

The effect of temperature on stigmatic receptivity in sweet cherry (Prunus avium L.)

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ABSTRACT

Plant reproduction is highly vulnerable to environmental conditions such as temperature and, consequently, planet warming may have significant consequences on the reproductive phase with serious implication in agricultural crops. While pollen tube growth is clearly affected by temperature, little information is available on its effect on the female side and on flower receptivity. In this work, the effect of temperature has been evaluated on stigmatic receptivity of sweet cherry *in vivo*, in the laboratory, and *in planta*, in the field. Results herein show that temperature has a clear effect on the duration of stigmatic receptivity. Thus, while high temperature reduced stigmatic receptivity, low temperature enlarged it. The stigma loses the capacity to offer support first for pollen penetration, second for pollen germination and, finally, for pollen adhesion. The effect of temperature was more pronounced on pollen germination and penetration than on pollen adhesion. High temperature reduced the germination capacity of the pollen as early as the first day after anthesis, a time when no apparent signs of stigma degeneration are apparent. This clear effect of temperature on stigmatic receptivity and pollen performance may have clear implication in crop performance and in establishing screening criterions of best-adapted genotypes.

KEYWORD INDEX

Female receptivity, gametophyte, pollen performance, sporophyte, reproductive phase, temperature stress.

INTRODUCTION

In Angiosperms, the stigma is the first female structure the pollen grains and pollen tubes have to face on their way to the female gametophyte to achieve double fertilization. The stigma provides an adequate environment for pollen grain germination and its morphology and structure has been studied with detail in a variety of species (Heslop-Harrison & Shivanna 1977; Knox 1984). One of the most important features of stigmas is stigmatic receptivity defined as the ability of the stigma to support pollen germination, which is a decisive stage in fertilization success and has a large variability among plant species (Heslop-Harrison 2000).

The length of stigmatic receptivity is variable depending on the species and is usually higher in wind-pollinated than in insect-pollinated species (Khadari *et al.* 1995). Thus, the stigma can be receptive for not much more than an hour or so, such as in Avena or Dactylis, to as long as several days, such as in other grass species (Pennisetum or Zea) or Eucalyptus where it can remain receptive for more than a week in particularly hostile environments (Heslop-Harrison 2000). From an agricultural perspective, stigmatic receptivity has also clear practical implications limiting floral receptivity, the effective pollination period (Guerrero-Prieto, Vasilakakis & Lombard 1985), and hence fruit set (reviewed in Sanzol & Herrero 2001). Moreover, in an ecological context, by altering stigmatic receptivity, flowering plants may influence the likelihood of fertilization by controlling indirectly the number and the quality of matings through the control of the number of pollen grains deposited and the time of germination (Cruden, Hermanutz & Shuttleworth 1984; Smith-Huerta & Vasek 1984; Primack 1985; Galen, Shykoff & Plowright 1986; Delph & Havens 1998).

While differences among species on stigmatic receptivity are apparent, much less is known on the possible effects that environmental conditions may have on the length of stigmatic receptivity within a species. It is well documented that the reproductive phase, especially from pollination to fertilization, is highly vulnerable to the prevailing environmental conditions including temperature (Hall 1992; Stephenson *et al.* 1992). Thus, planet warming may have significant consequences on the reproductive phase with serious implications in agricultural crops, mainly in species as cherries, which are in the southern border of their cultivation potential in Mediterranean regions. Pollen tube growth is clearly affected by temperature (Lewis 1942), and there is good evidence of genetic variability in pollen performance depending on temperature among species (Zamir, Tanksley & Jones 1981; Weinbaum, Parfitt & Polito 1984) or even among genotypes of the same species (Weinbaum *et al.* 1984; Polito, Luza & Weinbaum 1988). However little is known on the effect of temperature on the female structures. In fact, the effect of temperature on the pistil has been mainly focused on ovule longevity (Stösser & Anvari 1982; Postweiler, Stösser & Anvari 1985) and little has been done on its effect on the rest of the pistilar structures and their interaction with the male gametophyte. However, the progamic phase in higher plants is regulated by an intense pollen-pistil interaction (Linskens 1986) and while the pistil appears to be especially well designed to support pollen tube growth, it has mechanisms to encourage pollen competition and discriminate among male gametophytes at different steps of the progamic phase (Herrero 1992). Considering the existence of the intense and complex pollen-pistil interplay and the sensibility of these two components to temperature, the aim of this study was to analyze the effect of temperature on stigmatic receptivity in

controlled temperature chambers and in the he field. The response to temperature was evaluated both in the stigma and in pollen performance.

