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The Effect of Heat Stress on the Reproductive Structures of Peach

S. Carpenedo[1], M.C.B. Raseira[1,3], D.H. Byrne[2], and R.C. Franzon[1]

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Abstract

As in other areas of the world, global warming is also a reality in Southern Brazil, where the occurrence of temperatures above 25°C prior to blooming is becoming common, which is detrimental to the production of temperate climate fruit species. The aim of this work was to evaluate the effect of 30°C, during blooming, on pistil length, pollen number and viability of peach genotypes. Different genotypes as well as male and female parts of the flowers, responded differently to temperature. Among the assayed genotypes, 'BR1', 'Chimarrita', 'Tropic Beauty' and 'Atenas' showed higher tolerance to the high temperature condition.

In warm geographic zones, high temperature is the main environmental stress that limits growth, metabolism, and plant productivity worldwide (Hasanuzzaman et al., 2013). The most sensitive phase of plant development to extreme temperatures that dramatically affects the productivity of grains, vegetables and fruit crops is the flowering stage. As the flower is the organ that develops into a fruit, abiotic stress affects its capacity for fruit and seed production, leading to productivity loss (Hedhly, 2011). Very low temperatures during winter can damage buds by freezing, while high temperatures during pre-flowering and flowering leads to poor flower quality, a shortened flowering period and reduced effective pollination period (Hedhly et al., 2005). Poor fruit set is a serious problem for peach production under tropical and subtropical climatic conditions mainly due to warm temperatures during dormancy and bloom (Kozai et al., 2004). The reduced number of chilling hours associated with mild winter conditions, results in abnormal shoot growth patterns and poor plant development of temperate climate fruit trees in these regions. In addition, high temperatures, especially those above 25°C, before and during bloom can cause poor fruit set and low productivity.

Studies involving sexual reproduction are difficult because gamete development and fertilization are complex processes that occur in a short period of time and are mostly hidden by flower tissues (Zinn and Harper, 2010). Nevertheless, it is important to understand the effect of temperature on the reproductive phase of peach, since maximum temperatures above 25°C during the pre-flowering and flowering phases have been observed in peach production areas of Brazil.

The objective of this study was to evaluate the effect of two different temperatures during the pre-flowering stage on pistil length, number of pollen grains per anther (NPGA), and pollen viability in different peach genotypes.

Materials and Methods

The experiment was carried out over a three-year period (2011, 2012 and 2014) at Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil (2013 was not included due to data loss). Twelve peach genotypes

[1] Embrapa Clima Temperado, BR 392, Km 78, Caixa Postal 403, CEP 96010-971 Pelotas, RS, Brazil
[2] Texas A&M University, Department of Horticultural Sciences, College Station, TX, USA
[3] Corresponding author, Email: maria.bassols@embrapa.br
[*] The authors acknowledge the CNPq and Capes financial support.

Table 1. Chill hours (CH), average full bloom (FBD) and harvest dates (HD), average cycle (C), flesh color (FC) and purpose (PUR) of 12 peach genotypes, when grown in Pelotas, RS, Brazil.

Genotype	CH [x]	FBD	HD	C[z]	FC	PUR
Atenas	250	05/08	21/11	108	Y	D
Aurora 1	< 50 [y]	22/07	20/11	120	Y	F
BR 1	< 300	22/08	06/12	116	W	F
Cascata 1303	< 250	05/08	14/11	101	W	F
Chimarrita	350	22/08	04/12	104	W	F
Conserva 594	< 250	25/07	06/12	134	Y	P
Diamante	200	06/08	06/12	122	Y	D
Granada	300	12/08	18/11	108	Y	P
BRS Libra	< 200	10/07	24/10	106	Y	P
Maciel	< 300	27/07	10/12	135	Y	D
Tropic Beauty	< 50 [y]	18/07	09/11	114	Y	F
Turmalina	350	03/08	22/11	111	Y	P

[x] Chilling requirement in hours below 7.2°C (CH) data from the Embrapa peach breeding program.
[y] As reported by Pedro Junior et al., (2007).
[z] Average cycle calculated based on number of days from full bloom to harvest date (C); Flesh color yellow (Y) or white (W); Dual purpose (D) (processing and fresh), fresh market (F), or Processing (P).

