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EFFECT OF TEMPERATURE ON POLLEN TUBE KINETICS AND DYNAMICS IN SWEET CHERRY, *PRUNUS AVIUM* (ROSACEAE)[1]

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Prevailing ambient temperature during the reproductive phase is one of several important factors for seed and fruit set in different plant species, and its consequences on reproductive success may increase with global warming. The effect of temperature on pollen performance was evaluated in sweet cherry (*Prunus avium* L.), comparing as pollen donors two cultivars that differ in their adaptation to temperature. 'Sunburst' is a cultivar that originated in Canada with a pedigree of cultivars from Northern Europe, while 'Cristobalina' is a cultivar native to southeast Spain, adapted to warmer conditions. Temperature effects were tested either in controlled-temperature chambers or in the field in a plastic cage. In both genotypes, an increase in temperature reduced pollen germination, but accelerated pollen tube growth. However, a different genotypic response, which reflected the overall adaptation of the pollen donor, was obtained for pollen tube dynamics, expressed as the census of the microgametophyte population that successfully reached the base of the style. While both cultivars performed similarly at 20°C, the microgametophyte population was reduced at 30°C for Sunburst and at 10°C for Cristobalina. These results indicate a differential genotypic response to temperature during the reproductive phase, which could be important in terms of the time needed for a plant species to adapt to rapid temperature changes.

Key words: pollen tube dynamics; pollen tube kinetics; *Prunus avium*; Rosaceae; temperature stress.

Fertilization success in plants is the result of a sequence of processes that take place during the progamic phase. Environmental conditions affect steps such as pollen germination and pollen tube growth as well as development of the female structures (Stephenson et al., 1992; Delph et al., 1997). Among those environmental conditions, temperature is one of the most important factors that affect fruit and seed set. Temperature can affect different stages of the reproductive process such as stigmatic receptivity (Burgos et al., 1991; Egea et al., 1991; Cuevas et al., 1994; Kumar et al., 1995; Hedhly et al., 2003), ovule longevity (Thompson and Liu, 1973; Stösser and Anvari, 1982; Postweiler et al., 1985; Vasilakakis and Porlingis, 1985; Cerovic and Ruzic, 1992b; Burgos and Egea, 1993; Cerovic et al., 2000), or pollen germination and pollen tube growth (Lewis, 1942; Williams, 1970; Elgersma et al., 1989; Delph et al., 1997). Interest in the effect of temperature on reproductive processes is increasing because the global rise in temperature has affected plant populations by inducing a shift in several phenological traits such as flowering time (Parmesan and Yohe, 2003; Root et al., 2003). This is especially important in species such as sweet cherry that, in some regions such as the Mediterranean, are at the temperature limit of their cultivation potential. In fact, the results of simulations of global climate changes indicate that annual temperatures over Europe will increase at a rate between 0.1° and 0.4°C per decade and that the greatest increases are expected in southern and northeast Europe (IPCC, 2001).

Temperature has a clear effect on pollen tube kinetics, expressed as the time required for pollen germination and the rate of pollen tube growth. The results obtained on pollen germination vary among species and among cultivars of the same species but usually an optimum range of temperature parallels average temperatures at blooming time for pollen germination, as shown in several woody species such as avocado (Sedgley and Annells, 1981), almond and peach (Weinbaum et al., 1984), walnut (Luza et al., 1987), pistachio (Polito et al., 1988), apricot (Egea et al., 1992), pecan (Yates and Sparks, 1993), and mango (Sukhvibul et al., 2000). On the other hand, high temperatures accelerate pollen tube growth in herbaceous species such as *Oenothera* (Lewis, 1942), ryegrass (Elgersma et al., 1989), alfalfa (Katepa-Mupondwa et al., 1996), or groundnut (Kakani et al., 2002), as well as in woody species such as almond (Socías i Company et al., 1976), plum (Thompson and Liu, 1973; Jefferies et al., 1982; Keulemans and Van Laer, 1989), sour cherry (Cerovic and Ruzic, 1992a), apricot (Austin et al., 1998), apple (Petropoulou and Alston, 1998), pear (Lombard et al., 1972; Mellenthin et al., 1972; Vasilakakis and Porlingis, 1985), and *Betula* (Pasonen et al., 2000).

