



Short communication

Modelling impact of freezing temperatures on reproductive organs of deciduous fruit trees



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ABSTRACT

This study arises from the need for quantitative methods to assess risks of frost damage in agriculture. Classical mathematical functions were used to model damage due to freezing temperatures and a simple method based on experimental data is described to estimate the model parameters. Using survival data of vegetative and flower buds of deciduous fruit trees reported in the specialized literature, three sigmoidal functions were tested: logistic, Gompertz and Richards. The logistic function was chosen as the best model through the analysis of three comparative criteria: Root Mean Square Error (RMSE), statistical significance of parameters and Akaike Information Criterion (AIC). A parameter estimation method was developed based on a solution of a linear equation system of 2×2 fed only by two pairs of data, obtained from the simulated evolution of LT_{10} and LT_{90} for buds of apricot, cherry, apple and pear. The evolution of lethal temperatures (LT_{10} and LT_{90}) fit well to a monomolecular function (r^2 between 0.907 and 0.997, mean errors fluctuating between 0.5°C and 1.3°C). The method proved to be a good estimator of survival of buds and flowers, starting from published values of lethal temperatures. However, the model is highly sensitive to errors in the estimation of lethal temperatures during the flowering period. This study shows that it is possible to model crop response to freezing temperature, on the basis of limited data on freezing damage. Considering the simplicity of the method, it may be a useful tool to assess risks of frost damage and to improve models of climate change impacts.

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1. Introduction

Freezing injury is one of the main environmental stresses causing large economic losses in agriculture worldwide; it may also limit crop suitability in risky areas (Burke et al., 1976; Pearce, 2001; Snyder and Melo-Abreu, 2005; Rodrigo, 2000). Freezing temperatures in early spring occur frequently in temperate climate areas (Larcher, 1981). This phenomenon coincides with reproductive development (Farajzadeh et al., 2010; Salazar-Gutiérrez et al., 2016), which is the most sensitive phenological crop phase (Snyder and Melo-Abreu, 2005). Therefore spring frosts cause severe production losses, especially in temperate fruit trees (Kang et al., 1998; Larcher, 1981; Westwood, 1982), considering that after blooming no new flowers are produced during the same season (Rodrigo, 2000). Frosts may cause dramatic economic losses in temperate zones. Just one single event caused losses near 90% in stone fruit production in central Washington State (Proebsting, 1982).

Knowledge of risks of freezing damage facilitates decision-making in land use planning and management of crop systems. Quantification of potential losses due to frequency and intensity of freezing temperatures is important to evaluate the economic viability of technologies for frost damage prevention.

Risk assessment can be defined as the yield loss estimation (Araya and Stroosnijder, 2011; Chen et al., 2004; De Rouw, 2004; Richter and Semenov, 2005; Zhang et al., 2010); however, it is generally limited to the estimation of frost number and its intensity, without projecting the potential productive losses that each situation may cause. Some studies estimate probability of occurrence of days in which temperature is below a critical threshold of damage (Bennie et al., 2010; Farajzadeh et al., 2010; Linkosalo et al., 2000; Reinsdorf and Koch, 2013) or within a range associated with qualitative damage (Augsburger, 2013; Eccel et al., 2009; Geerts et al., 2006; Moeletsi et al., 2013; Rahimi et al., 2007). Other studies include probability estimation of events or years during which a fixed level of damage is exceeded. Thus, Höglind et al. (2013) and Vico et al. (2014) used LT_{50} (temperature killing 50% of plant tissues) for grass and wheat plants, respectively, while Cittadini et al. (2006) used LT_{90} for cherry reproductive organs.

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Having algorithms to estimate freezing damage on yield can help to improve crop models, which rarely consider the contribution of frosts to estimating the potential suitability of cultivated species. Few risk studies incorporate the degree of damage generated by intermediate temperatures because published information about resistance to freezing normally refers to lethal temperatures, mainly LT_{10} , LT_{50} y LT_{90} (Vitasse et al., 2014; Salazar-Gutiérrez et al., 2014, 2016). When data of temperature thresholds and freezing damage are limited, survival functions cannot be fitted by regression analysis. In these cases it is advisable to model the survival response of plants to freezing temperatures using a simple linear model that relies on two thresholds (Hijmans et al., 2003). However, literature has shown that the ratio of yield reduction to freezing temperatures is better represented by a sigmoidal equation than a linear function; this is valid for plant and organ survival, electrolyte leakage rate and impedance measurements (Verwijst and Von Fircks, 1994). According to the sigmoidal model, the response to freezing has two stages. In the first stage survival depends strongly on the physiological mechanism of resistance, whose effectiveness reduces as the temperature decreases. At the inflection point the exhaustion of this mechanism starts to be evident, and the effects of freezing temperatures reach severe damage. In the second phase survival depends greatly on the low temperature intensity, making it harder to recover from the damage as the temperature decreases.

The logistic function has mostly been used to describe this phenomenon (Fry et al., 1991; Leinonen, 1996; Luoraren et al., 2004; Mancuso, 2000; Salazar-Gutiérrez et al., 2014). However, it has been reported that this behavior may be better represented by asymmetric shape functions such as the Gompertz (Lim et al., 1998; Lindén et al., 2000) or the Richards function (AnisAko and Lindstrom, 1995; Verwijst and Von Fircks, 1994; Von Fircks and Verwijst, 1993). This phenomenon has been recently modelled by a Weibull function (Vico et al., 2014). Nevertheless, sigmoid functions are infrequently used in risk assessments because they are more complex and require greater experimental information than linear functions.

