

Acta Scientiarum. Agronomy  
ISSN: 1807-8621  
Editora da Universidade Estadual de Maringá -  
EDUEM

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Acta Scientiarum. Agronomy, vol. 42, 2020

Editora da Universidade Estadual de Maringá - EDUEM

**Available in:** <http://www.redalyc.org/articulo.oa?id=303062597021>

**DOI:** 10.4025/actasciagron.v42i1.42450

# Stigmatic receptivity of peach flowers submitted to heat stress

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Acta Scientiarum. Agronomy, vol. 42,  
2020

Editora da Universidade Estadual de  
Maringá - EDUEM

Received: 19 April 2018

Accepted: 03 June 2018

DOI: 10.4025/actasciagron.v42i1.42450

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**ABSTRACT.:** Because of climatic changes, the cultivation of temperate climate plants such as peach in subtropical climates has become a challenge. In these areas, temperatures exceeding 25°C often occur during the pre-flowering and flowering phases. The high temperature causes damages by acting during the early stages of pollen-pistil interaction processes. The objective of this work was to evaluate the stigmatic receptivity of peach flowers at 18°C and 30°C. The pollen adherence was evaluated as well as the germination and presence of pollen tubes in the transmitting tissue of the style. The genotypes responded differently to temperature. ‘Granada,’ ‘Diamante,’ and ‘Sensação’ had a stigmatic receptivity that was less affected when flowers were exposed to the higher temperature. Most genotypes showed a reduction in the number of pistils with pollen tubes growing in the style, particularly when pollination was delayed.

**Keywords:** *Prunus persica*, pollen viability, pollen tubes, flowering.

## Introduction

Global warming, as well as an increase in the intensity of climatic conditions such as extreme cold and hot periods, is an important threat to agricultural crops in some regions (IPCC, 2015), particularly those in which conditions are already marginal for the crops, which is the case of peach cultivation in subtropical regions (Souza et al., 2017a). The peach, because of the necessity accumulating chill hours, may be affected by a temperature increase, particularly in subtropical and tropical zones, that can affect the pollen grain germination and fruit setting rate (Souza, Pio, Tadeu, Zambon, & Reighard, 2017b).

Both high and low temperatures damage the reproductive tissues of plants by causing an advance or delay in flowering, asynchrony between the development of male and female floral structures, the formation of defective gametes and problems in fertilization (Zinn, Tunc-Ozdemir, & Harper, 2010). These consequently lower productivity. The levels

of susceptibility and tolerance, as well as the developmental stage in which the plant is most sensitive, varies among species and even among genotypes of the same species (Hatfield & Prueger, 2015; Hedhly, 2011). However, it is known that the reproductive stage is the most stress-sensitive phase, and the tolerance to this factor is a limiting characteristic of the plant's productivity (Hedhly, 2011; Zinn et al., 2010).

Floral receptivity plays a crucial role in the dynamics of pollination, reproductive success and consequently fruit production. The stigma is the first surface of the pistil to make contact with the pollen grain, and its adherence to the surface is followed by hydration and germination. Then, growth of the pollen tube continues through the stigma, style and finally ovary, where the ovule fertilization occurs (Hiscock & Allen, 2008; Losada & Herrero, 2012). Several studies have indicated that high temperature stress acts during the first stages of the pollen-pistil interaction, affecting pollen adherence to the stigma, pollen germination, and pollen tube growth along the transmitting tissue and increasing ovule degeneration (Hedhly, Hormaza, & Herrero, 2005; Snider & Oosterhuis, 2011; Vuletin Selak, Perica, Goreta Ban, & Poljak, 2013).

The aim of this work was to evaluate the stigmatic receptivity of the flowers of peach genotypes under a high (30°C) temperature compared to a moderate (18°C) temperature.

## Material and methods

Experiments were conducted during 2011, 2012, and 2014 to evaluate the stigmatic receptivity of peach genotypes. The work was conducted at the EMBRAPA Center for Temperate Climate Research, in Pelotas, Rio Grande do Sul State, Brazil (31°40' S and 52°26' W; 60 m altitude).

During 2011, the cultivars 'Atenas', 'Chimarrita', 'Diamante', 'Granada', 'Sensação', and 'Turmalina' and the selections 'Cascata 1303' and 'Conserva 594' were used. During 2012 and 2014, in addition to the previously mentioned genotypes, the cultivars 'Maciel' and 'Tropic Beauty' were included. Except for 'Tropic Beauty,' which was released jointly by Texas A&M University and the University of Florida (Gainesville), all genotypes were from the peach breeding program of Embrapa. 'Tropic Beauty' was chosen because of its adaptation to warm areas. 'Cascata 1303', 'Conserva 594' and 'Turmalina' are low-chill genotypes and well-adapted to subtropical regions. The other tested genotypes are commercial cultivars planted in southern Brazil.

Shoots containing flower buds at the balloon stage (1 day prior to blooming) were collected from the intermediate part of the adult peach plants in the orchards of Embrapa and taken to the laboratory. The shoots were divided into segments approximately 5 cm in length and containing from one to four flowers. Only flowers in the middle of each shoot were used. They were emasculated and affixed in phenolic foam (commonly used for seed germination) 2.5 × 2.5 × 3.8 cm in size (Green Up<sup>®</sup>; Floral Atlanta Ltd., São Lourenço, São Paulo, Brazil), previously washed in running water as recommended by the manufacturer. The shoots were

collected during July and August of each year, when most flowers were at the balloon stage, according to the cycle of each genotype.

The phenolic foam with the emasculated flowers was placed into plastic trays containing 1 cm of tap water and then submitted to a high-temperature stress treatment ( $30 \pm 1^\circ\text{C}$ ) in a heat chamber. For the control, the foams with the emasculated flowers were maintained in a room at  $18 \pm 2^\circ\text{C}$ . Both environments were maintained between 60 and 70% relative humidity with no light. The material remained either for 24, 48, 72, or 96h under the conditions previously described before the flowers were pollinated. Following pollination, they were maintained at  $18 \pm 2^\circ\text{C}$ .