While temperature affects pollen tube kinetics, information on the effect of temperature on pollen tube dynamics, expressed as the census of the microgametophyte population that succeeded to reach the base of the style, is missing. A temperature effect on the male gametophyte population is plausible because genetic variability in pollen performance depending on temperature has been reported among species (Zamir et al., 1981; Weinbaum et al., 1984; Patterson et al., 1987; McKee and Richards, 1998) and among genotypes of the same species for pollen germination (Weinbaum et al., 1984; Polito et al., 1988; Loupassaki et al., 1997; Srinivasan et al., 1999; Lankinen, 2001) and for pollen tube growth in vivo (Gawel and Robacker, 1986; Srinivasan et al., 1999; Pasonen et al., 2000; Sukhvibul et al., 2000). While some species have a reduced microgametophyte/ovule ratio (Herrera, 2002), in others

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TABLE 1. Maximum, minimum and mean temperatures (°C) outside and inside the polyethylene cage during the 5 d following anthesis.

Temperature characteristic	Outside	Inside
Mean maximum temperature	20.4	24.6
Mean minimum temperature	7.2	7.8
Mean temperature	13.8	16.2

the ratio is higher and provides an opportunity for pollen competition and selection (Levin, 1990; Niesenbaum, 1994). Attrition, a reduction in the microgametophyte population along the style, occurs in a number of unrelated species (Herrero and Dickinson, 1980; Cruzan, 1989, 1990, 1993; Herrero, 1992; Hormaza and Herrero, 1996, 1999; Smith-Huerta, 1997; Wang and Cruzan, 1998). This reduction in the number of pollen tubes growing along the style reflects pollen competition and could provide an opportunity for gametophytic selection (Mulcahy, 1979; Hormaza and Herrero, 1994). However, little information is available on the effect of temperature and other stress factors on pollen tube dynamics. If such an effect exists, it could be a valuable indicator of selection pressure during the gametophytic phase. To explore the implications of the effect of temperature on the reproductive phase, pollen tube kinetics and dynamics have been studied in sweet cherry (*Prunus avium* L.), under controlled conditions in temperature chambers and in the field. This behavior has been compared on two pollen donors with different genetic backgrounds. One, Sunburst, is a cultivar that originated in Canada with a pedigree of cultivars from Northern Europe. The other, Cristobalina, is a cultivar native to southeast Spain, adapted to warmer conditions.

MATERIALS AND METHODS

Plant material and experimental conditions—This work was carried out on sweet cherry cultivars at the SIA-DGA experimental orchards at the Campus de Aula Dei in Zaragoza, Spain. Sweet cherry is a temperate deciduous fruit tree species predominately self-incompatible although some cultivated genotypes are self-compatible. Two donor genotypes, Cristobalina, and Sunburst, and one female recipient, 'Summit' were chosen. The two donor genotypes have different flowering times: Cristobalina flowers in early March, while Sunburst flowers in early April. This is related to the different chilling requirements needed to flower that are lower for Cristobalina than for Sunburst. Neither cultivar shares *S*-alleles with 'Summit', and consequently, both are fully compatible with the genotype used as female recipient (A. Wünsch and J. I. Hormaza, EELM-CSIC Málaga-Spain, unpublished data).

Two experiments were performed. The first experiment was carried out in controlled temperature chambers at 10°, 20°, and 30°C with cut flowers over florist's foam. These temperatures cover the range of day/night temperatures normally occurring during cherry bloom. The second experiment was performed in the field using trees either outside or inside a polyethylene cage. On the day of anthesis, one Summit tree was covered with 0.178 mm thick polyethylene film placed on a metallic frame structure, and the other was left uncovered. This system has shown to be a better method to increase the temperature of trees in the field than other methods used (Rodrigo and Herrero, 2001). Temperatures inside and outside the plastic cage were monitored every 5 min with a data logger (Testostor 175–3, Testo, Lenzkirch, Germany) during the period of sequential pollination and fixation. While mean minimum temperatures remained unaffected, mean maximum temperatures increased 4.2°C, resulting in an increase of 2.4°C in the average temperature (Table 1).

