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Characterization of peach [*Prunus persica* (L.) Batsch.] cultivars for frost resistance

Caracterización de cultivares de duraznero [*Prunus persica* (L.) Batsch.] por resistencia a heladas

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Abstract

Spring frosts are one of main crop constraints for temperate fruit trees. Within a species exists variability in damage caused by subzero temperatures on flower organs during breaking winter rest. In five peach [*Prunus persica* (L.) Batsch.] and one nectarine cultivars, field frost damage was assessed, also recording full bloom date and bloom density. Additionally, mean lethal temperature (LT_{50}) of flower buds in the open flower state was determined through laboratory controlled thermal declines. Peach cultivars Maria Bianca and Summer Pearl had higher density of healthy flowers per cm of shoot, after field subzero temperatures. Field frost resistance was related mainly with high bloom density, combined, in some cases, with late bloom. Late bloom did not result in a resistance feature itself. Therefore, to select peach cultivars with less damage risk by subzero temperatures, it is important to consider more than one variable related with reproductive organs.

Key words: Acclimation, freezing, bloom density, phenology, LT_{50}, *Prunus persica*.

Resumen

Las heladas primaverales son una de las principales limitantes de la producción de frutales de clima templado. Dentro de una misma especie existe variabilidad en resistencia frente al daño en órganos florales ocasionado por temperaturas bajo cero durante la salida del reposo invernal. En cinco cultivares de duraznero [*Prunus persica* (L.) Batsch.] y uno de nectarino se evaluó el daño ocasionado por heladas y se determinaron la fecha de plena floración y la densidad de floración. Adicionalmente se determinó la temperatura letal media (TL_{50}) de las yemas florales en el estado de flor abierta, mediante descensos térmicos controlados en laboratorio. Los cultivares (cv) de duraznero Maria Bianca y Summer Pearl presentaron las mayores densidades de flores sanas por cm de ramo, luego de la ocurrencia de temperaturas bajo cero en campo. La resistencia a heladas en campo se relacionó principalmente con la elevada densidad de floración, en combinación, en algunos casos, con floración tardía. La floración tardía por sí sola no resultó una característica de resistencia; por tanto, para la elección de cultivares de duraznero con menor riesgo de daño por temperaturas bajo cero es importante tener en cuenta más de una variable relacionada con los órganos reproductivos.

Palabras clave: Aclimatación, congelación, densidad de floración, fenología, TL_{50}, *Prunus persica.*

Introduction

Distribution and productivity of the plant species are limited, among other factors by extreme temperatures (Fiorino and Mancuso, 2000). The successful behavior of a woody specie on a determined place implies the synchronization of the annual development of resistance to low temperatures with the seasonal changes of them (Lindén, 2002). The spring frosts are one of the main limiting factors for production of temperate fruit trees, however some species have evolved and developed resistance mechanisms, being this one of the most important characteristics to select crops, which can determine the feasibility to get acceptable harvests, reducing the input of caloric energy (Chaar, 2013). Quamme *et al.* (1982) in cultivated and native species of the *Prunus* genera found that the temperature for damage of the most susceptible tissues is strictly associated with the annual average of the minimum temperature in the limits of the geographical distribution of the studied plant materials. Kadir and Proebsting (1994) found that floral buds of 20 *Prunus* species showed different strategies under low temperatures. Even among the same species there is variability in the damage caused by subzero temperatures in floral organs during the breaking of the winter rest (Kodad and Socias i Company, 2005). The mean letal temperature (LT_{50}) is the subzero temperature at which 50% of the floral buds dies (Mathers, 2004). Cold resistance in the reproductive structures depends, among other factors, on the date of full flowering, the LT_{50} and flower density (Chaar, 2013). It is possible to evaluate the relative cold resistance of several plant materials under frost happening in the field (Granger and Rousselle, 1984; Lisek, 2007); therefore, the objective of this research was to evaluate the magnitude of the damage in flower buds and to quantify the variables of resistance of different peach cultivars after frosts in the field after the winter rest breaking in 2013 in Mendoza, Argentina.