Within a given year, the pollen used for pollinating all the flowers was from a cultivar that had high viability (70%), as determined by its *in vitro* germination. Pollen from the peach selection Conserva 1510 was used during 2011, and pollen 'Leonense' was used during 2012 and 2014. Twenty-four hours following pollination, the pistils were fixed in formalin:acid acetic:70% ethanol (1:1:18; FAA) (Johansen, 1940), and refrigerated until the evaluation.

After staining with lacmoid (Wilson & Brown, 1957), the pistils were placed on a glass slide with a drop of the dye and gently squashed under a cover slip. The pistils were evaluated for pollen adherence and germination and the presence of pollen tubes in the style with an optical microscope (Carl Zeiss do Brasil Ltda., São Paulo, São Paulo, Brazil) using a 10× ocular and 40× objective.

Pollen adherence and germination in the pistils were scored as follows: 1 = zero pollen grains adhered and/or germinated on the stigmatic surface, 2 = 1-25, 3 = 26-50, 4 = 51-75, 5 = 76 to 100, and 6 = more than 100 pollen grains adhered and/or germinated on the stigmatic surface. The presence of pollen tubes was also evaluated and expressed as the percentage of flowers with pollen tubes growing in the style. The evaluation of the pollen adherence and germination on the stigmatic surface and the presence of pollen tubes growing in the stylar tissue was completed using the same flowers.

Data related to the classes of adherence and germination of pollen grains on the stigmatic surface of flowers were transformed using  $\log(x)$ , while data related to the percentage of flowers with pollen tubes in the style were transformed using  $\arcsin(x/100)$ . The experimental design was completely randomized with an  $8 \times 4 \times 2$  factorial treatment structure (genotype  $\times$  time  $\times$  temperature) during 2011 and  $10 \times 4 \times 2$  during 2012 and 2014 with three replications of five flowers per plot.

ANOVA was performed using SISVAR statistical software (Ferreira, 2011), and when the interaction effect was significant, regression curves (time  $\times$  temperature) for each genotype during each year were calculated.

## Results and discussion

A significant interaction among genotype, time and temperature ( $p \leq 0.01$ ) was observed as well for the variables genotype, time ( $p \leq 0.01$ ) and

temperature ( $p \leq 0.05$ ) singularly during 2011. During 2012 and 2014, all the interactions were significant ( $p \leq 0.001$ ) as well as the singular variables (Table 1).

**Table 1**  
Summary of the analysis of variance, degree of freedom (DF) and the mean squares for pollen adherence (PAd) for the years 2011, 2012, and 2014.

Sources of variation	Mean Squares					
	2011		2012		2014	
	DF	PAd	DF	Pad	DF	PAd 2014
Genotype (G)	7	0.1116 **	9	0.0905 **	9	0.1403 **
Time (T)	3	0.1076 **	3	0.1027 **	3	0.8891 **
Temperature (°C)	1	0.0383 *	1	0.3824 **	1	0.3248 **
G x T	21	0.0103 ns	27	0.0533 **	27	0.0239 **
G x °C	7	0.0131 ns	9	0.0369 **	9	0.0443 **
T x °C	3	0.0413 **	3	0.1066 **	3	0.2158 **
G x T x °C	21	0.0195 **	27	0.0339 **	27	0.0416 **
Error	128	0.0068	160	0.0091	160	0.0070
C.V. (%)		12.8		19.0		17.3
Mean		0.6406		0.5038		0.4844

ns, \*\*, \* Nonsignificant or significant at  $p \leq 0.01$  or  $\leq 0.05$ , respectively.

During 2011, the cultivars 'Atenas', 'Chimarrita', 'Sensação', and 'Turmalina' and the selection 'Conserva 594' showed a reduction in pollen adherence when the flowers were exposed to 30°C compared to 18°C (Figure 1). The time of the reaction also varied with the cultivar, with 'Atenas' and 'Turmalina' showing less adherence over 24h, and 'Chimarrita', taking 53h to show a reduction in pollen adherence on the stigmatic surface at 30°C. At 18°C, 'Atenas', 'Chimarrita', and 'Conserva 594' showed consistent adherence for 96h, whereas 'Turmalina' and 'Sensação' showed a decrease in pollen adherence after approximately 60h. Two peach genotypes, 'Cascata 1303' and 'Diamante', did not have their pollen adherence expressively affected by the 30°C temperature, whereas 'Granada' showed a decrease in pollen adherence at both temperatures.

During 2011 and 2012, the cultivars 'Chimarrita' and 'Turmalina' showed a reduction in pollen adherence when the flowers were exposed to heat (Figure 2). The selection 'Cascata 1303', in contrast to its behavior of consistent pollen adherence at both temperatures during 2011, showed a decrease in pollen adherence during 2012.

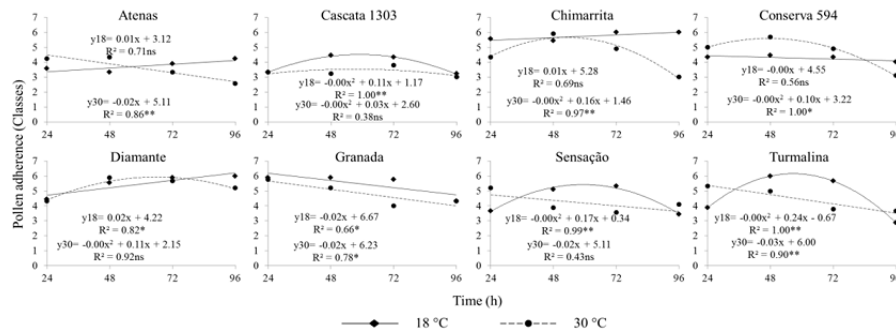


Figure 1

Adherence of pollen grains on the stigma (classes: 1 = zero; 2 = 1 to 25; 3 = 26 to 50; 4 = 51 to 75, 5 = 76 to 100, and 6 = over 100 pollen grains) of flowers at 18°C and 30°C for 24 to 96h during 2011. ns, \*\*, \* Nonsignificant or significant at  $p \leq 0.01$  or  $p \leq 0.05$ , respectively.