Pollination procedure—Pollen from the two donor genotypes was obtained from flowers collected 1 d before anthesis that under our conditions usually last for a few hours. Flowers of three individuals from each genotype were pooled and the anthers removed and dried on paper for 24–48 h at 20°C. Pollen was sieved through a 0.26-μm mesh to separate the pollen grains from anther debris and frozen at −20°C. The day prior to anthesis, the anthers and petals of the flowers of the recipient genotype were removed both in the tree and in the laboratory. In the laboratory, emasculated flowers were placed over soaked florist's foam at 20°C until the next day (anthesis) when they were pollinated with a brush and placed in controlled temperature chambers at 10°, 20°, and 30°C. The same procedure was followed in the field.

Fixation and microscopic observation—In all experiments, 10 flowers for each treatment were fixed daily in formalin : acetic acid : 70% ethanol (1 : 1 : 18 v/v; FAA; Johansen, 1940). Microscopic observations were made of squashed pistils washed 1 h in water three times, autoclaved for 10 min at 1 kg/cm^2 in 5% sodium sulfite (Jefferies and Belcher, 1974), and stained with 0.1% aniline blue in 0.1 N K_3PO_4 (Linskens and Esser, 1957). Preparations were examined under an Ortholux II light microscope (Leitz, Wetzlar, Germany) equipped with UV epifluorescence with a band pass 355–425 exciter filter and an LP 460 barrier filter.

Evaluation of pollen tube kinetics and dynamics—Pollen performance, expressed as pollen tube kinetics and dynamics, of the two donor genotypes was studied in controlled temperature chambers and in the field under the different temperature treatments. Pollen tube kinetics at the style level was evaluated during the 5 d after pollination as percentage of the style length traversed by the longest pollen tube (Lewis, 1942) and percentage of flowers with pollen tubes at their stylar base. Pollen tube dynamics was studied at the stigma by counting the number of adhered and of germinated pollen grains, then calculating the percentage germination. At the style, pollen tube dynamics was evaluated by counting the number of pollen tubes at the stylar base and expressed as the ''success ratio'' defined as the ratio of the number of pollen tubes reaching the stylar base to the number of germinated pollen grains. Statistical analyses were performed using SAS GLM v. 8 (SAS Institute, Cary, North Carolina, USA). Percentage data were arcsine transformed and then subjected to analysis of variance. Duncan's multiple range test (5%) for means separation (Duncan, 1955) was performed in cases of significant differences.

RESULTS

Pollen tube kinetics—Increasing temperatures in chambers accelerated pollen tube growth rate in the two crosses (Fig. 1a and b), reducing the length of time to reach the stylar base (Fig. 1c and d). This effect of temperature was highly significant ($P < 0.0001$) for days 1 and 2 after anthesis. During those two days, for the two donor genotypes, the increase of temperature from 10° to 20°C had a significant and more pronounced effect than the increase from 20° to 30°C. However, the two paternal genotypes behaved differently in the range 20°–30°C. While no significant differences were found at 20° and 30°C for Cristobalina pollen (Fig. 1b), resulting in a similar percentage of flowers with pollen tubes at the base of the styles (Fig. 1d), significant differences for Sunburst were found between 20° and 30°C (Fig. 1a); the percentage of flowers with pollen tubes at the base of the style increased (Fig. 1c).

In spite of the relatively small differences in temperature inside and outside the plastic cage (Table 1), the results in the field (Fig. 2) had the same pattern as those in controlled temperature chambers. For days 1 and 2 after anthesis, a slight increase in temperature significantly accelerated pollen tube growth rates (Fig. 2a and b) and shortened the time required to reach the base of the style (Fig. 2c and d).

