periods of storage at approximately  $-20^{\circ}\text{C}$ , being this effect genotype dependent.

Low temperature storage of pollen has been studied in some species. Martínez-Gómez *et al.* (2000) indicated that pollen of two almond cultivars was viable during 8 weeks when was stored at  $4^{\circ}\text{C}$ . This results were confirmed later with four different almond cultivars (Martínez-Gómez *et al.*, 2002) and also the authors found that storage conditions below  $0^{\circ}\text{C}$  ( $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$ ) did not affect pollen germination after one year. In a recent work, Lora *et al.* (2006) observed that germination of cherimoya pollen stored at sub-zero temperatures ( $-20$ ,  $-80$  and  $-196^{\circ}\text{C}$ ) was progressively reduced with conservation time at three temperatures studied, reaching a minimum after 90 days of storage. No differences in pollen germination among temperatures were observed for up to 30 days.

From results in this work it can be concluded that storage at  $-20^{\circ}\text{C}$  during one year does not affect pollen viability of the cultivars 'Cristobalina', 'Brooks', 'Somerset' and 'Ruby', whereas viability was still relatively high for the rest of cultivars.

Although there are some previous studies on the storage of pollen from cherry cultivars for short or long

periods of time at different temperatures, to our knowledge, cultivars studied here have been tested for the first time since they are relatively new selections from breeding programmes or self-compatible and early-ripening local Spanish cultivars. A procedure to appropriately conserve pollen, maintaining a good viability, may allow a better planning of controlled crosses and also provide a way of exchanging pollen between breeding programmes.

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