A 50% decrease in pollen adherence (96-h vs. 24-h time period) was observed for ‘Cascata 1303’, ‘Chimarrita’ and ‘Maciel,’ when these cultivars were grown at 30°C.

Stigmas of ‘Atenas’, ‘Chimarrita’, ‘Tropic Beauty’, and ‘Turmalina’ did not lose the capacity to support pollen adherence over 96h when maintained at 18°C, but were negatively influenced by the temperature of 30°C. ‘Cascata 1303’ showed a considerable decrease in adherence at both temperatures. ‘Maciel,’ although it had reduced adherence at both temperatures, the reduction was higher at 30°C than at 18°C. In contrast, in stigma receptivity studies performed using different peach genotypes, ‘Maciel’ did not show negative behavior when the flowers were submitted to a temperature of 29°C (Zanandrea, Raseira, dos Santos, & da Silva, 2011).

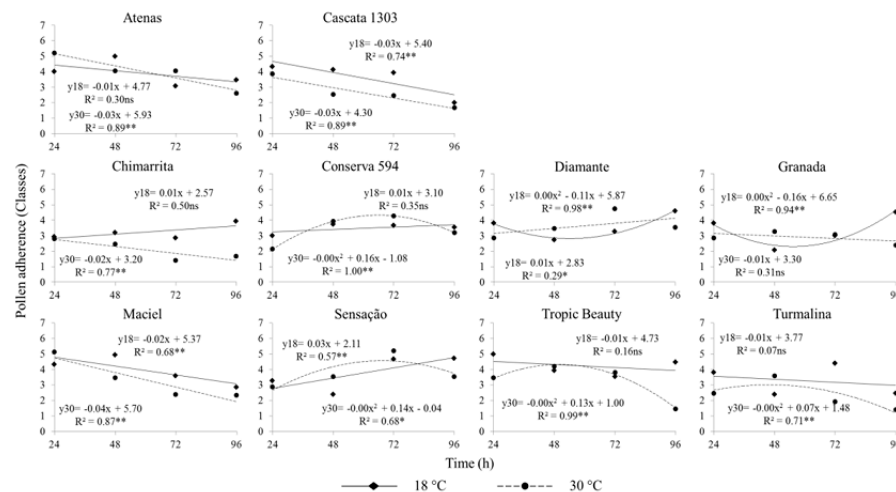


Figure 2

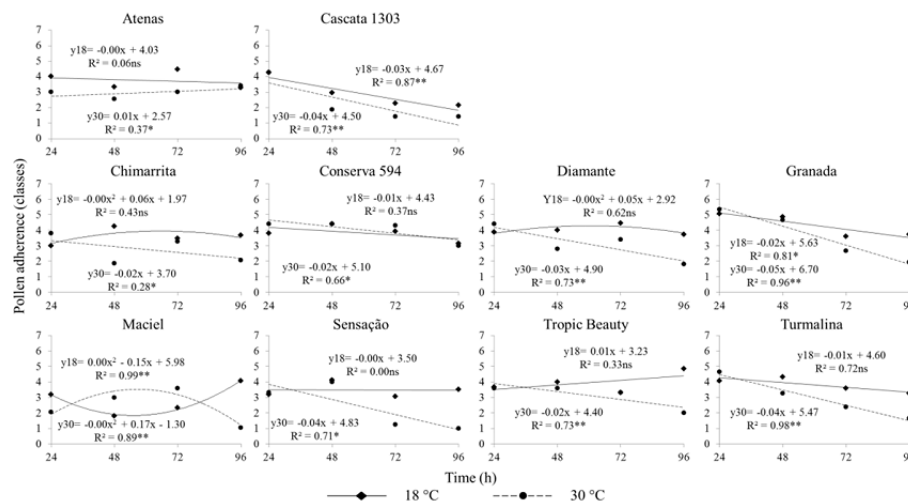
Adherence of pollen grains on the stigma (classes: 1 = zero; 2 = 1 to 25; 3 = 26 to 50; 4 = 51 to 75, 5 = 76 to 100, and 6 = over 100 pollen grains) of flowers at 18°C and 30°C for 24 to 96h, during 2012. ns, \*\*, \* Nonsignificant or significant at  $p \leq 0.01$  or  $p \leq 0.05$ , respectively.

In addition, ‘Tropic Beauty’ was negatively influenced by the high temperature (Figure 2), as well as ‘Turmalina’, both of which similarly



behaved when exposed to heat. However, for ‘Turmalina’, this reduction occurred earlier than that of ‘Tropic Beauty’ when compared to the temperature of 18°C.

During 2014, except for ‘Atenas’ and ‘Conserva 594’, all of the other genotypes showed a reduction in pollen adherence to the stigma when the flowers were exposed to 30°C (Figure 3). This loss was not observed for the flowers that remained at 18°C, with the exception of ‘Cascata 1303’ and ‘Granada’.



**Figure 3**

Adherence of pollen grains on the stigma (classes: 1 = zero; 2 = 1 to 25; 3 = 26 to 50; 4 = 51 to 75, 5 = 76 to 100, and 6 = over 100 pollen grains) of flowers at 18°C and 30°C for 24 to 96h during 2014. ns, \*\*, \* Nonsignificant or significant at  $p \leq 0.01$  or  $p \leq 0.05$ , respectively.