Pollen tube dynamics—At the stigma level, germination percentage on the two consecutive days after pollination did

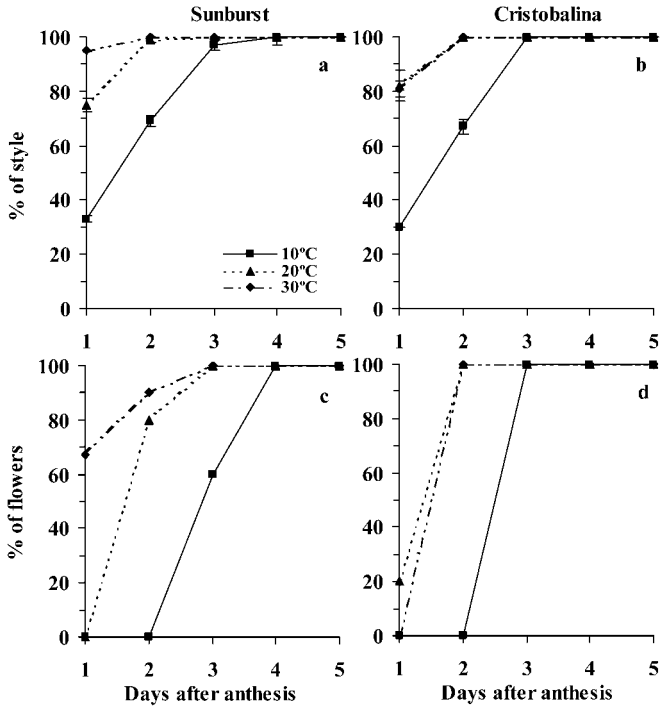

Fig. 1. Pollen tube kinetics in controlled temperature chambers at 10°, 20°, and 30°C expressed as the percentage of the style traveled by the longest pollen tube (a and b) and the percentage of flowers with pollen tubes in their stylar bases (c and d) for the two pollen donor genotypes Sunburst (a and c) and Cristobalina (b and d) (means ± 1 SE).

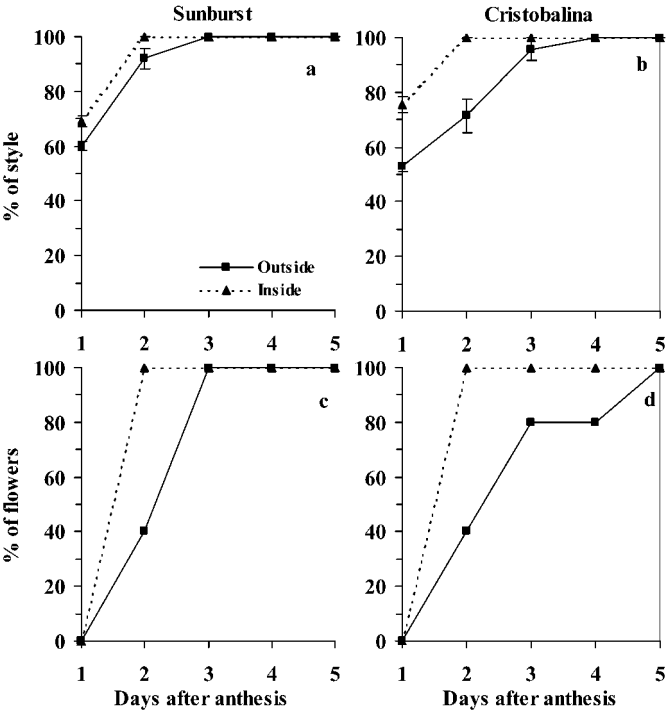

Fig. 2. Pollen tube kinetics in the field outside and inside the plastic cage expressed as the percentage of the style traveled by the longest pollen tube (a and b) and the percentage of flowers with pollen tubes at their stylar bases (c and d) for the two pollen donor genotypes Sunburst (a and c) and Cristobalina (b and d). (means ± 1 SE).