MATERIALS AND METHODS

Plant material

This work was carried out on a sweet cherry collection maintained at the SIA-DGA experimental orchards located at the Campus de Aula Dei in Zaragoza, Spain. The cultivar ‘Summit’ was used as a female parent and the cultivar ‘Sunburst’ as a male parent. Two experiments were performed, one with cut flowers in the laboratory and the other with whole trees in the field. Cut flowers were maintained on moist florist foam at controlled temperatures of 10°C, 20°C and 30°C. For the field experiment, two trees of ‘Summit’ were chosen. On the day of anthesis of the flower population studied, one of the two chosen trees (warm treatment) was covered with 0.178 mm thick polyethylene film with a metallic structure and the other (control) was left uncovered. Such a system has proven as a valuable method to increase temperature in the field without negatively affecting other parameters (Rodrigo & Herrero 2001). Temperature inside and outside the plastic cage was monitored every 5 min. with a data logger (Testostor 175-3, Testo, Germany) throughout the period of sequential pollination and fixation.

Pollination procedure

Pollen was obtained from flowers of ‘Sunburst’ collected just one day before anthesis (balloon stage); anthers were removed and left to dry on a piece of paper for 24-48 hours at room temperature. Pollen was sieved through a 0.26 µm mesh and frozen at 20°C until required. The duration of stigmatic receptivity was evaluated through the capacity of the stigma to support pollen germination. For this purpose, flowers were emasculated one day before anthesis and hand-pollinated the day of anthesis and thereafter every subsequent day. This experiment was performed in controlled

temperature chambers in the laboratory and in the warm and control treatments in the field. For the laboratory experiment, emasculated flowers were maintained in trays with soaked florist foam at room temperature until the following day when they were placed in the controlled temperature chambers at 10°C, 20°C and 30°C. This day, considered as anthesis, a batch of 10 flowers was pollinated and, subsequently, 10 flowers were pollinated every day during 4 days at 30°C, 6 days at 20°C, and 10 days at 10°C (the maximum lifetime of flowers at each temperature). The same procedure was followed in the field with the trees inside and outside the polyethylene cage and the pollination was carried out until style abscission occurred.

Microscopic observation

In all the treatments the pistils were fixed 24 hours after pollination in formalin: acetic acid: 70% ethanol (1: 1: 18; FAA) (Johansen 1940). Pollen performance was monitored in squash preparations after washing out the fixative with distilled water 3 times, one hour each, softening the pistil in 5% sodium sulphite in the autoclave during 10 minutes at 1 kg.cm⁻² (Jefferies & Belcher 1974), and staining with 0.1% aniline blue in 0.1 N K₃PO₄ (Linskens & Esser 1957). Preparations were examined under an Ortholux II microscope equipped with UV epifluorescence with a Band Pass 355-425 exciter filter and an LP 460 barrier filter. Statistical analyses were performed using the SAS GLM (V. 8, SAS Institute, Inc., Cary, N.C.). Percentage data were subjected to arcsine root square transformation and analysis of variance was performed. The differences between means were analysed by the Fisher's least significant difference (LSD) test at the 0.05 level of significance.

RESULTS

Stigmatic receptivity has been evaluated both in controlled temperature chambers and in the field on the basis of three parameters, which have been shown to represent three consecutive processes in pear (*Pyrus communis* L.) flowers (Sanzol, Rallo & Herrero 2002): pollen adhesion to the stigma, pollen germination, and pollen tube penetration to the transmitting tissues. The effect of temperature has been evaluated in the female and the male side. In the female side stigmatic receptivity was evaluated as percentage of flowers capable of supporting pollen germination. In the male side the response of the pollen population was evaluated as percentage of pollen grains able to perform each one of the three steps required for a successful *in vivo* germination.

Evaluation of stigmatic receptivity *in vivo* in controlled temperature chambers

The capacity of the stigma to support pollen germination

Stigmatic receptivity was lost gradually in three consecutive steps. Thus, the flowers lost the capacity to support first the penetration of pollen tubes into the transmitting tissue, then the germination of pollen grains and, finally, the adhesion to the stigma. This occurred at the three temperatures studied, 10°C, 20°C and 30°C, but was most conspicuous at 10°C (Fig. 1).