Materials and methods

Location and experimental material

The experiments were performed in the National Institute for Agricultural Technology (INTA), Agricultural Experimental Station Junín, province of Mendoza (Argentina) at 33° 6’ 57.5” south, 68° 29’ 4” East, at 653 MASL, in a cultivar collection (CV) of peach for fresh consumption, including the cvs Flavorcrest, Milenio INTA, Maria Bianca, Summer Pearl and Compact Red Haven, and the nectarine cv Zee Gold. From each cultivar three adult plants were evaluated, semipalmete grown in north-south direction and watered by gravity.

Determination of the LT_{50}

At the breaking of the autumn-winter rest of 2013, the LT_{50} was determined in the lab by thermal decreases in a freezer, simulating the occurrence of frost in the field. Since the frost tolerance depends on the developmental state of the buds, to compare the results, the cultivars were evaluated at the same phenological state (Dale, 1987; Kodad *et al.*, 2010). From each evaluated cultivar four mixed branches per cold level were used, each one with 10 or more buds at the ‘F’ state (open flower, with totally expanded petals) (Baggiolini, 1952; Reig *et al.*, 2013). The assays were performed between 13 and 18 September 2012, depending on the availability of 'F' state flowers of each cultivar evaluated. Simultaneously and in each assay were subjected to artificial freezing three cultivars. The branches were collected on the same day of applying cold treatments in the laboratory, and kept in plastic bags. Frost simulation was performed on a freezer trademark Neba with an Ako temperature controller. Because of the importance of simulating the natural frost in the lab assays (Gusta *et al.*, 2003) the temperature was reduced at 2 °C/h rate, removing the different cold treatments after 1 h at constant temperature of –2, –3 and –4 °C.

To prevent air stratification and achieve homogenization of temperature on the volume, a fan Mark Morris (220 V, 3000 rpm and 18 cm in diameter) was added. As control for each cultivar a sample was placed at room temperature. Air temperature in the freezer was recorded during experiments with Hobo model U12 sensor, with an interval of 2 seconds between measurements. After cold treatments, branches remained at room temperature for 16h in the laboratory with water at the base of the bottles to prevent dehydration. For the determination of chilling injury the gynoecium were observed using a Zeiss stereomicroscope model Stemi DV4 (32 X), considering as damaged the tissues with brown coloring (Szalay *et al.*, 2010). The TL_{50} was determined graphically by the vertical projection of the intersection between the damage curve according to temperature and the horizontal line corresponding to 0.5 proportion of damaged gynoecium.

Phenology

The phenology was determined in one branch per cultivar during the breaking of the winter rest of 2013; this was located at the middle height of the east side of the plants with similar exposition to solar radiation. For the phenology recording the number of floral buds corresponding to each stage of the Baggiolini scale (1952) (Reig*et al.*, 2013), expressing the results of each date as percentages. The date of full flowering of each cultivar was when 50% of the floral buds were at the 'F' state.

Flower density

The number of flower buds was determined at the end of winter 2013 by taking six branches – three of each side, west and east- per cultivar (two in each one of three plants). For the calculation of the flowering density the number of flower buds was divided by the length of the branch.

Recording of the frosts in the field and evaluation of the damage caused

At the end of winter rest of 2013, the air temperature was recorded every 30 minutes with an I-Button digital sensor, placed in a weather shelter inside the crop frame at 1.5 m height. In three plants of each cultivar under study, six branches of the treetop from the east and west sides at 1.5 m height were collected on September 25, 2013. The rating of the damage in each cultivar was made in 20 randomly chosen flower buds in different growth stages. To determine the damage the same criteria used in the frost simulation in the lab was used (Szalay *et al.*, 2010). Additionally, the flower type of each cultivar was recorded, highlighting the pinky flower with large and colorful petals and, the bell shape flower with small petals and protruding stamens (UPOV, 2014). For the discussion, the results are expressed as survival (number of healthy flowers, cm of branch), calculated from the percentage of healthy flowers and the flower density.

Experimental design and statistical analysis

To determine the LT_{50} in the lab a completely randomized design was used, with four replicates (branches) per treatment; whereas for the determination of the flower density the same design with six replicates was used. The statistical analysis of the data was performed in with InfoStat/Professional software (Universidad Nacional de Córdoba, 2006). For flower density of the cultivars an analysis of variance was performed and, to calculate the significance of the mean differences, the Duncan ($P < 0.05$) was used. The damages by frost in the field were analyzed by tables of contingency, evaluating 20 flower buds per cultivar according to damage presence or absence.