In a climate change context, it is also necessary to generate functions or models of crop response to climate variables. When these functions are incorporated into crop simulation models, they allow assessing the impact of weather events on agriculture in a changing climate.

Therefore, the purpose of the current study is to provide a method to adjust a sigmoid function to estimate freezing damage due to lethal temperatures, together with its parameters, when only two lethal temperatures are known. Applications are shown for apple, pear, cherry and apricot.

2. Materials and methods

Published experimental information in different fruit species was used to model survival of buds and flowers, and the evolution of lethal temperature from bud swell to fruit set. There is no one single species with all the experimental information to build a model including both processes; for this reason information on several species was combined to deduce a general shape of plant response to freezing temperature.

2.1. Selecting the best model of plant response to freezing temperatures

To assess the degree of damage due to freezing temperatures, this paper proposes to model the survival of buds, flowers and small fruit once a freezing temperature event has occurred. Survival is expressed as the proportion of buds or flowers that survive (S_{LT}) after being submitted to a lethal temperature (LT). Survival

is expressed by a numerical scale from 0 (all buds or flowers are completely killed) to 1 (no damage observed).

To model this, three mathematical functions were used which have 1 as asymptote, which facilitates the fitting process by reducing the number of parameters to be estimated, that is, two parameters in the case of the logistic (Eq. (1)) and Gompertz (Eq. (2)) functions and 3 parameters for the Richards function (Eq. (3)).

These mathematical functions were tested to find the best fit function representing freezing damage and organ survival in tree species. Although the logistic function is extensively used, the Gompertz function has been recommended for electrolyte leakage (Lim et al., 1998; Lindén et al., 2000) and the Richards function for survival data (Verwijst and Von Fircks, 1994; Von Fircks and Verwijst, 1993). The three functions to estimate S_{LT} are given below to allow a better description of the role of parameters in the process of freezing sensitivity:

$$S_{LT} = \frac{1}{1 + e^{-\beta(LT-\gamma)}} \quad (1)$$

$$S_{LT} = \frac{1}{e^{(e^{-\beta(LT-\gamma)})}} \quad (2)$$

$$S_{LT} = \frac{1}{(1 + e^{-\beta(LT-\gamma)})^\delta} \quad (3)$$

For the logistic and Gompertz functions, β is the maximum damage rate and corresponds to the curve slope at the inflection point, which is reached at a temperature γ . In the case of the logistic function, γ corresponds to the temperature that causes 50% death (LT_{50}). In the case of the Gompertz function this temperature causes approximately 83% damage (Bolker, 2008). The Richards function is more flexible, and the inflection point varies depending on the parameter δ , which defines the curve shape.

To establish a general shape of plant survival response to freezing temperatures, survival data of reported fruit species (pistachio, apricot, kiwi and grape) were used. Survival data of these species were fitted to the three sigmoid functions mentioned above for different phenological stages: bud swell, green tip, tight cluster, full bloom and post-bloom in pistachio (Pakkish et al., 2011); dormant buds, first bloom (Hewett, 1976) and full bloom (Gil-Albert, 1998; Meng et al., 2007) in apricot; dormant buds and flower buds of the season in kiwi (Hewett and Young, 1981) and dormant buds in grapes (Kovács et al., 2002). From the published data by Pakkish et al. (2011), only high fruit load trees data were used. From the apricot and grape cultivars evaluated by Hewett (1976) and Kovács et al. (2002), we used the Moorpark and Vignoles cultivars, respectively.

The best model was selected on the basis of three criteria. Goodness of fit was evaluated by the root mean square error (RMSE); the model capacity to represent the phenomenon of freezing sensitivity was evaluated by testing statistical significance of its parameters. Finally, the relative model quality was evaluated in terms of balance between the degree of fit with observed data and model complexity. For this the Akaike Information Criterion (AIC) was adopted. The regression analyses were performed using Infostat Statistical Software, 2014 version, produced by the Universidad Nacional de Córdoba, Argentina.

2.2. Setting sigmoid function parameters with few data

Once the best function was selected, a method was developed to estimate its parameters starting from limited survival data. In order to obtain a general evolution model for lethal temperatures from bud swell to fruit set, LT_{10} and LT_{90} values from species having good experimental support were used. These species were apple (Ballard et al., 1998), cherry (Ballard et al., 1997), apricot (Ballard et al., 1994) and pear trees (Ballard et al., 1993). These data were collected in the

Table 1
Numerical adaptation of the Fleckinger and Baggiolini phenological scales.

Fruit type	Phenological phase	Scale	Code	Assigned Value (F) ^a		
Pome fruits	Bud swell	Fleckinger	B	1–4		
	Bud burst		C	5–8		
	Leaves developing and flower button exposed		D	9–12		
	Floral buttons developing		E	13–16		
	Blossom		F	17–20		
	Petals fall		G and H	21–24		
	Fruit set		I	25–28		
	Stone fruits (apricot/cherry)		Bud swell	Baggiolini	B	1–4
			Bud burst/Tight cluster		C	5–8
White or pink tips/Open cluster		D	9–12			
Stamens exposed		E	13–16			
Blossom		F	17–20			
Petals fall		G	21–24			
Fruit set		H	25–28			

^a Each phase is divided into four sub-stages.