During 2011, the average score of the pollen adherence was higher (4.53) than that observed during 2012 (3.38) and 2014 (3.31). The average annual temperature for both years (2011 and 2012) during July was very similar but lower than that during 2014. During August, the average of the medium temperature during 2012 was higher by 4.2 and 2.8°C than that during 2011 and 2014, respectively. This pre-exposure of flowers to high temperature mainly during July and August (prior to blooming) may have negatively influenced the stigma of the flower, causing an earlier loss of the ability to support pollen adherence. In our research, most of the peach stigmas were receptive 24h after emasculation, but this factor may not depend only on the genotype but also on the climatic conditions during the pre-anthesis phase (Kodad, Messaoudi, Mamouni, Lahlou, & Socias, 2013).

Although these temperature variations seem small, it is known that depending on the species and the phenological stage of the plant in which these temperatures occur, they can cause a considerable decrease in fruiting (Hatfield & Prueger, 2015). Reductions in fruit setting were recorded in apricot when there was an increase of 3°C in the average daily temperature during the week before the anthesis (Rodrigo & Herrero, 2002).

In general, regarding the adherence of pollen grains to the stigma during at least two of the three years of the evaluation, the most influenced genotypes by flower exposure to 30°C as compared to 18°C were 'Chimarrita' during 2011 and 2012 and 'Tropic Beauty' during 2012 and 2014. The least influenced genotypes were 'Diamante' and 'Conserva 594'. For all of the genotypes, there were variations among years of lower or higher intensity.

Many studies have documented the negative influence of high temperatures in the pre-anthesis and anthesis phase for several species, such as peach (Couto, Raseira, Herter, & Silva, 2010; Zanandrea et al., 2011), cotton (Snider, Oosterhuis, Skulman, & Kawakami, 2009), and olives (Vuletin Selak et al., 2013). This is considered to be the most sensitive phase for the plants because of the number of involved processes and the short time in which they occur (Hedhly, Hormaza, & Herrero, 2009; Zinn et al., 2010). However, this response is highly variable among the studied genotypes, as well as the environmental conditions in which the plants are exposed over time (Hatfield, Boote, & Kimball, 2011; Hedhly, 2011).

For the germination of pollen grains on the stigma, a significant interaction ( $p \leq 0.01$ ) among genotype, time and temperature occurred during the three years of evaluation, and all of the interaction was significant ( $p \leq 0.01$ ), except for the interaction between the genotype and temperature during 2011 (Table 2).

**Table 2**  
Summary of the analysis of variance, degree of freedom (DF) and the mean squares for pollen germination (PGe) for the years 2011, 2012, and 2014.

Sources of variation	Mean Square					
	2011		2012		2014	
	DF	PGe	DF	PGe	DF	PGe
Genotype (G)	7	0.1862 **	9	0.2133 **	9	0.3121 **
Time (T)	3	0.2057 **	3	0.2921 **	3	0.8004 **
Temperature (°C)	1	0.1241 **	1	0.6500 **	1	0.5468 **
G x T	21	0.0216 **	27	0.0303 **	27	0.0534 **
G x °C	7	0.0164 ns	9	0.0926 **	9	0.0709 **
T x °C	3	0.0835 **	3	0.0599 **	3	0.1505 **
G x T x °C	21	0.0188 **	27	0.0248 **	27	0.0441 **
Error	128	0.0083	160	0.0083	160	0.0056
C.V. (%)		18.3		14.2		12.4
Mean		0.4977		0.6414		0.6059

ns, \*\*, Nonsignificant or significant at  $p \leq 0.01$  respectively.

During 2011, the behavior of the germination was very similar to that of adherence during the same year. In general, most of the studied genotypes showed a reduction in pollen germination on the stigma when the flowers were submitted to a temperature of 30°C (Figure 4). 'Chimarrita', 'Diamante', 'Granada', and 'Turmalina' suffered a reduction in pollen germination when pollination occurred on the flowers at 30°C. In contrast, 'Atenas', 'Cascata 1303', 'Conserva 594', and 'Sensação' were only slightly affected.



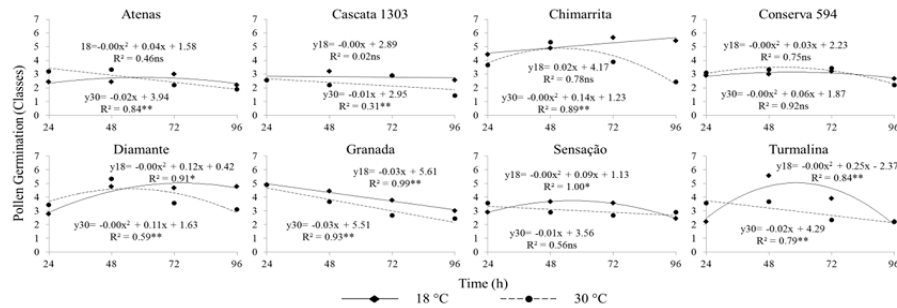


Figure 4

Germination of pollen on the stigma (classes: 1 = zero; 2 = 1 to 25; 3 = 26 to 50; 4 = 51 to 75, 5 = 76 to 100, and 6 = over 100 pollen grains) in peach flowers at 18°C and 30°C for 24 to 96h during 2011. ns, \*\*, \* Nonsignificant or significant at  $p \leq 0.01$  or  $p \leq 0.05$ , respectively.

During 2012, ‘Chimarrita’, and ‘Cascata 1303’ showed a sharper reduction in pollen germination than other genotypes, almost completely losing their ability for pollen germination after 72h at 30°C (Figure 5), although there was some adherence up to 96h during 2012 (Figure 2). This indicates that even though the stigma provides conditions for pollen adherence, it does not ensure pollen germination.