Results

Mean lethal temperature in the laboratory

Flower damage determination was done with the method proposed by Szalay *et al.* (2010) using a stereomicroscope and allowed the clear differentiation between healthy and damaged gynoecium by temperatures subzero (Figure 1).

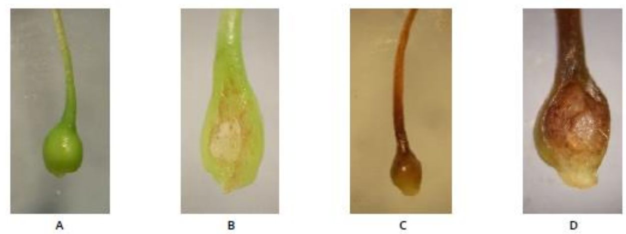

Figure 1. Healthy (A and B) and damaged (C and D) of nectarine Zee Gold cv under the stereomicroscope.

The damaged gynoecium had a brown coloring, whereas the healthy ones stayed green 16 h after the application of the cold treatments. For each temperature level, the proportion of damaged gynoecium varied according to the cultivar. The observed values for LT_{50} in the 'F' state allow to differentiate cultivars by their relative resistance of the flower buds to temperature subzero (Figure 2).

Phenology and flower density

The date of full flowering vary widely between peach and nectarine cultivars evaluated, with an occurrence of 50% flowers open between 9 and 29 September (Table 1).The flower density showed a wide range between the evaluated cultivars (0.25 to 0.55 flowers/cm). The Compact Red Haven and Milenio INTA cvs had the lowest flower density (0.25 and 0.33 flowers/cm, respectively), while the Maria Bianca and Summer Pearl cvs showed the highest values (0.55 and 0.5 flowers/cm, respectively). It should be mentioned that the highest flower density promotes increased production of fruits facing similar percentages of frost damage.

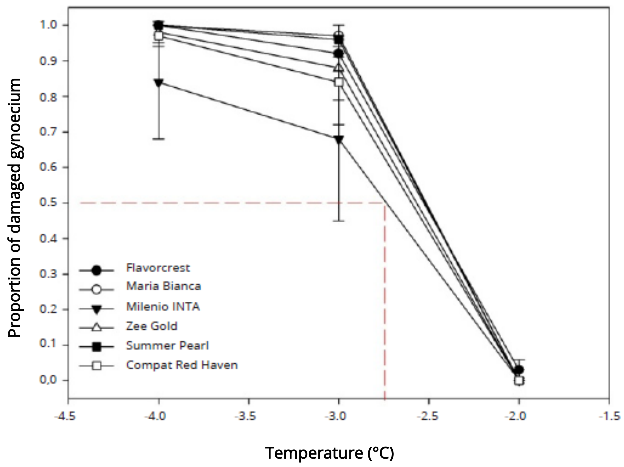

Figure 2. Rate of damaged gynoecium in flowers of evaluated peach and nectarine cultivars.
Experiments in freezer, 2012 (the pointed line indicates the freezing temperature for the 50% damage, LT_{50}).

Table 1. Dates for full flowering and flower density of the peach and nectarine cultivars evaluated during the 2013-2014 season.

Cultivar	Full flowering	Flower density (flowers/cm)	
		Mean	S.D.
Compact Red Haven	19-9	0.25 a*	0.12
Milenio INTA	9-9	0.33 a	0.08
Flavorcrest	9-9	0.35 ab	0.11
Zee Gold	29-9	0.37 ab	0.14
Summer Pearl	11-9	0.5 bc	0.10
Maria Bianca	24-9	0.55 c	0.15

* Values in the same column followed by same letters do not differ statistically (P < 0.05), according to Duncan's test.