Washington State University Prosser Irrigated Agriculture Research and Extension Center. The Fleckinger phenological scale was used for apple and pears, and the Baggiolini scale for apricot and cherry. A numerical phenological scale is necessary for modelling purposes. Therefore a numerical scale of 7 phenological phases was adopted for both scales. Each phase was divided into 4 stages: 25, 50, 75 and 100% expression of the phase (Table 1). A simplification was made in the Fleckinger scale by grouping the phases G (fall of the first petals) and H (last petals fall) into one step.

The selected model was used to simulate the evolution of lethal temperatures and the results were evaluated by comparing the determination coefficient (r^2) and the root mean square error (RMSE) between simulated and observed data. Validation was performed with a set of data not used during the model development phase. These data consisted in twelve years of experimental observations by collecting reproductive organs in the field and subjecting them to artificial freezing (Proebsting and Mills, 1978). During the validation process, the model efficiency (EF) and the root mean square error (RMSE) were determined.

Finally, the method for constructing the sigmoid function on the basis of the observed and estimated LT_{10} and LT_{90} was validated by comparing the estimated and observed survival of flowers in the states of first bloom (Hewett 1976) and full bloom in apricots (Gil-Albert, 1998; Meng et al., 2007).

3. Results and discussion

3.1. Survival function of floral organs

According to the RMSE values the Richards function is the most appropriate model in most cases (in 9 out of 11 cases), followed by the logistic function, which had the best fit in the remaining 2 cases. The RMSE of the logistic function tied with the function of Richards on 3 occasions, and had the second lowest RMSE in the remaining cases (Table 2).

Although the Richards function obtained the best fit to the observed data, in about half of these cases the additional parameter included in this function (δ) was not statistically significant. These results indicate that the parameter δ does not supply additional information to explain the response to freezing temperatures, and can be excluded from the model. In the other half of the cases, AIC values of the Richards function were greater than those of the logistic function (Table 2, Fig. 1), which suggests that increasing the number of parameters in order to improve the goodness of fit is not advisable.

The logistic function is a particular case of the Richards function, where the δ parameter is set to 1 (Eqs. (1) and (2)). When there is doubt about the convenience of including this parameter

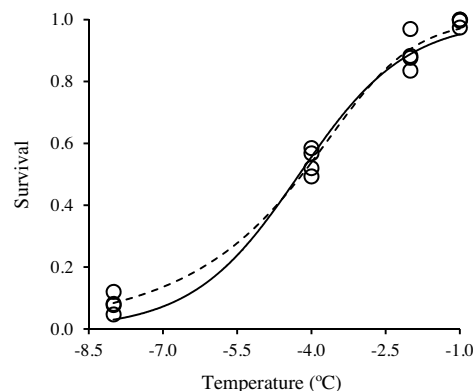


Fig. 1. Survival of floral organs to freezing temperature in tight cluster stage of pistachio (extracted data from Pakkish et al., 2011). Logistic (solid line) and Richards (dashed line) models.

in the model construction, the logistic function is the model that best describes the plant response to freezing temperatures.

In cases where the Richards function gave a very good fit, the logistic function tended to underestimate survival particularly at both ends of the response curve, that is, for temperatures close to 0 °C and extreme negative temperatures. Verwijst and Von Fircks (1994) reported the same type of difference. The tight cluster stage of pistachio showed the greatest difference between the logistic and Richards functions (Fig. 1).

The absolute errors reached by the logistic function fluctuated between 0 and 0.16 in the survival scale of 0–1; only 2% reached values over 0.10, while 75% were below 0.05. These error magnitudes were similar to those of the Richards function, for which the absolute errors ranged from 0 to 0.15, with 3% of errors greater than 0.10, while 81% had values less than 0.05. Considering the acceptable level of errors and its simplicity, the logistic model seems to be the most suitable for simulation models aimed at evaluating freezing impact on floral organs of deciduous fruit species.

The results showed that floral organ survival to freezing temperatures presents a sigmoid behavior, which is well represented by the logistic function. The use of this function is less recommended in cases where the response to freezing temperatures is asymmetric. In this paper, these cases were shown to be represented by the Richards function, which is more complex to adjust. This function is useful when there is a large data set indicating a clear asymmetric behavior of plant response. In case of limited data, where there is less certainty that the plant response is asymmetrical, the logistic function is more advantageous due to a greater facility to estimate

Table 2
Statistical parameters for evaluating the Logistic (**L**), Gompertz (**G**) and Richards (**R**) functions.