were used in this study (Table 1). They were grafted on 'Aldrighi' peach rootstock and established in pots. All genotypes are from the peach breeding program of Embrapa except 'Tropic Beauty' which was released in a partnership between Texas A&M University and the University of Florida , and 'Aurora 1', which originated from the Instituto Agronômico de Campinas breeding program, São Paulo, Brazil. 'Tropic Beauty' was chosen because of its adaptation to warm areas, 'Aurora 1' was developed for planting in subtropical areas, Cascata 1303 and Conserva 594 are selections from the Embrapa breeding program, considered as very low chill and being adapted to subtropical regions like 'Turmalina'. The other tested cultivars are largely planted in Southern Brazil.

Before bud swelling (June), eight to 10 plants of each genotype, were placed in a cold room at 4°C, 70% average humidity and no light, for 360 h, aiming to accumulate enough chill hours (hours below 7.2°C) for dormancy completion of all the genotypes. After this period, plants were kept in a greenhouse at 14°C, until buds reached the desirable flowering stage. It is interesting to note that due to genetic differences, the phenological behavior of the genotypes were different so the temperature treatments started at different times for each genotype. When most of the buds in each genotype began to swell, or reached the B stage, according to the Baggiolini scale (Baggiolini, 1952), four to five plants of that genotype were placed in a heat chamber at 30°C, for 48 h, in absence of light, whereas others remained at 14°C in a greenhouse under natural light. Both environments were kept at 70% relative humidity. After the 48 h in the heat chamber, the plants were returned to the greenhouse (with natural light) until bloom. Four replications of five flowers recently opened were randomly collected from each genotype and treatment, and in random positions of the plant, and their pistil lengths were measured in mm, with a ruler.

For number of pollen grains per anther (NPGA), the experimental design was completely randomized with four replications and five flowers per plot. These flowers were randomly collected from the plants exposed

to 14°C or 30°C. From each plot, five anthers were detached, giving a total of 25 anthers per replication. The anthers were placed in vials and, when they were dry, 1 ml of lactic acid was added to the vial. The number of pollen grains per anther (NPGA) was counted according to Tuite (1969), using a Neubauer chamber.

For in vitro pollen viability, remaining anthers of the same flowers used for NPGA were removed and dried on a piece of paper, at room temperature for two days. Immediately after drying, the viability was measured by scattering the pollen on a solidified germination medium (sucrose 10%, agar 1% dissolved in distilled water) on slides adapted to this purpose, and left to germinate during three hours at 24°C (Couto et al., 2010). The pollen was considered germinated when the pollen tube length exceeded the pollen grain size.

NPGA had two years of data whereas the other parameters were observed for three years.

For statistical analysis, NPGA and pollen viability data were transformed to the proportion of the square root of the arc sin respectively. The experimental design was completely randomized with a 12 x 2 factorial treatment structure (genotype-temperature) with four replications. Data were analyzed by analysis of variance (ANOVA), and means were compared by Scott-Knott test using the SISVAR statistical software (Ferreira, 2011).

Results and Discussion

Significant genotype-temperature interaction was observed for NPGA in 2011 and 2012 (Table 2). Two cultivars, Tropic Beauty and Chimarrita, were not affected either year whereas the selections Cascata 1303, Conserva 594 and 'BRS Libra' had lower NPGA when the plants were exposed to the 30°C temperature, compared to 14°C, for both years of evaluation. The selection Conserva 594 had the highest reduction, 52.9% and 68.8% in 2011 and 2012, respectively. Other cultivars had reduced NPGA in only one of the two years. These included 'Diamante'

Table 2. Number, percentage loss and average number of pollen grains per anther for 12 peach genotypes exposed to 14ºC and 30ºC, during pre-bloom in Years 2011 and 2012, Pelotas, RS, Brazil.