Record of frost in the field and damage evaluation

Figure 3 shows the temporal variation of the air temperature in the experimental plot observed during the end of winter rest in the different evaluated peach cultivars. The events of subzero temperatures are shown in Table 2. It was observed that the most severe frosts were also generally more extensive (Figure 4), however the linear regression adjustment was low (R^2=0.33). A relationship between the cultivar and the damage observed in the field was found (P <0.0001), varying between 35 and 100% of the observed floral buds (Table 3). The flowers of peach and nectarine can be rosacea type or bell shape, being the first larger and most colorful (Figure 5). The materials with lower percentage of damage have, mostly, bell shape flowers. The exception was the cv. Milenio INTA with bell shape flowers and 100% of damaged flowers (Figure 6).

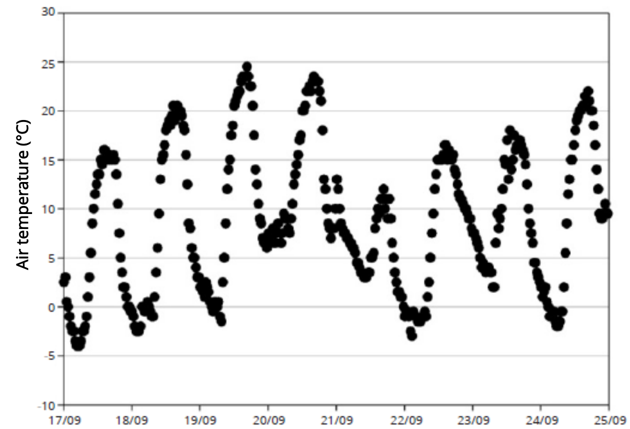

Figure 3. Evolution of the air temperature (ºC) during the frosts in the field. INTA Agricultural Experimental Station Junín, province of Mendoza, 2013.

Table 2. Characterization of the frosts in the field that happened during the breaking of the winter rest of 2013. INTA Agricultural Experimental Station Junín, province of Mendoza.

Date	Minimum temperature (ºC)	Duration of the freezing temperature (h)
17-09	-4	6
18-09	-2.5	8
19-09	-1.5	3
22-09	-3	7.5
24-09	-2	4.5

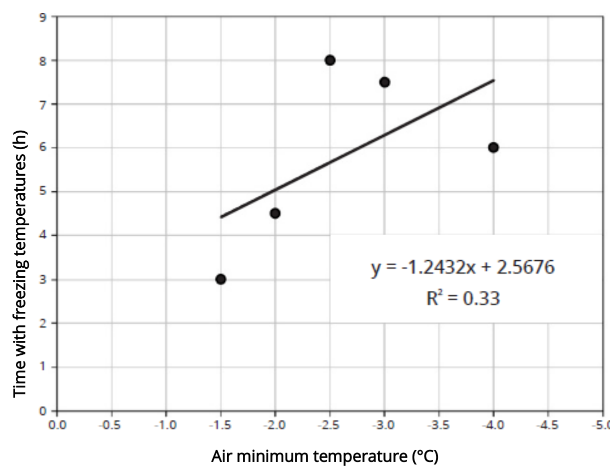

Figure 4. Relationship between the time with freezing temperatures and the air minimum temperature (ºC) reached by the frosts in the field.
Season 2013-2014. INTA Agricultural Experimental Station Junín, province of Mendoza.

Discussion

Phenology and flower density

In the different cultivars of canning peach, Chaar *et al.* (2011) also observed a wide variability in the average date of full flowering, which varied between August, 28 and September, 11.

Table 3. Damage by frosts in the field. Statistics: Pearson Chi-square; value: 30.43; d.o.f: 5; P < 0.0001.

Cultivar	Damage (relative frequency)
Flavorcrest	0.95
Maria Bianca	0.50
Milenio INTA	1.00
Zee Gold	0.75
Summer Pearl	0.55

Figure 5. Flowers of peach of the rosacea (Flavorcrest cv) and of bell shape type (Milenio INTA and Maria Bianca cvs).