Specie	Stage	Function	β	γ	δ	RMSE	AIC	Source of data
Grape	Dormant bud	L	0.543	-15.257		0.066 ^a	-58.29	Kovács et al. (2002) (n ^d = 24)
		G	0.396	-16.237		0.079	-49.68	
		R	0.668	-14.074	0.604 ^{ns}	0.066 ^a	-57.52 ^c	
Pistachio	Bud swell	L	0.509	-10.515		0.044 ^a	-51.07	Pakkish et al. (2011) (n = 16)
		G	0.342	-11.926		0.048	-47.96	
		R	0.443	-12.031	1.630 ^{ns}	0.044 ^a	-50.28 ^c	
	Green tip	L	0.586	-8.446		0.047 ^a	-48.75	Pakkish et al. (2011) (n = 16)
		G	0.394	-9.662		0.050	-46.66	
		R	0.507	-9.850	1.680 ^{ns}	0.047 ^a	-47.79 ^c	
	Tight cluster	L	0.925	-4.243		0.051 ^b	-46.15 ^c	Pakkish et al. (2011) (n = 16)
		G	0.936	-4.517		0.059	-41.09	
		R	1.322	-2.872	0.366	0.040 ^a	-56.89	
	Full bloom	L	2.046	-1.999		0.044 ^b	-64.38 ^c	Pakkish et al. (2011) (n = 20)
		G	1.941	-2.173		0.048	-60.73	
		R	3.057	-1.308	0.320	0.035 ^a	-72.60	
Post bloom	L	1.703	-2.098		0.041 ^b	-66.56 ^c	Pakkish et al. (2011) (n = 20)	
	G	1.691	-2.259		0.051	-58.61		
	R	2.361	-1.462	0.424	0.035 ^a	-72.27		
Apricot	Dormant bud	L	0.997	-12.271		0.037 ^b	-19.07 ^c	Hewett (1976) (n=6)
		G	0.679	-12.969		0.060	-13.09	
		R	1.433	-11.274	0.453 ^{ns}	0.033 ^a	-20.12	
	First bloom	L	0.981	-5.604		0.030	-21.85	Hewett (1976) (n=6)
		G	0.667	-6.281		0.022 ^a	-25.08 ^c	
		R	0.758	-7.583	3.246 ^{ns}	0.022 ^a	-25.15	
	Full bloom	L	1.572	-4.381		0.021 ^a	-20.75	Gil-Albert (1998) (n=5)
		G	1.129	-4.790		0.047	-12.89	
		R	1.647	-4.250	0.870 ^{ns}	0.026 ^b	-18.96 ^c	
Kiwi	Dormant bud	L	0.873	-11.577		0.066 ^a	-16.96	Hewett and Young (1981) (n=8)
		G	0.552	-12.368		0.073	-15.38	
		R	1.243 ^{ns}	-10.655	0.512 ^{ns}	0.071 ^b	-15.21 ^c	
	Flower bud	L	7.227	-2.386		0.020 ^b	-24.64 ^c	Hewett and Young (1981) (n=6)
		G	4.307	-2.476		0.046	-16.40	
		R	10.450	-2.180	0.350	0.004 ^a	-45.34	

ns Non-significant estimation (p-value > 0.05).

^a Best model considering the lowest root mean square error.

^b Second model considering the lowest root mean square error.

^c Lower AIC value between models with lower root mean square error.

^d n is number of experimental observations.

its parameters and their acceptable level of error, similar to that achieved by Richards function.

3.2. Logistic model fitting

The fitting process of the logistic function is done on the basis of two properties of this model. The first is that this function can be linearized to establish its exponent, and the second consists of fixing the asymptote at 1 so as to require only two parameters. The model can be expressed by the following linear equation:

$$-\beta(LT - \gamma) = \ln\left(\frac{1}{S_{LT}} - 1\right) \quad (4)$$

Because this function requires two parameters, they can be found by solving a system of linear equations (Eqs. (5) and (6)), for which only two threshold temperatures (LT_1 and LT_2) and their respective levels of survival (S_{LT1} and S_{LT2}) are needed:

$$\beta LT_1 - \beta \gamma = -\ln\left(\frac{1}{S_{LT1}} - 1\right) \quad (5)$$

$$-\beta LT_2 + \beta \gamma = \ln\left(\frac{1}{S_{LT2}} - 1\right) \quad (6)$$

Combining Eqs. (6) and (7) the β parameter may be derived:

$$\beta = \frac{[\ln\left(\frac{1}{S_{LT2}} - 1\right) - \ln\left(\frac{1}{S_{LT1}} - 1\right)]}{LT_1 - LT_2} \quad (7)$$

By replacement of β in the linear expression (Eq. (4)), γ may be obtained:

$$\gamma = \frac{\ln\left(\frac{1}{S_{LT2}} - 1\right)}{\beta} + LT_2 \quad (8)$$

In physiological terms, γ (LT_{50}) represents the temperature threshold at the maximum rate of injury, while β represents the organ sensitivity once the damage is initiated. Freezing resistance studies have used the logistic function to fit survival data and to estimate LT_{10} , LT_{50} or LT_{90} as resistance indices (Salazar-Gutiérrez et al., 2014, 2016; Vitasse et al., 2014); LT_{50} is the most common descriptor of resistance (Ershadi et al., 2016; Lenz et al., 2013; Thorsen and Höglind, 2010). However, tissue response to freezing temperature is not completely defined by only lethal temperatures. When damage begins, the rapidity of tolerance loss can be very different among species, cultivars, tissues and phenological stages. For example, a given species may be highly resistant to start being injured, but after the initial damage the resistance may be lost quickly as the temperature decreases.

Therefore, it is very important to include one parameter associated with the shape of the curve to represent better the freezing resistance of a specific organ. Few studies have published β values of logistic curves describing freezing survival (Salazar-Gutiérrez et al., 2014, 2016). Some authors have provided methods to simulate or estimate β when only LT_{50} is known. Leinonen (1996), working with young pine trees enclosed by open chambers in the field, observed a nonlinear relationship between β and LT_{50} , proposing a monomolecular function to estimate the slope (β)

Table 3
Monomolecular equations, r^2 and RMSE for the evolution of LT_{10} and LT_{90} .