	2011				2012			
Genotype	14 °C	30 °C	Loss (%)	Average	14 °C	30 °C	Loss (%)	Average
Atenas	800 bA[z]	560 bA	30.0	680 b	1260 bA	530 cB	57.9	895 c
Aurora 1	570 bA	400 bA	29.8	485 b	1220 bA	800 bB	34.4	1010 b
BR 1	840 bA	1240 aA	- 47.6	1040 a	1850 aA	1420 aB	23.2	1635 a
Cascata 1303	1090 aA	610 bB	44.0	850 b	1340 bA	790 bB	41.0	1065 b
Chimarrita	780 bA	570 bA	26.9	675 b	210 eA	190 eA	9.5	200 d
Conserva 594	1020 aA	480 bB	52.9	750 b	160 eA	50 gB	68.8	105 e
Diamante	980 aA	400 bB	59.2	690 b	170 eA	260 eA	- 52.9	215 d
Granada	930 aA	490 bB	47.3	710 b	130 eB	250 eA	- 92.3	190 d
BRS Libra	1470 aA	960 aB	34.7	1215 a	670 dA	400 dB	40.3	535 d
Maciel	980 aA	1030 aA	- 5.1	1005 a	230 eA	60 gB	73.9	145 e
Tropic Beauty	1270 aA	840 aA	33.9	1055 a	910 cA	700 bA	23.1	805 c
Turmalina	1120 aA	370 bB	67.0	745 b	175 eA	140 fA	20.0	157 d
Average	988 A	663 B	31.1	825.0	694 A	466 B	20.6	579.8
CV (%)	17,2				13,3			

[z] Means followed by the same lowercase letters in the colum and uppercase letters in the row do not differ by Scott-Knott test at $p<0.05$. Mean comparisons were made only within years.

(59.2%), 'Granada' (47.3%) and 'Turmalina' (67.0%) with decreases in NPGA in 2011, and 'Atenas' (57.9%), 'Aurora 1' (34.4%), 'BR1' (23.2%) and 'Maciel' (73.9%), in 2012. Interesting to note that 'Granada' and 'Diamante' had higher NPGA at 30°C, in 2012.

'BR 1' had a reduction in 2012, for plants exposed to 30°C, but still had the highest pollen production among the evaluated genotypes.

The production and germination of pollen is affected by both genetic and environmental factors (Camposeo et al., 2008; Mert, 2009). Differences in pollen grain production among years were found in other *Prunus* species such as sour cherry (Davarynejad et al., 2008), peach (Nava et al., 2009) and apricot (Gallotta et al., 2014). The NPGA differences between years could also be due to the pretreatment conditions of the potted plants (which were grown outside before the cold room treatment, thus exposed to natural conditions). The temperatures (maximum, average and minimum) in May, were higher in 2012 than in 2011 (Table 3).

High temperatures during dormancy to the pre-bloom period can negatively influence the production of pollen grains or lead to male gametophyte sterility (Kozai et al., 2004). In our case, May temperatures in 2012 were warmer than in 2011, by 2.7°C, 1.6°C and 0.7°C for the maximum, average and minimum temperature respectively.

Genotype-temperature interaction was significant for pollen viability for the three studied years (Table 4). 'Atenas' and 'BR1' were not negatively affected by exposure to 30°C, whereas 'Aurora 1', 'BRS Libra', 'Maciel', 'Turmalina' and the selection Cascata 1303 had pollen viability reduced in two out of three years, indicating that these genotypes were more sensitive to high temperatures. A similar temperature effect on pollen germination with varied cultivar response was reported for citrus (Distefano et al., 2012) and strawberry (Ledesma and Sugiyama, 2005).

The most adapted cultivars to subtropical-tropical climates should produce 1000-2000 pollen grains per anther with a viability ranging usually from 60% to 95% (Barbosa et al., 1989). Only 'BR1' fulfilled these requirements. In general, an average percentage of germination over 50%, regardless of the year and temperature, is considered satisfactory (Scorza and Sherman, 1995). None of genotypes exposed to high temperature treatment had pollen viability lower than 50% for the three years of evaluation, except 'Diamante' and 'Turmalina', with the latter one in two out of three years of study. 'Diamante' did not have reduced pollen viability from the temperature stress in the second and third years of evaluation, but had the lowest average viability among the studied genotypes in all years except 'Chimarrita' in 2012.

This fact did not appear to have much consequence since a single pollen grain can fertilize the ovule. However, it may also indirectly serve as an indicator of higher or lower tolerance of genotypes to high temperatures at the pre-bloom stage.