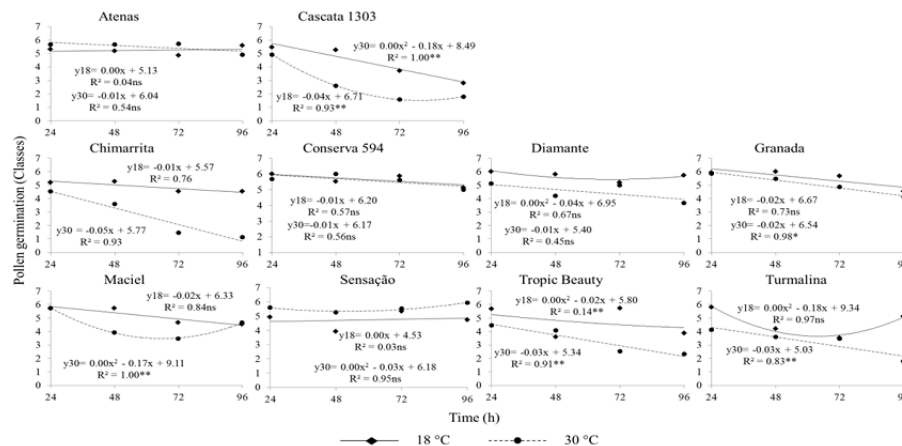


Figure 5

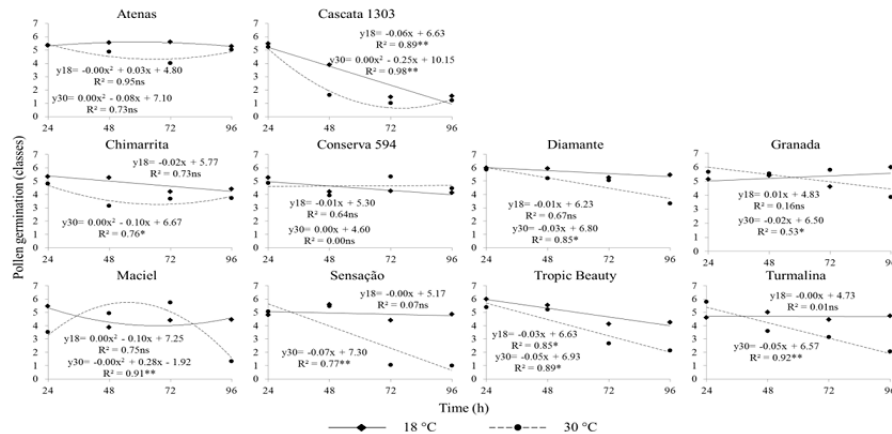
Germination of pollen on the stigma (classes: 1 = zero; 2 = 1 to 25; 3 = 26 to 50; 4 = 51 to 75, 5 = 76 to 100, and 6 = over 100 pollen grains) in peach flowers at 18°C and 30°C for 24 to 96h during 2012. ns, \*\*, \* Nonsignificant or significant at  $p \leq 0.01$  or  $p \leq 0.05$ , respectively.

The cultivar ‘Granada’, at 30°C, presented a small reduction in the stigmatic capacity of the flowers in providing conditions for the pollen germination.

‘Diamante’, ‘Tropic Beauty’ and ‘Turmalina’ also had the ability to provide conditions for pollen germination harmed in the flowers that were kept at 30°C. However, the cultivars ‘Chimarrita’, ‘Diamante’, ‘Maciel’, ‘Sensação’, and ‘Turmalina’ (Figure 5) showed very little change for the pollen germination when the flowers remained at 18°C before pollination. The cultivar ‘Sensação’, in the same way as for adherence during 2012 (Figure 2), had a higher germination of pollen grains on the stigma when the flowers were submitted to 30°C (Figure 5), and there

was no reduction over time. The genotypes 'Conserva 594' and 'Atenas', which did not suffer when the flowers were exposed either to 18 or 30°C for the periods of evaluation, also stand out during this year.

During 2014, it was observed that flowers of any of the genotypes remained at 18°C, except 'Cascata 1303' and 'Tropic Beauty,' presented an expressive loss in the ability of pollen germination (Figure 6), as occurred with most genotypes during 2012.



**Figure 6**

Germination of pollens on the stigma (classes: 1 = zero; 2 = 1 to 25; 3 = 26 to 50; 4 = 51 to 75, 5 = 76 to 100, and 6 = over 100 pollen grains) in peach flowers at 18°C and 30°C for 24 to 96h during 2014. <sup>ns</sup>, \*\*, \* Nonsignificant or significant at  $p \leq 0.01$  or  $p \leq 0.05$ , respectively.

For the 'Sensação' cultivar, in contrast to its behavior during previous years, the pollen germination on the stigma of flowers submitted to 30°C suffered a marked reduction during 2014. Pollen germination was also impaired by heat during 2014 for 'Cascata 1303', 'Tropic Beauty,' 'Maciel' and 'Turmalina'. The last was also affected by heat during the previous years of the evaluation.

Over the 3 years, the most heat-sensitive genotypes with respect to pollen germination were 'Cascata 1303', 'Chimarrita', 'Tropic Beauty', and 'Turmalina', and the most heat-tolerant peaches were 'Atenas', 'Conserva 594', 'Diamante', 'Granada', and 'Sensação.' Again, for all genotypes, there were variations at different grades among the studied years.

A significant interaction among genotype, time and temperature for the percentage of pistils with the presence of pollen tubes in the style was found ( $p \leq 0.05$  for 2011 and  $p \leq 0.01$  for 2012 and 2014) (Table 3).

During 2011, 'Cascata 1303', 'Atenas', and 'Sensação' showed no reduction in the percentage of pistils with pollen tubes in the transmitting tissue of the style of flowers submitted to 30°C (Figure 7).

Table 3

Summary of the analysis of variance, degree of freedom (DF) and the mean squares for the percentage of pollen tubes in the style (PPTS) for the years 2011, 2012, and 2014.

Sources of variation	Mean Square					
	2011		2012		2014	
	DF	PGe	DF	PGe	DF	PGe
Genotype (G)	7	0.1862 **	9	0.2133 **	9	0.3121 **
Time (T)	3	0.2057 **	3	0.2921 **	3	0.8004 **
Temperature (°C)	1	0.1241 **	1	0.6500 **	1	0.5468 **
G x T	21	0.0216 **	27	0.0303 **	27	0.0534 **
G x °C	7	0.0164 ns	9	0.0926 **	9	0.0709 **
T x °C	3	0.0835 **	3	0.0599 **	3	0.1505 **
G x T x °C	21	0.0188 **	27	0.0248 **	27	0.0441 **
Error	128	0.0083	160	0.0083	160	0.0056
C.V. (%)		18.3		14.2		12.4
Mean		0.4977		0.6414		0.6059

ns, \*, \*\*, Nonsignificant, significant at  $p \leq 0.01$  or  $p \leq 0.05$ , respectively.