Species	Function	RMSE	r^2
Apricot	$LT_{10Apricot} = -2.304 (1 + 3.4204 e^{(-0.1271F)})$	0.50	0.971
	$LT_{90Apricot} = -3.249 (1 + 7.2513 e^{(-0.1218F)})$	0.84	0.981
Cherry	$LT_{10Cherry} = -2.458 (1 + 3.6914 e^{(-0.4730F)})$	0.33	0.980
	$LT_{90Cherry} = -3.491 (1 + 3.8902 e^{(-0.1786F)})$	0.26	0.997
Apple	$LT_{10Apple} = -1.290 (1 + 7.7189 e^{(-0.1417F)})$	0.99	0.907
	$LT_{90Apple} = -2.431 (1 + 7.0012 e^{(-0.1388F)})$	1.15	0.954
Pear	$LT_{10Pear} = -1.318 (1 + 6.9364 e^{(-0.1063F)})$	0.43	0.982
	$LT_{90Pear} = -1.5300 (1 + 12.1810 e^{(-0.0917F)})$	1.29	0.962

using the value of LT_{50} . Similarly, [Vico et al. \(2014\)](#) proposed to model the survival of wheat plants to freezing temperatures using a Weibull function, which is also defined by LT_{50} and a parameter representing the slope (α). The authors indicate that there is a co-evolution of these parameters during the phenological development of wheat. However, α was established considering the ratio α/LT_{50} as a constant because data were not enough to model this phenomenon. The requirement of sufficient data to estimate the slope can be overcome by using the method proposed by this work, which propose to build the logistic function starting from two points by using a simple calculation technique. The method is flexible in terms of the type and quantity of required data. The model can be fit on the basis of the effect of any two of the temperatures, not necessarily LT_{50} . If LT_{50} is known, only one more critical temperature and its associated damage is required to estimate β , with no need for a set of data to relate the two parameters.

For modelling purposes in assessment of frost damage risk, it is necessary to know several critical temperatures that can allow the evaluation of different damage levels. If survival has not been measured and only two critical temperatures are known, then the plant response to freezing temperatures can be modeled as a linear function ([Hijmans et al., 2003](#)). Additionally, this paper provided a tool to reconstruct logistic functions using two lethal temperatures.

If lethal temperatures are available for only some stages, then a simulation model based on the Mitscherlich equation can be used to estimate lethal temperatures of the stages with no data.

3.3. Modelling lethal temperature evolution during floral development

The resistance to freezing temperatures of the reproductive organs shows a steady decrease after bud swell, reaching maximum sensitivity at fruit set, just a few days after flower fecundation. According to this behavior, the monomolecular Mitscherlich function (Eq. (9)) showed good fit to estimate the evolution of LT_{10} and LT_{90} between bud swell and fruit set (stages 1 and 28 in the numerical scale) ([Fig. 2](#)). This function is defined by three parameters: (i) the highest lethal temperature at the most sensitive phase (LT_{high}), equivalent to the asymptote of the curve, (ii) the maximum rate of dehardening (k), that represents the speed to reach the highest sensitivity, and (iii) B , which is a constant of integration without any biological meaning.

$$LT_x = LT_{high} (1 - B e^{-kF}) \quad (9)$$

This function showed a high degree of fit, with r^2 ranging between 0.907 and 0.997 ([Table 3](#)). Errors in the estimation of LT_{90} were generally greater than those achieved in the estimation of LT_{10} . The smaller errors were observed in the stone species cherry and apricot, with RMSE lower than 0.5 °C in the case of LT_{10} and 0.9 °C for LT_{90} . Errors in pome fruit trees approached 1 °C in the case of LT_{10} and 1.3 °C in the case of LT_{90} .

Monomolecular dynamics of lethal temperatures agree with patterns of LT_{50} found in some previous studies of deacclimation after a period of winter resistance. [Kalberer et al. \(2007\)](#) and

[Rowland et al. \(2008\)](#) used the linear-log model to simulate LT_{50} values of azalea and blueberry floral buds as function of days of deacclimation at a determined temperature. Both monomolecular and linear-log models describe a maximum rate of loss resistance at the beginning of the process in spring; then dehardening occurs at a decreasing rate until the minimum resistance is reached. Moreover, *Hydrangea* and some blueberry varieties have exhibited a Gompertz evolution of LT_{50} in buds as a function of days of deacclimation ([Pagter et al., 2011](#); [Rowland et al., 2008](#)).

Greater errors in the estimation of the LT_{90} than the LT_{10} may be caused by greater natural variability of LT_{90} values. Authors of lethal temperature data used in this study did not publish dispersion values, nevertheless other publications provide information on the variability of LT_{10} and LT_{90} . [Miranda et al. \(2005\)](#) assessed lethal temperatures during flower bud development of peach, sweet cherry, almond, Japanese plum and blackthorn. Confidence intervals of LT_{90} reached values about double those of LT_{50} intervals. According to lethal temperatures of eight walnut cultivars evaluated by [Aslamaraz et al. \(2010\)](#) in fall and early winter, the ranges of LT_{90} were 1–2.7 times wider than ranges of LT_{10} for buds. Values of standard deviation for lethal temperatures published by [Proebsting and Mills \(1978\)](#) indicate greater variability of LT_{90} for flower buds of apple, pear, peach, apricot and sweet cherry cultivars.