Table 3. Average maximum (Max.), medium (Med.) and minimum (Min.) temperature (°C) occurred on May, June, July and August of 2011, 2012, and 2014 at the Embrapa Clima Temperado, Pelotas, RS, Brazil.

	Average Temperature (°C)[z]								
	May			June			July		
Year	Max.	Med.	Min.	Max.	Med.	Min.	Max.	Med.	Min.
2011	19.0	14.4	10.9	15.6	11.7	8.3	14.9	10.6	7.1
2012	21.7	16.0	11.6	17.2	12.0	7.7	14.5	9.7	5.5
2014	19.9	15.6	12.3	12.3	18.0	14.4	18.7	14.3	11.2

[z] Data collected from the Agrometeorological Station of Embrapa Clima Temperado, Pelotas, RS, Brazil.

Table 4. Pollen viability (%) and average pollen viability for 12 peach genotypes exposed to 14ºC and 30ºC during pre-blooming time in the years of 2011, 2012 and 2014, Pelotas, RS, Brazil.

Genotype	2011			2012			2014		
	14 °C	30 °C	Average	14 °C	30 °C	Average	14 °C	30 °C	Average
Atenas	29 dB[z]	55 bA	42 c	63 aA	67 aA	65 b	77 bA	73 aA	75 a
Aurora 1	89 aA	54 bB	72 b	62 aA	55 bA	59 b	85 aA	74 aB	80 a
BR 1	74 bA	63 bA	69 b	78 aA	75 aA	77 a	48 dA	60 aA	54 c
Cascata 1303	84 bA	56 bB	70 b	76 aA	76 aA	76 a	84 aA	72 aB	78 a
Chimarrita	87 bA	40 cB	64 b	29 bA	36 bA	33 d	67 bA	62 bA	65 b
Conserva 594	80 bA	46 cB	63 b	37 bA	49 bA	43 c	61 cA	68 bA	65 b
Diamante	51 cA	28 cB	39 c	39 bA	31 bA	35 d	35 eA	30 cA	33 d
Granada	89 aA	56 bB	73 b	48 bA	51 bA	50 c	74 bA	65 bA	70 b
BRS Libra	88 aA	84 aA	86 a	71 aA	50 bB	61 b	90 aA	67 bB	79 a
Maciel	82 bA	67 bB	75 b	75 aA	50 bB	63 b	79 bA	75 aA	77 a
Tropic Beauty	84 bA	62 bB	73 b	55 bA	49 bA	52 c	78 bA	72 aA	75 a
Turmalina	89 aA	39 cB	64 b	70 aA	44 bB	57 b	72 bA	78 aA	75 a
Average	77.2 A	54.2 B	66	58.6 A	52.8 B	56	70.8 A	66.3 B	69
CV(%)	10.9			16.4			11.5		

[z] Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ by Scott-Knott test at $p \leq 0.05$. Mean comparisons were made only within years.

Table 5. Pistil length and average pistil length (cm) of peach genotypes exposed to the temperatures of 14ºC and 30ºC, during pre-blooming time in the years 2011, 2012 and 2014, Pelotas, RS, Brazil.