These three processes were reduced significantly ($p < 0.05$) as the temperature increased (Fig. 2). The adhesion of pollen grains to the stigma (Fig. 2a) started to decline 2 days after anthesis at 30°C; this occurred one day later at 20°C, while at 10°C stigmas were able to adhere pollen for up to 10 days after anthesis. The effect of

temperature was more evident for pollen germination (Fig. 2b). Indeed, the capacity of stigmas to support pollen germination was completely lost at 3, 5 and 8 days after anthesis at 30°C, 20°C, and 10°C respectively. This effect was paralleled but more severe by pollen tube penetration to the transmitting tissue (Fig. 2c). At 30°C this capacity was lost 2 days after anthesis. At 20°C, despite the low value obtained after 3 days (28%), it persisted until day 5. At 10°C it was maintained up to 4 days to fall then slowly until 8 days after anthesis when it was totally lost.

Pollen performance

To evaluate to which extent pollen behaviour was simply a reflection of the stage of degeneration of the stigma, or, alternatively, if there was a differential genotypic response of the different pollen grains placed on a single stigma, pollen performance was evaluated as the percentage of pollen grains capable to accomplish the three subsequent steps of adhesion, germination, and penetration. While the cessation of activity closely paralleled degeneration of the stigma, pollen performance proved to be a far more sensitive system to reflect the effect of temperature (Fig. 3).

Increasing temperatures also affected negatively these three processes practically following the same patterns in the three temperatures studied (Fig. 3). Pollen adhesion was higher at 30°C than at 20°C or 10°C for the first day of pollination. However, the number of pollen grains adhering to the stigma decreased following 1, 3 and 10 days after anthesis at 30°C, 20°C, and 10°C respectively. A differential response of pollen grains to temperature was most conspicuous for both germination and penetration, since for the first day of pollination at anthesis, when no effect on the stigmatic tissues was observed, the percentage of germinated pollen grains and pollen tubes penetrating the

transmitting tissue was significantly affected by temperature ($p < 0.05$). Indeed, at 10°C all adhered pollen could germinate and penetrate to the transmitting tissues, while at 20°C we registered 90% germination and penetration, and at 30°C only 27% germination and 22% penetration. The germination capacity was lost after 3 days at 30°C, 5 days at 20°C, and 8 days at 10°C (Fig. 3).

Evaluation of stigmatic receptivity *in planta* in the field

To evaluate to which extent these differences observed in controlled temperature chambers may have a reflection in field conditions, stigmatic receptivity was evaluated in the field with trees located inside and outside a plastic cage. The polyethylene cage produced an increase of the mean maximum temperatures of 5.6°C (from 19.6 °C to 25.2 °C), while the mean minimum temperatures were not altered (7°C). This resulted in a 2.8°C increase in the mean temperatures (from 13.3°C to 16.1 °C). Although this temperature increase is small, when compared to the range imposed in controlled temperature chambers, it was sufficient to affect both the stigma and pollen.

The capacity of the stigma to support pollen germination

While results are not significant within the short range of temperature registered between the two treatments, the stigma showed a trend to lose the ability to support pollen germination first inside and then outside the plastic cage (Fig 4). Similar to the results obtained in controlled chambers, the stigma lost the capacity to support first pollen tube penetration to the transmitting tissue, then pollen germination, and finally pollen grain adhesion.

The parameter less affected by temperature was the capacity of flowers to support the adhesion of pollen grains since it only decreased at the end of the experiment (Fig. 4a). However, the capacity of flowers to support germination started to decrease four days after anthesis, in the warm treatment, and 2 days later in the control. The fifth day after anthesis, while all the flowers were able to support germination in the control, only 43% did it in the warm treatment. From day 6 after anthesis the percentage of flowers capable of supporting germination was very low in both warm and control conditions (Fig. 4b). Pollen tube penetration to the transmitting tissue paralleled pollen germination. Four days after anthesis, while all flowers supported penetration to the transmitting tissues in the control treatment, only 28% supported the penetration in the warm treatment. This capacity was lost 6 days after anthesis (Fig. 4c).

Outside the plastic cage, with a mean temperature of 13.3°C, the response was close to that obtained in controlled chambers at 10°C. Inside the plastic cage, with a mean temperature of 16.1°C, the response was intermediate between those obtained in controlled chambers at 10°C and at 20°C.

Pollen performance

The effect of temperature on pollen performance was also evaluated under warm and control conditions. Pollen adhesion, germination, and penetration followed a similar trend to that observed in controlled temperature chambers although the effect was significant ($p < 0.01$) only for pollen tube penetration. The differences between the two treatments occurred mainly during the first 6 days after anthesis; after that period few pollen grains could achieve the three consecutive processes.