The role of genetic control of cold hardiness was indicated by [Ferguson et al. \(2014\)](#) and [Sakai and Larcher \(1987\)](#), but its dynamics in acclimation and deacclimation is mainly driven by temperatures ([Charrier and Améglio, 2011](#); [Salazar-Gutiérrez et al., 2016](#)). Other factors also influence the level of freezing resistance, such as rootstock ([Köse, 2006](#); [Tsipouridis and Thomidis, 2005](#)), vigor ([During, 1997](#)), training system, health status, yield in previous years and cultural practices ([Szalay et al., 2010](#)).

The cited studies suggest that the initial temperatures of damage may be more related to genetic characteristics and would be less dependent on environmental factors. External factors would affect principally the ability to tolerate decreasing temperatures once the freezing damage has begun.

According to [Kadir and Proebsting \(1994\)](#) and [Sakai and Larcher \(1987\)](#), the level of cold hardiness at a given time ranges between two genetically determined levels of hardiness, the minimum and potential cold hardiness of each development stage. The potential cold hardiness is the maximum attainable hardiness ([Sakai and Larcher, 1987](#)), while the minimum cold hardiness is a basal resistance to freezing temperatures ([Hatch, 1967](#); [Slater et al., 1991](#)). Thus these concepts indicate specific limits to hardening and dehardening ([Proebsting, 1978](#)). The level of cold hardiness achieved between the minimum and the potential limit depends on the environmental variables ([Hofmann et al., 2015](#)). However, species or cultivars differ in the ability to respond to environmental changes ([Coleman et al., 1992](#); [Kalberer et al., 2006](#)).

The limits of hardiness and the capacity to acclimate could explain the variability and the errors in estimations of lethal temperatures in stone and pome fruit species. Pome fruits species would have a larger range of hardiness levels and a faster rate of deacclimation than stone fruit species in response to environmental stimuli.

The validation process showed that estimated lethal temperatures with the monomolecular model correlated well with the LT_{10} and LT_{90} values reported by [Proebsting and Mills \(1978\)](#). This correlation was greater for LT_{10} than for LT_{90} . In the case of LT_{90} the model is a better predictor in the more sensitive stage close to flowering, however, it tends to overestimate the threshold of damage at the earliest stages which are less sensitive (these stages are shown at the bottom of [Fig. 3c](#) and [d](#)). Other authors have reported less variability or estimation error of lethal temperatures in late stages of bud development ([Proebsting and Mills, 1978](#); [Szalay et al., 2010](#)).

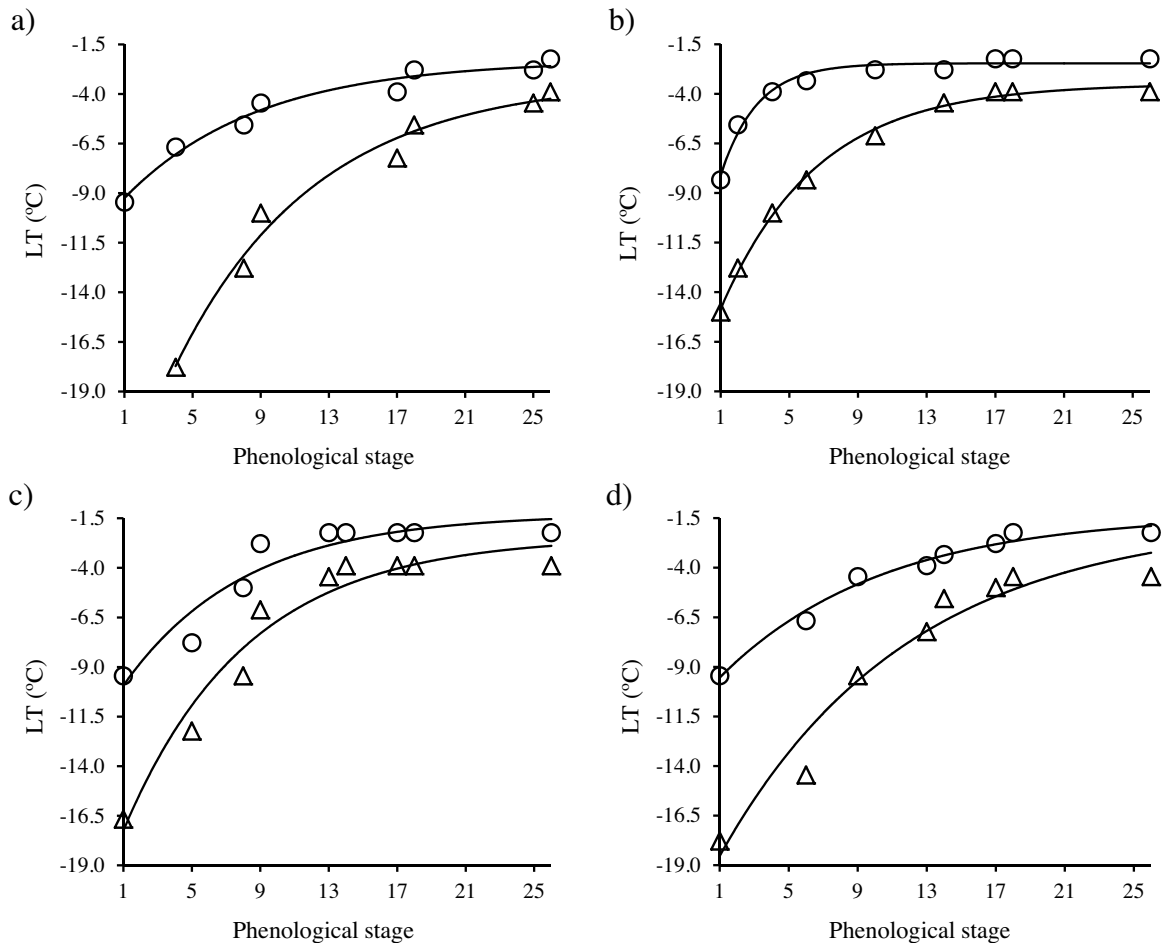


Fig. 2. Monomolecular model of the dynamics of lethal temperature during the reproductive phase of (a) apricot (data obtained from Ballard et al., 1994), (b) cherry (from Ballard et al., 1997), (c) apple (from Ballard et al., 1998) and (d) pear (from Ballard et al., 1993). LT_{10} (circles) and LT_{90} (triangles).