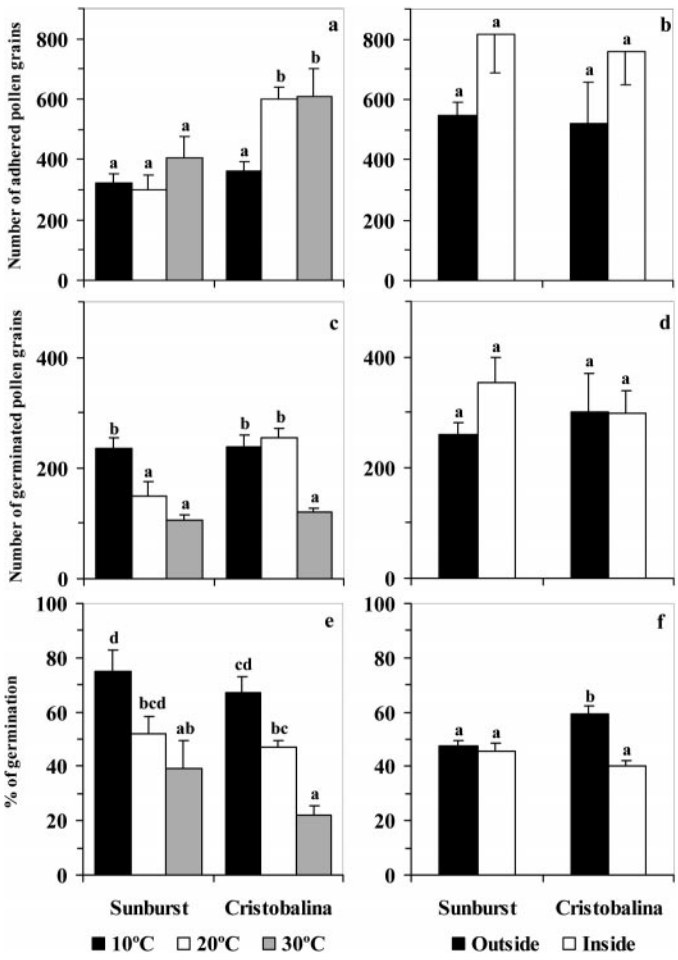

Fig. 3. Pollen performance at the stigma. Pollen grain adhesion and germination for the two pollen donor genotypes Sunburst and Cristobalina expressed as the number of adhered pollen grains (a and b), the number of germinated pollen grains (c and d), and the percentage of germination (e and f) in controlled temperature chamber at 10°, 20°, and 30°C (a, c, and e) and in the field outside and inside the plastic cage (b, d, and f) (means ± 1 SE).

not differ; thus, results are presented for only 1 day after pollination. The number of adhered pollen grains tended to increase with temperature in both conditions (Fig. 3a and b), but this effect was significant only for Cristobalina in controlled temperature chambers. In spite of increased adhesion, the number of germinated pollen grains decreased with increasing temperature (Fig. 3c and d). But, this reduction was significant only in temperature chambers for Sunburst between 10° and 20°C and for Cristobalina between 20° and 30°C. The percentage of germination was significantly lower at 30°C for both cultivars (Fig. 3e). In the field, with mean temperatures of 13.8° and 16.2°C, significant differences were recorded only for Cristobalina (Fig. 3f).

In the style, the number of pollen tubes gradually decreases. While high temperature accelerated pollen tube growth, the number of pollen tubes that reached the base of the style reached a maximum at a particular time and did not increase with time. No differences in success ratio [(number of pollen tubes at the base of the style/number of germinated pollen grains) × 1000] were noted between pollen donors at 20°C (Fig. 4a) or in the field (Fig. 4b). However, temperatures of 10° and 30°C had an effect that was genotype dependent. High

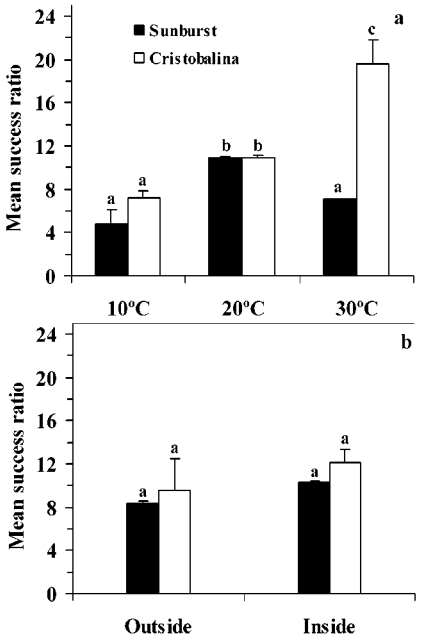

Fig. 4. Mean success ratio, expressed as (number of pollen tubes at stylar base/number of germinated pollen grains) × 1000, for the two pollen donor genotypes Sunburst and Cristobalina in controlled temperature chambers at 10°, 20°, and 30°C (a) and in the field outside and inside the plastic cage (b) (means ± 1 SE).

temperature (30°C), while reducing the number of pollen tubes at the base of the style in Sunburst, increased them in Cristobalina. Thus, an optimum success ratio was obtained for Sunburst at 20°C, while this optimum was 30°C for Cristobalina, which presented a success ratio of 7.2 at 10°C, but of 19.6 at 30°C.