Genotype	2011			2012			2014		
	14 °C	30 °C	Average	14 °C	30 °C	Average	14 °C	30 °C	Average
Atenas	1.49 aA[NS]	1.37 aA	1.43 a	1.91 aA[z]	1.94 aA	1.93a	1.73 aA	1.67 aA	1.7 a
Aurora 1	1.37 aA	1.26 aA	1.32 a	1.63 cA	1.51 cA	1.57 c	1.26 cA	1.32 cA	1.29 d
BR 1	1.57 aA	1.52 aA	1.55 a	1.59 cA	1.28 dB	1.44 c	1.60 aA	1.31 cB	1.46 c
Cascata 1303	1.48 aA	1.52 aA	1.50 a	1.71 bA	1.74 bA	1.73 b	1.57 aA	1.45 bB	1.51 b
Chimarrita	1.36 aA	1.25 aA	1.31 a	1.45 cA	1.57 cA	1.51 c	1.47 bA	1.47 bA	1.47 c
Conserva 594	1.52 aA	1.61 aA	1.57 a	1.45 cA	1.58 cA	1.52 c	1.47 bA	1.50 bA	1.49 c
Diamante	1.51 aA	1.15 aA	1.33 a	1.58 cA	1.42 cA	1.50 a	1.45 bA	1.36 cA	1.41 c
Granada	1.31 aA	1.17 aA	1.24 a	1.64 cA	1.47 cA	1.56 c	1.46 bA	1.35 cA	1.41 c
BRS Libra	1.47 aA	1.34 aA	1.41 a	1.55 cA	1.50 cA	1.53 c	1.63 aA	1.45 bB	1.54
Maciel	1.59 aA	1.35 aA	1.47 a	1.92 aA	1.73 bB	1.83 c	1.57 aA	1.56 aA	1.57 b
Tropic Beauty	1.53 aA	1.51 aA	1.52 a	1.65 cA	1.62 bA	1.64 c	1.53 aA	1.60 aA	1.57 b
Turmalina	1.54 aA	1.52 aA	1.53 a	1.76 bA	1.65 bA	1.71 b	1.64 aA	1.48 bB	1.56 b
Average	1.48 A	1.37 B		1.65 A	1.58 B		1.53 A	1.46 B	
CV (%)	19.6			7.8			5.7		

[NS] Non significant at $p \leq 0.05$.

[z] Means followed by the same lowercase letters in the column and uppercase letters in the row do not differ by Scott-Knott test at $p \leq 0.05$. Mean comparisons were made only within years.

For pistil length, the genotype-temperature interaction was significant for the years 2012 and 2014 but not for 2011 (Table 5). Overall the 30°C treatment caused a shortening of the pistils, in this study. This shortening along with abnormal development of ovarian tissue was also observed in apricot during the last week of flower development when temperature was increased (Rodrigo and Herrero, 2002), and may be related to an acceleration of anthesis (Zinn et al., 2010) which does not allow the reproductive structures to completely develop before the flower opens.

In our study, pistil length of 'Atenas', 'Aurora 1', 'Chimarrita', Conserva 594, 'Diamante', 'Granada' and 'Tropic Beauty' were not negatively affected by high temperature.

Analyzing the data together, for the male flower parts there was no reduction in NPGA for 'Chimarrita' and 'Tropic Beauty', and the cultivars Atenas and BR1 had no reduction in pollen viability, when plants were exposed to 30°C for 48 hours. 'BR1', even with the reduction in the number of pollen grains per anther, in 2012, produced more pollen grains than those produced by other genotypes.

For the female part of the flower evaluated, in this case the pistil length, genotypes not negatively affected by high temperature were 'Atenas' 'Aurora 1', 'Chimarrita', Conserva 594, 'Diamante', 'Granada' and 'Tropic Beauty'. However, there are other important variables not considered in this study such as stigma receptivity and ovule longevity, among others.

Genotypes that were superior to the others in at least two of the variables studied were 'Chimarrita', 'Atenas', and 'Tropic Beauty'.

Overall, there was a reduction in number of pollen grains per anther, pollen viability, and pistil length for plants subjected to 30°C as compared to those maintained at 14°C. However, peach genotypes differed dramatically in their responses with the most tolerant of the genotypes assayed, being 'BR1', 'Chimarrita', 'Tropic Beauty' and 'Atenas'. In spite of the differences between years, this indicates that it is possible to develop peach cultivars with enhanced tolerance to high temperatures during blooming.

Literature Cited

Baggiolini, M. 1952. Stades Repères Du Pêcher. Revue Romande D'agriculture, de Viticulture et D'arboriculture 4: 32–33.

Barbosa,W., F.A. Campo-Dall'orto, and M. Ojima. 1989. Comportamento vegetativo e reprodutivo do pessegueiro IAC Tropical. Campinas, Instituto Agronômico, (boletim científico).

Camposeo, S., G. Ferrara, M. Palasciano, and A. Godini. 2008. Varietal Behaviour according to the Superintensive Oliveculture Training System. Acta Hort. 791:271–74.