The best fit was observed in cherry (Fig. 3b) and the worst fit in the case of apricot sensitivity, with RMSE of 1.2 °C and EF of 0.90 for LT_{10} , and RMSE of 2.2 °C and EF of 0.93 for LT_{90} (Fig. 3a). These errors are of similar magnitude to those reported in the validation of models of lethal temperatures in grape (Ferguson et al., 2011; Ferguson et al., 2014).

3.4. Evaluation of the method to estimate function parameters

Using only two critical temperatures, LT_{10} and LT_{90} , freezing survival functions for apricot flowers were adjusted using the method proposed in Section 3.2. These functions were evaluated by comparing the estimated survival with survival data published by Hewett (1976) at first bloom and Gil-Albert (1998) and Meng et al. (2007) at full blossom.

The model was a good predictor for flower survival using lethal temperatures reported by Ballard et al. (1994) (Fig. 4). The model tended to underestimate slightly survival in full bloom, with absolute errors between 0.016 and 0.086; at the beginning of flowering survival was underestimated, however at this phenological moment errors were less than 0.090.

Using the monomolecular model to estimate LT_{10} and LT_{90} at full bloom, survival was estimated with errors less than 0.044. Estimations of LT_{10} and LT_{90} at the beginning of flowering worsened compared to observed data, having errors of -0.7 and -1.0 °C (Fig. 2a). This led to increased errors of survival estimations at this stage, reaching absolute errors up to 0.27 (Fig. 4a). According to the results, errors of under- or overestimation of about 1 °C in pre-

dicting lethal temperatures can induce a high degree of error in the estimation of survival with the logistic function adjusted with these temperatures. Therefore, the fitting degree of the lethal temperatures model is critical to shape plant freezing survival with limited data. For this reason it is necessary to generate information on lethal temperatures with more data to improve the degree of fit and to calibrate the model for different varieties of the same species.

Lethal temperatures are determined in growth chambers, by cooling at a fixed rate down to a predetermined temperature. Once the extreme temperature is reached, it is maintained for 30 min (Snyder and Melo-Abreu, 2005). Lethal temperatures refer to plant tissue temperature (Proebsting and Mills, 1978) but are likely to differ from air temperature (Snyder and Melo-Abreu, 2005); this may be another source of error. Thus, for estimating frost damage, critical temperatures obtained in the laboratory should be validated with field observations (Matzner et al., 2016). In addition, because of their high variability, lethal temperatures cannot be considered as biological constants (Proebsting and Mills, 1978). Therefore lethal temperatures can be only used as a guideline for decision making (Snyder and Melo-Abreu, 2005; Miranda et al., 2005).

Empirical models are useful to establish a reference behavior for crop species. Thus the monomolecular and logistic models reproduce a mean behavior of lethal temperatures and freezing damage in buds of fruit trees. In this study freezing survival was simulated using lethal temperatures, while lethal temperatures were modelled as a function of phenological stage. The fluctuations of lethal temperatures and freezing damage due to other environmental