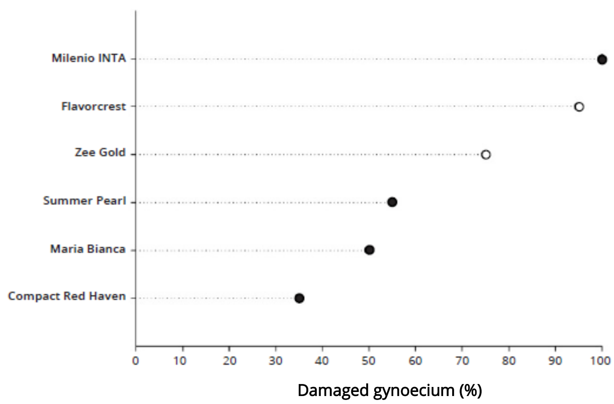

Figure 6. Damage percentage caused by frost in the field on flower buds of peach and nectarine according to cultivar and flower type (• = rosacea; ° = bell shape).
INTA Agricultural Experimental Station Junín, province of Mendoza, 25-9-

Okie and Werner (1996) observed that the variety effect in the flower density in peach and nectarine was strongly higher the environmental and their interaction. Contrary, Kodad and Socias i Company (2008) observed for 3 consecutive years in almond (*Prunus dulcis* [Mill.] D. A. Webb) that the variance due to the year was the main component of the total variance on the flower density.

Mean lethal temperature in the laboratory

Buyukyilmaz and Kester (1976) observed in longitudinal cuts of almond buds and flowers, that the pistil was the most susceptible element and used the brownish coloring as indicator of the death of the reproductive organ. Similarly, Flinn and Ashworth (1994) determined the freezing damage in blueberry buds (*Vaccinium corymbosum* L.) from the black color in the ovary.

The relative location of the different materials in respect to the LT_{50} cannot be stated as general for the other phenological stages. There is evidence of temporal changes in the relative tolerance to freezing temperatures in wild cherry (*Prunus cerasus* L.) (Mathers, 2004), grape (*Vitis vinifera* L.) (Ferguson *et al.*, 2011) and almond (Viti *et al.*, 1994). However, in temperate areas as the productive oasis in Mendoza, the critical temperatures for freezing damages happen at the end of the winter rest, when the plants are no longer acclimated (Szalay *et al.*, 2010), therefore, the LT_{50} parameter during the F state is a good indicator of the relative resistance of flower buds of the different cultivars.

Characterization of the resistance to freezing temperatures

Maria Bianca and Summer Pearl cvs showed the highest values for healthy flowers/cm of branch (flower survival) after the freezing temperatures in the field; whereas María Blanca cv was characterized for high flower density (0.55 flowers/cm) and late flowering, as shown its full flowering in September 24, 2013. The Summer Pearl cv only showed high flower density (0.5 flowers/cm) (Figure 7). The Compact Red Haven showed an acceptable behavior under freezing temperatures (Figure 7) and was associated with a middle LT_{50} (-2.6 °C) (Figure 2) and late flowering, due to its full flowering date September 19, 2013 (Table 1). The low percentage of damage in the field (35%, Table 3) was compensated by its low flower density (0.25 flowers/cm, Table 1).

The Milenio INTA cv was the less resistant with 100% of flowers damaged. Although it showed a lower LT_{50} (high tolerance: -2.75 °C) (Figure 7), its flowering as early (Table 1) together with Flavorcrest cv, also with high damage percentage (Figure 6). Due to its early flowering, the floral buds were more susceptible to freezing. Chaar *et al.* (2011) considered that using late flowering materials of canning peach can be an advantage in zones with high risk of spring frosts. In this sense, Rowland *et al.* (2005) observed in blueberry buds a reduction in cold resistance towards the opening stages of the flower. However, late flowering alone is not a characteristic for resistance; for instance, the Zee Gold cv had a later flowering that the María Bianca cv (Table 1) but, the mean LT_{50} (-2.5 °C) and the mean flower density (0.37 flowers/cm) of the first one are associated with a high damage in the field (75%) and moderately initial number of flowers, resulting in a survival