DISCUSSION

Pollen tube kinetics, defined as the rate of pollen germination and pollen tube growth rate, and pollen tube dynamics, defined as the changes in the census of the microgametophyte population as they grow along the pistil, were affected by temperature in controlled temperature chambers with a range of 20°C and in the field with a slight temperature range (4.4°C for the maximum and 2.2°C for the average temperatures). Furthermore, the two genotypes tested differed in their response to temperature mainly at the style level.

Temperature effect—The optimum temperatures for pollen germination, pollen tube kinetics, and dynamics differ, suggesting an independent control. While warmer temperatures decreased the percentage of germination, they accelerated pollen tube growth rate in the style. Finally, pollen tube dynamics, evaluated through the final number of pollen tubes that reach the base of the style, was affected by temperature, in a genotype-dependent manner, which reflected the temperature adaptation of the pollen donor.

The decrease in germination in both paternal genotypes as temperature increased may be explained by an overall adaptation of sweet cherry to lower temperatures. The number of adhered pollen grains, however, increased with temperature. This could be explained by an acceleration of stigma maturation resulting in a higher stigmatic secretion that retains more pollen grains (Hedhly et al., 2003). This would not necessarily result in an increase in the number of germinated pollen grains, and the two processes, pollen grain adhesion and germination, might be independently regulated as observed in pear (Sanzol et al., 2003).

Increasing temperature in the chambers and field accelerated pollen tube growth rate in the two crosses. This reduced the time needed to reach the style base fitting observations in a range of species (Lewis, 1942; Williams, 1970; Delph et al., 1997). The results obtained in the field, in spite of relatively small differences in temperature, had the same pattern as those obtained in controlled temperatures. This supports previous work in plum showing that cut flowers can be a good predictor of the expected results in the field, allowing better control of environmental conditions and more replications in a reduced space (Jefferies et al., 1982).

The number of pollen tubes was reduced as they grew through the style. While temperature accelerated pollen tube growth rate, the number of pollen tubes getting to the base of the style remained constant and did not increase with time. This number was affected by both the genotype and the temperature. While accurate pollen tube attrition studies in relation to temperature have not been performed yet, an effect of temperature on the number of tubes reaching the stylar base has been recorded in other species. In sour cherry, Cerovic and Ruzic (1992a) obtained the highest number of pollen tubes at intermediate (15°–20°C) temperatures and found fewer pollen tubes at higher (25°C) and lower (5°–10°C) temperatures. However, in avocado the number of tubes reaching the stylar base 24 h after pollination did not differ significantly at 17°/12°C, 25°/20°C 33°/28°C, day/night (Sedgley and Annells, 1981). Thus, this effect depends upon the species and the strength of the temperature stress. Results here show that this response depends also on genotype of the pollen donor.

Subjecting pollinated flowers to different temperature treatments affects the success ratio in a genotypic-dependent manner. Unlike other studies (Cruzan, 1993), there was no correlation between number of pollen tubes at the base of style and pollen adhesion or germination. This may be related to the wide range in number of pollen tubes reaching the base of the style among different species, a characteristic related to the number of ovules. For example, an average of 78 pollen tubes reached the stylar base in *Erythronium grandiflorum* (Cruzan, 1989) and 390 in *Petunia hybrida* (Cruzan, 1993). In sweet cherry, only 1–6 pollen tubes reached the base of the style, which is too small a number to infer a significant correlation with those present at the stigma level.