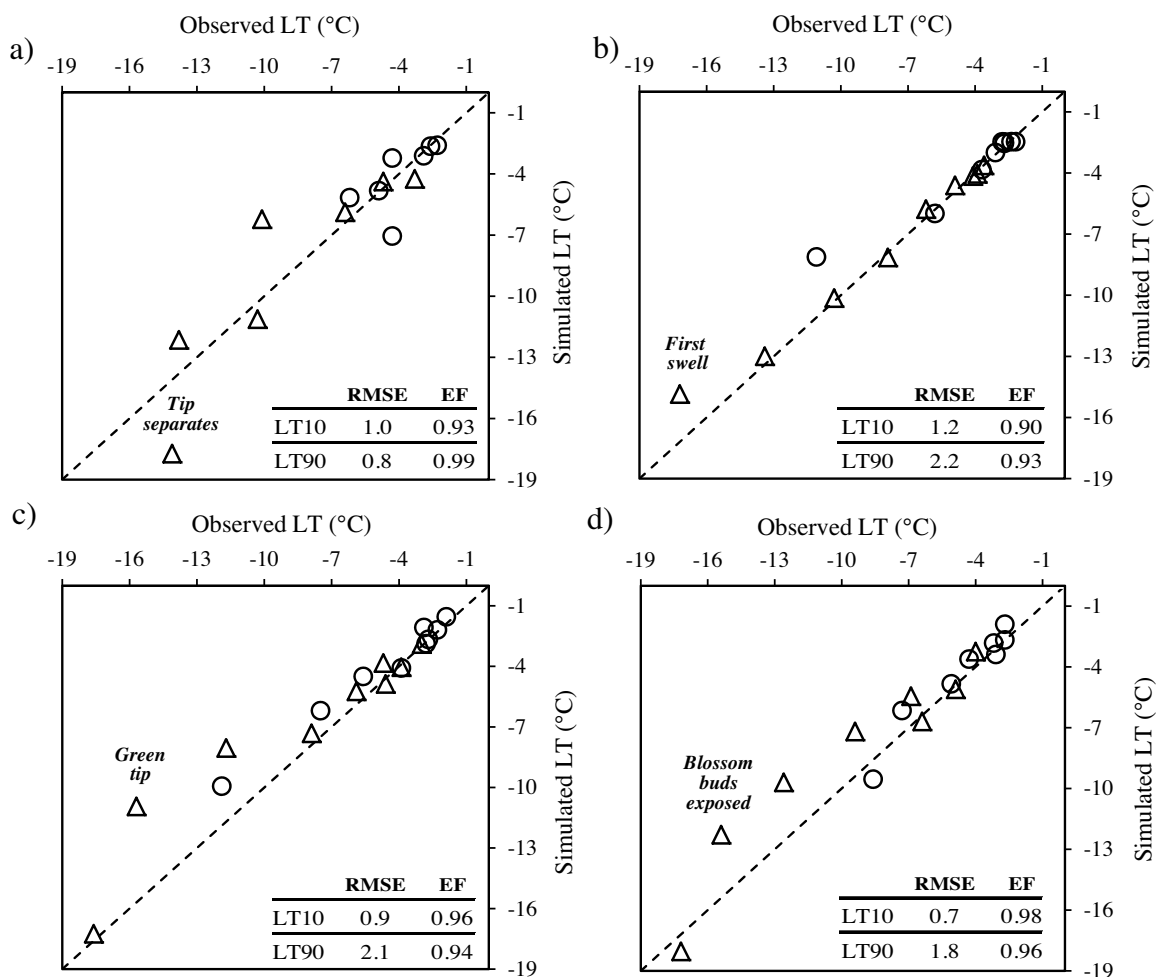


Fig. 3. Simulated lethal temperature threshold vs. observed data of Proebsting and Mills (1978). LT₁₀ (circles) and LT₉₀ (triangles). (a) Apricot. (b) Cherry. (c) Apple. (d) Pear.

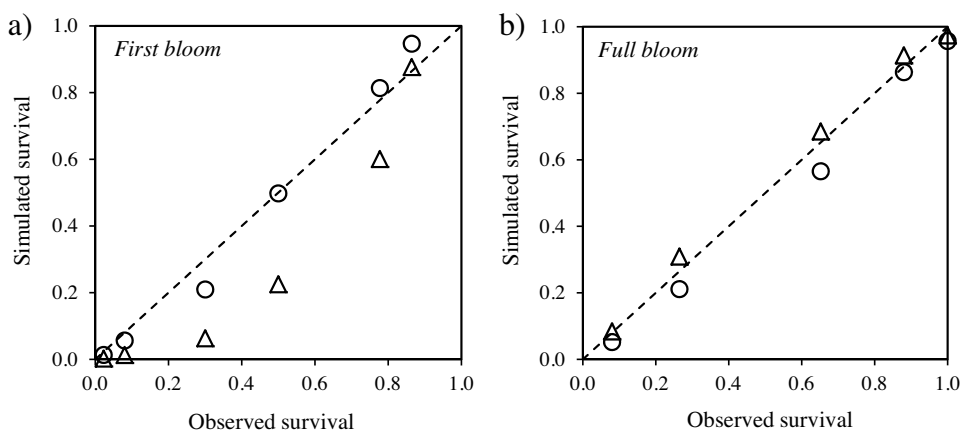


Fig. 4. Comparison between observed survival of apricot floral organs and simulated by the logistic function. Circles: calculated with a logistic function adjusted using LT₁₀ and LT₉₀ published by Ballard et al. (1994). Triangles: calculated with a logistic function adjusted using LT₁₀ and LT₉₀ calculated by the apricot monomolecular model of lethal temperatures as a function of phenological stages.

variables can be assessed by incorporating these factors into more complex models. In addition, if the differences between tissue and air temperatures in several environmental contexts are evaluated, these could be incorporated to more accurate frost damage models.

This study showed that freezing damage can be estimated when few lethal temperatures are known. The proposed models and methodology are an initial step to incorporate sigmoidal models of freezing damage in crop models and frost risk assessment in

fruit species having limited data on freezing survival and lethal temperatures.

4. Conclusions

When data of freezing survival are limited, the developed methodology in this paper allows the construction of logistic curves using two lethal temperatures, LT₁₀ and LT₉₀. The values of LT₁₀

and LT_{90} can be obtained from a monomolecular model of lethal temperatures when these are unknown. Because of its simplicity and low demand of experimental data, this method is readily applicable to other crop species and can be incorporated in crop simulation models, allowing the quantification of expected damages due to freezing temperatures in different locations having data on minimum temperatures. This modelling exercise showed that with available experimental information it is possible to model survival of reproductive organs, which are the most sensitive to freezing temperatures. This methodology is a first step to incorporate sigmoid models in frost risk assessment in cases or species with limited quantitative information. This approach is a good starting point to build quantitative models of frost injury in fruit species, which is essential for economic evaluation of frost damage. In addition, this study provides a useful tool for assessing climate risks in the context of current and future climatic scenarios.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: [10.1016/j.agrformet.2016.05.002](https://doi.org/10.1016/j.agrformet.2016.05.002).

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