The cultivars 'Diamante' and 'Granada' showed the same behavior, and a slight reduction was observed for this variable when exposed to 30°C when compared to 18°C. 'Chimarrita', 'Turmalina', and 'Conserva 594' had a large decrease in the percentage of pistils with pollen tube growth in the styles at 30°C in comparison to those at 18°C.

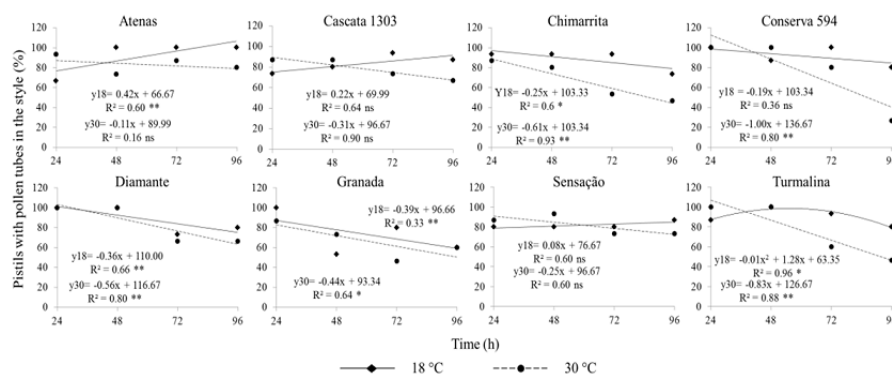


Figure 7

Percentage of pistils with pollen tubes in the style of peach flowers (%) at 18°C and 30°C for 24 to 96h during 2011. ns, \*\*, \* Nonsignificant or significant at  $p \leq 0.01$  or  $p \leq 0.05$ , respectively.

During 2012, the 'Granada' cultivar (Figure 8) showed similar behavior to the previous year, as well as 'Sensação' and 'Turmalina'.

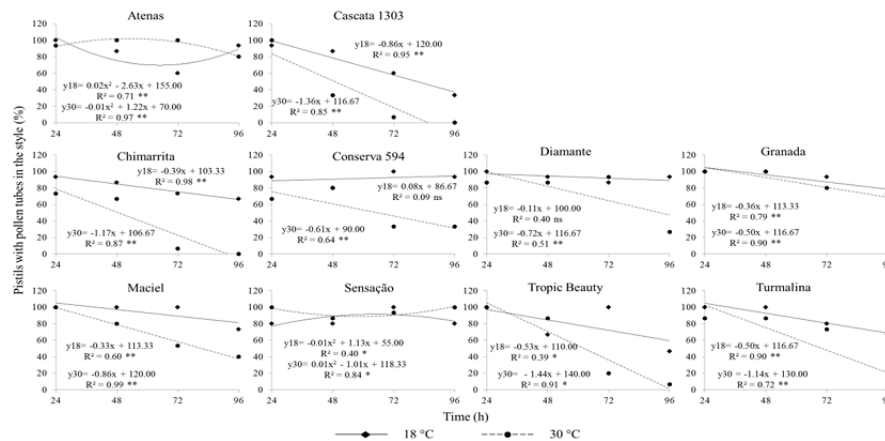


Figure 8

Percentage of pistils with pollen tubes in the style of peach flowers (%) at 18°C and 30°C for 24 to 96h during 2012. ns, \*\*, \* Nonsignificant or significant at  $p \leq 0.01$  or  $p \leq 0.05$ , respectively.

'Cascata 1303', 'Chimarrita', 'Conserva 594', 'Maciel', 'Tropic Beauty', and 'Turmalina' (Figure 8) were the most negatively influenced genotypes by heat in terms of the presence of pollen tubes in the style during 2012. 'Diamante' was only negatively influenced after 72h exposed to 30°C.

The 2014 results were consistent with the 2012 results for 'Cascata 1303', 'Chimarrita', 'Tropic Beauty', and 'Turmalina', showing a decrease in the percentage of flowers with pollen tubes in the styles at both temperatures, and for 'Maciel' and 'Diamante', which showed minimal or no change at 18°C but were negatively affected at 30°C (Figure 9).

In contrast, 'Sensação' and 'Atenas', which during previous years showed consistent pollen tube presence in the style, showed a negative response to the temperature stress during 2014.

For the 3 years of evaluation, a decrease in the presence of pollen tubes in the transmitting tissue of style when the flowers were maintained under 30°C compared to 18°C was observed in most of the studied genotypes.

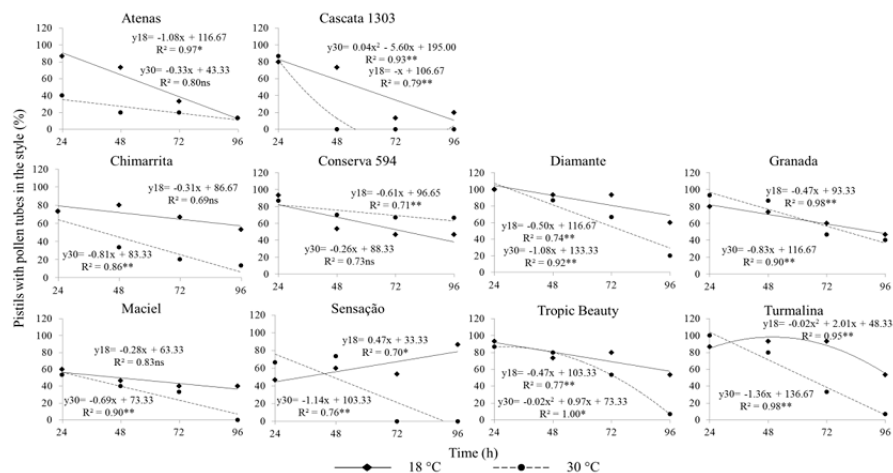


Figure 9

Percentage of pistils with pollen tubes in the style of peach flowers (%) at 18°C and 30°C for 24 to 96h during 2014. ns, \*\*, \* Nonsignificant or significant at  $p \leq 0.01$  or  $p \leq 0.05$ , respectively.