Couto, M., M.C.B. Raseira, F.G. Herter, and J.B. Silva. 2010. Influence of High Temperatures at Blooming Time on Pollen Production and Fruit Set of Peach 'Maciel' and 'Granada'. Acta Hort. 872:225-230.

Davarynejad, G. H., Z. Szabó, J. Nyéki, and T. Szabó. 2008. Phenological Stages, Pollen Production Level, Pollen Viability and in Vitro Germination Capability of Some Sour Cherry Cultivars. Asian J. of Plant Sci. 7:672-676.

Distefano, G., A. Hedhly, G. Las Casas, S. La Malfa, M. Herrero, and A. Gentile. 2012. Male-Female Interaction and Temperature Variation Affect Pollen Performance in Citrus. Sci. Hort. 140:1–7.

Ferreira, D.F. 2011. Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia. 35:1039-1042.

Gallotta, A., M. Palasciano, A. Mazzeo, and G. Ferrara. 2014. Pollen Production and Flower Anomalies in Apricot (*Prunus Armeniaca L.*) Cultivars. Sci. Hort. 172:199–205.

Hasanuzzaman, M., K. Nahar, M. M. Alam, R. Roychowdhury, and M. Fujita. 2013. Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants. Intl. J. Mol. Sci. 14:9643-9684.

Hedhly, A., J.I. Hormaza, and M. Herrero. 2005. The Effect of Temperature on Pollen Germination, Pollen Tube Growth, and Stiggmatic Receptivity in Peach. Plant Biol. 7:476–83.

Hedhly, A. 2011. Sensitivity of Flowering Plant Gametophytes to Temperature Fluctuations. Environ. Expt. Bot. 74:9–16.

Kozai, N., K. Beppu, R. Mochioka, U. Boonprakob, S. Subhadrabandhu, and I. Kataoka. 2004. Adverse Effects of High Temperature on the Development of Reproductive Organs in 'Hakuho' Peach Trees. J. Hort. Sci. and Biotechnol. 79: 533–537.

Ledesma, N. and N. Sugiyama. 2005. Pollen Quality and Performance in Strawberry Plants Exposed

to High-Temperature Stress. J. Amer. Soc. Hort. Sci.130: 341–347.
Mert, C. 2009. Temperature Responses of Pollen Germination in Walnut (*Juglans Regia L* .). J. Biol. Environ. Sci. 3:37-43.
Nava, G.A., G.A. Dalmago, H. Bergamaschi, R. Paniz, R. P. dos Santos, and G.A.B. Marodin. 2009. Effect of High Temperatures in the Pre-Blooming and Blooming Periods on Ovule Formation, Pollen Grains and Yield of 'Granada' Peach. Sci Hort. 122:37-44.
Pedro, Jr., M.J., W. Barbosa, G. de S. Rolim, and J. L. de Castro. 2007. Época de florescimento e Horas de frio para pessegueiros e nectarineiras. Revista Brasileira de Fruticultura. 29:425-430.
Rodrigo, J. and M. Herrero. 2002. Effects of Pre-Blossom Temperatures on Flower Development and Fruit Set in Apricot. Scientia Hort. 92:125–135.
Scorza, R. and W. B. Sherman. 1995. Peaches, p. 325-440. In: J. Janik and J.N. Moore (eds). Fruit Breeding. I. John Wiley and sons, INC., New York.
Tuite, J. 1969. Use the Spencer Hematocytmeter, p.182-185. In: J. Tuite (ed.). Plant Pathological Methods Fungi and Bacteria. Burgess Publishing Company, Minneapolis.
Zinn, K.E., M. Tunc-Ozdemir, and J. F. Harper. 2010. Temperature Stress and Plant Sexual Reproduction: Uncovering the Weakest Links. J. Ex. Bot. 61: 1959–1968.

About The Cover:

There is rich diversity in the genus *Actinidia*, as show in the cover photograph.Considerable variation exists in internal color as well as size, external color and shape. Breeding for improved size, flavor, color and storage life is progressing but the crop is based on only a very few cultivars internationally. Commercially, kiwifruit was originally reliant on only the green-fleshed type but gold-fleshed cultivars have recently become very popular, especially in Asian markets.

Photo courtesy of Dr A. R. Ferguson,
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