As in controlled temperature chambers, the pollen first lost the capacity to penetrate the transmitting tissue, then the capacity of germination and, finally, the capacity to adhere to the stigma. Pollen adhesion, although low, was maintained in both conditions up to 9 days after anthesis. The number of pollen grains adhered to the stigma was higher in the warm than in the control treatment; thus, during the 6 days following anthesis adhered pollen grains averaged 550 and 380 pollen in warm and in control treatments, respectively. Both germination and penetration were very low 6 days following anthesis and, on the whole, lower in the warm treatment (Fig 5a and 5b).

DISCUSSION

Results herein show that temperature has a clear effect on the duration of stigmatic receptivity. While high temperature reduced stigmatic receptivity, low temperature enlarged it. This was apparent in the controlled temperature experiment, but it was also clear in field despite the slight increase of 2.8°C in the mean temperature. But temperature also had an added direct effect on pollen germination, and high temperature reduced the germination capacity of the pollen.

Temperature effect on the female side: the stigma

Temperature clearly affected the duration of stigmatic receptivity. The methodology of pollinating flowers of different ages, which proved to be a useful method to assess the duration of stigmatic receptivity (Gonzalez, Coque & Herrero 1995), is also valid to assess the effect of temperature on stigmatic receptivity. Results in the field corresponded well to the behaviour of cut flowers placed in controlled temperature chambers. The results obtained in the field outside the plastic cage (13.3°C) were close to those of the 10°C chamber, while the results obtained inside the plastic cage (16.1°C) were intermediate between the 10°C and the 20°C chambers. *In vivo* cut flowers of plum (*Prunus cerasifera* L.) have proven to be a good predictor of the response obtained in the field for pollen tube growth in the style (Jefferies *et al.* 1982). Results herein show that this method is also valuable for the prediction of temperature effects on stigmatic receptivity. This method has a clear interest for future studies since the method described here with cut flowers placed in controlled temperature chambers allows a better control of the environmental conditions in a reduced space and without the influence of additional environmental factors.

Three different processes take place in the stigma before pollen tubes grow in the style: pollen grain adhesion, pollen germination, and pollen tube penetration into the transmitting tissue. Recent results obtained in pear (Sanzol *et al.* 2002) show that the ability to achieve these steps is lost gradually; thus, the stigma loses the capacity to offer support first for pollen penetration, second for pollen germination and, finally, for pollen adhesion. The results obtained in this work show that these steps are conserved in cherry and that all three processes are negatively affected by increasing temperatures. The effect of temperature, especially in controlled temperature chambers, was higher on the capacity of flowers to support pollen grain germination and pollen tube penetration to the transmitting tissue than on their capacity to allow pollen adhesion. Indeed, at 10°C, pollen germination and initial pollen tube growth lasted to 9 days after anthesis, while at 20°C and 30°C, this capacity was lost in 5 days and 2-3 days after anthesis, respectively.

The results obtained in the field were consistent with those of controlled temperature chambers, in spite of the small difference between the mean temperatures registered outside and inside the plastic cage (2.8°C) compared to those with the controlled temperature chambers (interval of 20°C). This could be explained by a major effect of the mean maximum temperatures since the difference registered between the two treatments was 5.6°C, while mean minimum temperatures were not altered. This result proves the sensibility of these processes to small variation of temperature, and can contribute to explain records of reduced fruit set under excessively warm springs. Likewise, they are in accordance with records observed in the field of a reduced stigmatic receptivity under these conditions (Burgos, Egea & Dicenta 1991; Egea *et al.*, 1991). In fact, small variations in temperature can be observed even among flowers in

the same tree (Landsberg, Powell & Butler 1973), which could explain the variation in response registered even within the same treatment in the field.

Behaviour of the male side: Pollen germination

High temperature also had a direct effect on pollen performance per se, since pollen responds to temperature, independently of the effect registered in the stigma. This was most apparent in flowers pollinated at anthesis, when all the flowers were shown to be equally receptive. In these conditions, clear differences were registered within the pollen population present in one stigma in the ability to germinate. While all the adhered pollen grains could germinate and penetrate to the transmitting tissue at 10°C, at 20°C the germination was 90% and at 30°C only 27% could germinate and only 22% could penetrate. Thus, pollen performance could be considered as a more sensitive test to study temperature effect as we registered clear differences in pollen performance as early as the first day after anthesis, a time when all the stigmas were equally receptive. A reduction in the percentage of pollen germination could have an effect on pollen selection. Similarly to what has been observed in other species (reviewed in Hormaza & Herrero 1994) genotype x temperature interactions occur for pollen germination in sweet cherry (Hedhly, Hormaza & Herrero, unpublished data). Consequently, pollen selection could occur even during pollen germination in the stigma since the most adapted gametophytes to particular temperatures would have an advantage in pollen germination regardless of the later effect of temperature during pollen tube growth in the pistil. The question remains whether this selection may have a reflection in the genetic composition of the next sporophytic generation.