The most negatively affected genotypes at 30°C compared to 18°C regarding the percentage of pistils with pollen tubes in the transmitting tissue of style were 'Cascata 1303', 'Chimarrita', 'Conserva 594', 'Diamante', 'Maciel', 'Tropic Beauty', and 'Turmalina'. Whereas the less affected types were 'Atenas', 'Granada' and 'Sensação'.

In the same manner as observed for adherence and germination, in general, the 30°C treatment was more harmful to pollen tube penetration in the style, and it also seems that this parameter was more sensitive to heat; the decrease in the percentage of flowers with pollen tubes in the style seems to occur more rapidly and to a greater extent than the decrease that occurs in terms of pollen adherence and germination.

Studies regarding floral receptivity indicate that pollen adherence and germination are less sensitive to high temperature than pollen growth in the style and into the ovary. The loss of stigmatic receptivity occurs first with the loss of the capacity of pollen tubes to penetrate into the style, followed by the loss of the capacity of the flower stigma to support the pollen germination, and finally the loss of the stigma capacity to support the pollen adherence. This behavior has also been observed in others' studies of peach (Hedhly et al., 2005) and olives (Vuletin Selak et al., 2013).

The highest sensitivity of pollen tube penetration to heat was also verified in the present experiment. The genotypes suffered a major loss in the presence of pollen tubes in the style, followed by a reduction in pollen germination and the adherence of pollen in the stigma of the flower. For example, selection 'Cascata 1303', particularly during 2012 and 2014, after 72 and 48h of heat exposure, although it had no pollen tubes in the transmitting tissue of the style, pollen germination was still observed and to a greater degree pollen adherence. The cultivars 'Chimarrita', 'Tropic Beauty', and 'Turmalina' and the selection 'Conserva 594' also showed similar behavior.

The genotypes 'Chimarrita' and 'Cascata 1303' at 72h had already showed very few or no pistils with pollen tubes in the style, particularly during 2012 and 2014, indicating a high susceptibility to elevated temperature. This sensitivity can explain why 'Chimarrita', although considered a low chill (200-300h  $\leq$  7.2°C) peach in Brazil, is not cultivated in peach-producing regions in which the temperature is high during the flowering time, such as Southeast Brazil.

Other cultivars such as 'Tropic Beauty' and 'Turmalina' also showed a reduction in the percentage of pistils with pollen tubes in the style; however, only after 72h of exposure to 30°C.

Although most of the studied genotypes suffered when exposed to high temperature to some extent, some genotypes such as 'Atenas', 'Diamante' (2011 and 2012), 'Conserva 594' (2011 and 2014), and 'Tropic Beauty' showed some tolerance to heat stress. In addition, although they showed a reduction in the percentage of flowers with the presence of pollen tubes in the style for at least 2 years, the main decrease occurred only after the pistils had remained in this condition for 96h subject to the higher temperature.

‘Sensação’ also appears to show some degree of tolerance to high temperature because during 2 (2011 and 2012) out of the 3 years of evaluation, it did not show any signs of having undergone negative heat action during any of the steps of stigmatic receptivity.

A different behavior from other genotypes was observed in the cultivar ‘Granada’, which basically behaved in the same manner during all of the studied years for all parameters and decreased during all of the evaluation for both temperatures over time. However, there are literature reports that this cultivar shows a reduction in effective fruiting when high temperatures occur during the flowering period (Couto et al., 2010). Thus, it is not possible to attribute this poor fruitification to the influence of heat on the stigma receptivity of this cultivar, which suggests that egg or pollen viability and their sensitivity to heat may also be factors in the determination of the fruit set of this cultivar as is supported by a study by Nava et al. (2009). This study indicated that high temperature decreased the viability of embryo sacs and lowered the production of normal pollen grains in the cultivar ‘Granada’.

In the present study, we determined that a temperature of 30°C negatively affected stigmatic receptivity (pollen adherence and germination and pollen tube growth), particularly in some genotypes, such as ‘Cascata 1303’, ‘Conserva 594’, ‘Chimarrita’, ‘Maciel’, ‘Tropic Beauty’, and ‘Turmalina’. Considering that the loss of the capacity of pollen tube penetration in the style transmitting tissue occurs first and that no fecundation is possible without it, these genotypes are more susceptible to high temperature, even if they still have pollen adherence and germination. However, ‘Atenas’, ‘Granada’, and ‘Sensação’ seem to have some degree of heat tolerance.

For all the evaluated parameters, differences in the behavior of the genotypes among the studied years were found. Differences in stigmatic receptivity for the same genotypes among years have also been found in cherry (Kodad & Socias i Company, 2013). The differences in our study were probably because of differences in climatic conditions during the years and the pre-flowering conditions as the plants were maintained in the field under natural conditions, which emphasizes the importance of a knowledge of the materials to be grown in a specific climatic area.

## Conclusion

The stigmatic receptivity was more injured at 30°C in comparison to that at 18°C. The pollen tube in the stigma was the most heat-sensitive among the studied parameters. Most of the genotypes showed a reduction in pollen tubes in the style over 72h at 30°C. ‘Cascata 1303’, ‘Conserva 594’, ‘Chimarrita’, ‘Maciel’, ‘Tropic Beauty’, and ‘Turmalina’ were the most-heat susceptible genotypes. ‘Atenas’, ‘Granada’, and ‘Sensação’ showed some degree of heat tolerance.