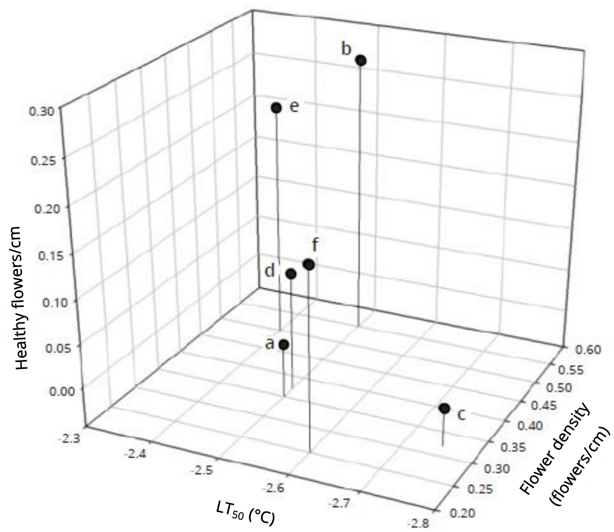

Figure 7. Flower survival (healthy flowers/ cm of branch) after frost events in the field, in function to the mean letal temperature (LT_{50}) and the flower density in branches of different peach cultivars.

(a: Flavorcrest; b: Maria Bianca; c: Milenio INTA; d: Zee Gold; e: Summer Pearl; f: Compact Red Haven).

as low as 0.0925 flowers/cm of branch (Figure 7). Mather *et al.* (1980) observed in blackcurrant (*Ribes nigrum* L.) that in years of late frostings, the cultivars with late flowering as only characteristic for resistance were severely damaged. Lamb (1982) observed in pear (*Pyrus communis* L.) a higher trend of damage by field frosts in cultivars with early flowering but, in the same flowering time there were cultivars with different degree of flower survival. According to Imani and Khani (2011) the most effective indirect method to avoid frost damage in almond is by selecting less susceptible cultivars at the same phenological stages. Kodad and Socias i Company (2005) mentioned that in almond the high potential to produce flowers can compensate the damage of frosts and together with high intrinsic cold resistance, can keep fruiting at commercially acceptable levels.

The mechanisms responsible for frost resistance can be divided in avoidance and tolerance. The avoidance or scape is the capability of the plant to avoid ice formation in the tissues, for example, by over-cooling. Tolerance is the capability of the plant to survive the formation of extracellular ice and the consequently cellular dehydration without irreversible damage (Cary, 1985; Jacobsen *et al.*, 2007). It is important to notice that the results of this research showed a combination of tolerance and avoidance in peach, since the resistant materials did not show as only characteristic the late flowering. Similarly, Kodad and Socias I Company (2005) cited that in almond the late flowering is not enough to surpass the frost damages, therefore the intrinsic resistance can be considered as well as selection target. Both, avoidance and tolerance, are under genetic control and have evolved in response to selective pressures (Palonen and Buszard, 1997; Gusta and Wisniewski, 2013). However, the resistance mechanisms of a particular organ are not constant during the annual cycle but, vary temporarily; in this respect, Pramsohler and Neuner (2013) observed in apple (*Malus domestica* Borkh.) buds extra-organ frost during the winter rest, whereas, during the bud breaking there was over-cooling, which was not detected later during full flowering. Ashworth *et al.* (1989) proposed that the spatial separation of the ice inside the dormant peach buds is consequence of the bud shape and the vascular development. During the winter rest the vascular tissues in the flowers are not totally differentiated and the xylem continuous between the floral organs and the subjacent tissues is not yet established.

According to Arora and Rowland (2011) the predicted global climate change can have major effects in the degree and location of cold damage, due to the likely increase on the meteorological phenomena in the future. Plants that are capable of resisting a premature de-acclimatization and to acclimate quickly when the cold is back, can have higher chances to survive intense winters. In this sense, Chaar and Astorga (2012) mentioned that the cultivars with more plasticity, because they can get acceptable harvests in diverse geographic areas, are those with low cold requirement and high heat requirement. While the low cold requirement can be satisfied even in zones with not cold winters, the high heat requirement is seen as late flowering, which helps the avoidance of late frosts.

Conclusions

In the studied materials of peach for direct consumption, the frost resistance in the field was mainly associated with high flower density, in combination, in some cases, with late flowering.

Late flowering alone did not resulted as a resistance characteristic, therefore, to select peach cultivars with less risk of damage for subzero temperatures, is important to take into account more than one variable associated with reproductive organs, like density and flowering date and the mean lethal temperature.

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