In the most distant days from anthesis, pollen performance paralleled female aging and temperature affected the three processes evaluated. Indeed, while high temperature induced an increase in pollen adhesion, at the same time it reduced pollen germination and penetration. Pollen germination was lost after 8 days at 10°C, after 4 days at 20°C, and after 2 days at 30°C. No differences were detected between germination and penetration, suggesting that pollen germination is the key factor in pollen performance in the stigma and also the most vulnerable step to high temperature.

A clear relationship between temperature and pollen germination has been recorded in other species although the optimum temperature for pollen adhesion and germination is variable depending on the species. Thus, in almond (*Prunus dulcis* (Mill.) D.A. Webb), adhesion and germination were found to be higher at 22°C than at 15°C (Vezavaei 1997). Similarly, in avocado (*Persea americana* Mill.), Sedgley & Annells (1981) found higher pollen grain germination and pollen tube penetration at 25°C/20°C (day/night) and at 33°C/28°C than at 17°C/12°C. These species differences may be a reflection to the adaptation of different species to the prevailing temperatures during flowering time. Indeed, the adaptation of pollen germination to different temperatures appears to reflect the adaptation of these species to particular climates (Zamir *et al.* 1981; Weinbaum *et al.* 1984; Polito *et al.* 1988). The negative correlation between germination and temperature in sweet cherry registered in this experiment could be explained by a higher adaptation of sweet cherry pollen to lower temperatures.

High temperatures are detrimental for the female part by reducing the length of stigmatic receptivity, as in this work, and accelerating ovule degeneration (Postweiler *et al.* 1985; Cerovic, Ruzic & Micic 2000). However, at the same time they are advantageous for the pollen hastening its tube growth rate (Lewis, 1942), which could

signify an adaptation of the male part to a short female receptivity. On the other hand, low temperatures may act against the pollen by reducing its germination and growth rate, which could limit the fertilization success (Thompson & Liu 1973; Jakobson & Martens 1994). In this case, the pistil seems to compensate the effect on pollen by enlarging stigmatic receptivity and delaying style and ovule degeneration (Stösser & Anvari 1982). The result of the effect of temperature on the male and on the female side is that fertilization can be ensured in a wider range of temperatures. Pollen-stigma interaction is the first step of pollen-pistil interaction during the reproductive process in Angiosperms. Work is in progress to assess the relevance of temperature on the rest of the interaction steps that take place before fertilization occurs.

ACKNOWLEDGEMENTS

A.H. was supported by an AECI and a SIA-DGA fellowships and financial support for this work was provided by INIA (project grant RTA 01-103).

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FIGURE LEGENDS

Figure 1. Stigmatic receptivity at 10°C expressed as percentage of flowers with pollen grains adhered to the stigma, germinated pollen grains and pollen tubes penetrating to the transmitting tissues.

Figure 2. Effect of temperature on adhesion, germination and penetration of pollen on the stigma at 10°C, 20°C and 30°C expressed as percentage of flowers with pollen grains adhered to the stigma (a), with germinated pollen grains (b) and with pollen tubes penetrating the transmitting tissues (c). Vertical bar represents LSD at the 0.05 level of significance.

Figure 3. Effect of temperature on germination and penetration of pollen on the stigma at 10°C, 20°C and 30°C, expressed as the percentage of germinated pollen grains (mean \pm SE) (a) and the percentage of pollen tubes (mean \pm SE) penetrating to the transmitting tissues (b).

Figure 4. Effect of temperature on adhesion, germination and penetration of pollen on the stigma in both control and warm treatment, expressed as the percentage of flowers with pollen grains adhered to the stigma (a), with germinated pollen grains (b) and with pollen tubes penetrating to the transmitting tissues (c). Vertical bar represents LSD at the 0.05 level of significance.

Figure 5. Effect of temperature on germination and penetration of pollen on the stigma in the control and warm treatment, expressed as the percentage of germinated pollen

grains (mean \pm SE) (a) and percentage of pollen tubes penetrating to the transmitting tissues (mean \pm SE) (b).

ILLUSTRATIONS