## Acknowledgements

The authors acknowledge the Capes financial support

## References

- Couto, M., Raseira, M. C. B., Herter, F. G., & Silva, J. B. (2010). Influence of high temperatures at blooming time on pollen production and fruit set of peach “Maciel” and “Granada”. *Acta Horticulturae*, 872(1), 225-230. DOI: 10.17660/ActaHortic.2010.872.30
- Ferreira, D. F. (2011). SISVAR: A computer statistical analysis system. *Ciencia e Agrotecnologia*, 35(6), 1039-1042. DOI: 10.1590/S1413-70542011000600001
- Hatfield, J. L., & Prueger, J. H. (2015). Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes*, 10(1), 4-10. DOI: 10.1016/j.wace.2015.08.001
- Hatfield, J., Boote, K., & Kimball, B. (2011). Climate impacts on agriculture: implications for crop production. *Agronomy Journal*, 103(2), 351-370. DOI: 10.2134/agronj2010.0303
- Hedhly, A. (2011). Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environmental and Experimental Botany*, 74(1), 9-16. DOI: 10.1016/j.envexpbot.2011.03.016
- Hedhly, A., Hormaza, J. I., & Herrero, M. (2005). The effect of temperature on pollen germination, pollen tube growth, and stigmatic receptivity in peach. *Plant Biology*, 7(5), 476-483. DOI: 10.1055/s-2005-865850
- Hedhly, A., Hormaza, J. I., & Herrero, M. (2009). Global warming and sexual plant reproduction. *Trends in Plant Science*, 14(1), 30-36. DOI: 10.1016/j.tplants.2008.11.001
- Hiscock, S. J., & Allen, A. M. (2008). Diverse cell signalling pathways regulate pollen-stigma interactions: The search for consensus. *New Phytologist*, 179(2), 286-317. DOI: 10.1111/j.1469-8137.2008.02457.x
- IPCC. (2015). *Climate Change 2014: Synthesis Report*. Geneva, SW: IPCC.
- Johansen, D. A. (1940). *Plant microtechnique*. New York and London: McGraw-Hill Book Company Inc
- Kodad, O., & Socias i Company, R. (2013). Flower age and pollenizer could affect fruit set in late-blooming self-compatible almond cultivars under warm climatic conditions. *Scientia Horticulturae*, 164(1), 359-365. DOI: 10.1016/j.scienta.2013.09.049
- Kodad, O., Messaoudi, Z., Mamouni, A., Lahlou, M., & Socias, R. (2013). Stigma receptivity is limiting fruit set in almond in warm climates. *Acta Horticulturae*, 976(1), 325-332. DOI: 10.17660/ActaHortic.2013.976.44
- Losada, J. M., & Herrero, M. (2012). Arabinogalactan-protein secretion is associated with the acquisition of stigmatic receptivity in the apple flower. *Annals of Botany*, 110(3), 573-584. DOI:10.1093/aob/mcs116
- Nava, G. A., Dalmago, G. A., Bergamaschi, H., Paniz, R., Santos, R. P., Marodin, G. A. B. (2009). Effect of high temperatures in the pre-blooming and blooming periods on ovule formation, pollen grains and yield of

- 'Granada' peach. *Scientia Horticulturae*, 122(1), 37-44. DOI: 10.1016/j.scienta.2009.03.021
- Rodrigo, J., & Herrero, M. (2002). Effects of pre-blossom temperatures on flower development and fruit set in apricot. *Scientia Horticulturae*, 92(2), 125-135. DOI: 10.1016/S0304-4238(01)00289-8
- Snider, J. L., & Oosterhuis, D. M. (2011). How does timing, duration, and severity of heat stress influence pollen-pistil interactions in angiosperms? *Plant Signaling & Behavior*, 6(7), 930-933. DOI: 10.4161/psb.6.7.15315
- Snider, J. L., Oosterhuis, D. M., Skulman, B. W., & Kawakami, E. M. (2009). Heat stress-induced limitations to reproductive success in *Gossypium hirsutum*. *Physiologia Plantarum*, 137(2), 125-138. DOI: 10.1111/j.1399-3054.2009.01266.x
- Souza, F. B. M., Pio, R., Barbosa, J. P. R. A. D., Reighard, G. L., Tadeu, M. H., & Curi, P. N. (2017a). Adaptability and stability of reproductive and vegetative phases of peach trees in subtropical climate, *Acta Scientiarum Agronomy*, 39(4), 427-435. DOI: 10.4025/actasciagron.v39i4.32914
- Souza, F. B. M., Pio, R., Tadeu, M. H., Zambon, C. R., & Reighard, G. L. (2017b). Boric acid in germination of pollen grains and fruit set of peach cultivars in subtropical region. *Revista Ciência Agronômica*, 48(3), 496-500. DOI: 10.5935/1806-6690.20170058
- Vuletin Selak, G., Perica, S., Goreta Ban, S., & Poljak, M. (2013). The effect of temperature and genotype on pollen performance in olive (*Olea europaea* L.). *Scientia Horticulturae*, 156(1), 38-46. DOI: 10.1016/j.scienta.2013.03.029
- Wilson, J. A., & Brown, S. O. (1957). Differential staining of pollen tubes in grass pistils. *Agronomy Journal*, 49(4), 220-222. DOI: 10.2134/agronj1957.00021962004900040018x
- Zanandrea, I., Raseira, M. C. B., Santos, J., & Silva, J. B. (2011). Receptividade do estigma e desenvolvimento do tubo polínico em flores de pessegueiro submetidas à temperatura elevada. *Ciencia Rural*, 41(12), 2066-2072. DOI: 10.1590/S0103-84782011001200005
- Zinn, K. E., Tunc-Ozdemir, M., & Harper, J. F. (2010). Temperature stress and plant sexual reproduction: Uncovering the weakest links. *Journal of Experimental Botany*, 61(7), 1959-1968. DOI: 10.1093/jxb/erq053

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