The fact that temperatures for higher pollen germination in the stigma were not coincident with temperatures for a higher success ratio in the style suggests that the two processes are independent and that there are separate grounds for separate evaluation of different functions. Differences in the optimum temperatures for pollen germination and for pollen tube growth have also been recorded in other species (Mckee and Richards, 1998; Kakani et al., 2002). While pollen germination in the stigma occurs in an autotrophic way, pollen tube growth in the style is heterotrophic (Herrero and Dickinson, 1981). Germination at the stigmatic level might depend on the pollen itself, and further pollen tube growth will also depend on the interaction with the pistil (Herrero, 1992; Hormaza and Herrero, 1999). These mechanisms could operate separately or in

combination in the upper part of the style (Ockendon and Gates, 1975; Sedgley, 1976; Winsor and Stephenson, 1995), in the lower part (Cruzan, 1989), or along the entire length of the style (Hormaza and Herrero, 1999). These different responses to temperature in the progamic phase may explain the complexity of the response recorded in other species (Lankinen, 2001).

Genotypic behavior—Except for the increased adhesion at higher temperature registered for Cristobalina, no consistent differences were observed between the two genotypes at the stigmatic level. However, higher temperatures accelerated pollen tube growth rate, and genotypic differences were observed in the style, suggesting a finely tuned pollen selection as the reproductive process progresses. Thus, pollen tube attrition in the style was affected by temperature, but the response was genotype-dependent and reflected the temperature adaptation of the pollen donor. Cristobalina pollen tubes were faster than Sunburst in reaching the stylar base of all flowers at the three temperatures tested. However, while pollen tubes of Cristobalina grew at similar rates at 20° and 30°C, Sunburst significantly increased its growth rate at 30°C. Although studies on the effect of varying temperatures on the performance of different genotypes are scarce and most work has been done in pollen germination in vitro, differences among genotypes in pollen tube growth rate depending on temperature have been reported in unrelated species such as *Gossypium hirsutum* (Gawel and Robacker, 1986), *Cicer arietinum* (Srinivasan et al., 1999), *Mangifera indica* (Sukhvibul et al., 2000), and *Betula pendula* (Pasonen et al., 2000).

Concerning the number of pollen tubes reaching the stylar base, a differential genotypic response to temperature was recorded, maximum values for Sunburst were obtained at 10°–20°C, while for Cristobalina these were recorded at 30°C. The success ratio confirms this genotypic behavior, and while no differences were recorded for the two genotypes in their success ratio at 10° and 20°C, the success ratio of Cristobalina at 30°C was 2.8 times that of Sunburst. These results are in concordance with the predominant temperatures in their area of distribution. Cristobalina is an early-flowering cultivar originating in southeastern Spain, while Sunburst is a late-flowering cultivar originated from a breeding program in Canada from crosses among cultivars from Northern Europe and consequently is adapted to a cooler climate.

Studies on pollen tube attrition have mainly concentrated on censusing the microgametophyte population (Herrera, 2002) and on pollen tube attrition that occurs in some species due to their mating system (Plitmann, 1993; Smith-Huerta, 1997) or as an isolating reproductive mechanism (Wang and Cruzan, 1998). However, evaluation of microgametophytic populations under a potential selective pressure has been neglected, although the evaluation of post-pollination mechanisms affecting seed paternity (Marshall, 1988) reveals that under stress conditions maternal plants become more selective.

Because temperature affects pollen tube dynamics and there are differences in performance between genotypes, temperature during the reproductive phase could act as a selective pressure agent for genotypes better adapted for pollen tube growth in the style. While this point needs to be evaluated in intraspecific pollen mixtures, it does occur in mixed pollination, with pollen from different species presenting different tolerances to temperature (Zamir et al., 1981). The lack of a genotypic advantage at the standard temperature conditions and the genotype–environment interaction recorded here could promote the maintenance of genetic variation in pollen performance (Gillespie and Turelli, 1989; Mulcahy et al., 1996; Delph et al., 1997; Lankinen, 2001), avoiding fixation of the genes controlling pollen tube growth rate (Mulcahy et al., 1996). However, results here suggest that, under strong selection pressure, those pollen donors and particular microgametophytes that are better adapted to the selection pressure are favored, which could be important in terms of the time needed for a plant species to adapt to rapid temperature changes.

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