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Changing of the flower bud frost hardiness in three Hungarian apricot cultivars

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Abstract

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Hungary lies near the northern border of the apricot growing area, so frost hardiness is a decisive factor for the reliability of production. Both the development and loss of frost hardiness take place gradually in the overwintering organs, depending on the hereditary traits of the cultivars and the prevailing environmental conditions. Among the overwintering organs the flower buds are the most sensitive to frost. The frost hardiness of the flower buds of three Hungarian cultivars (Ceglédi bíborkajszi, Gönci magyar kajszi and Rózsakajszi C. 1406) was determined using artificial freezing tests during the dormancy period in 11 years. Mathematical models were developed to describe changes in frost hardiness of the flower buds in each cultivar. Ambient temperatures have a significant effect on the hardening and dehardening of flower buds, so it is important to study this trait as many years as possible. Based on the 11 years data characteristic features of frost hardiness of 3 apricot cultivars could be described accurately. Based on the results obtained the hardening process in the flower buds of apricot cultivars can be divided into two distinct phases. Tendencies in the changing of frost hardiness of 3 studied cultivars were similar, but significant differences were detected between them.

Keywords: overwintering organs; LT_{50}; apricot; mathematical model

Hungary is situated near the northern range of apricot production area, so frost hardiness is a decisive factor for the reliability of production (Pénzes, Szalay 2003). The frost hardiness of the overwintering organs of deciduous trees in the temperate zone changes continually during the dormancy period, gradually developing/increasing then gradually diminishing (Smith et al. 1994; Lindén 2002). The development of frost hardiness is determined fundamentally by the inherited genetic traits, so there are substantial differences between the cultivars. Actual level of frost hardiness, is greatly influenced by environmental factors, therefore the development of frost hardiness for a given cultivar may differ over the years and at different locations, so it is important to study this trait of genotypes as many years as possible (Pénzes, Szalay 2003). The frost hardiness of the overwintering organs can be investigated by potassium exosmosis analysis (Werner et al. 1993), by differential thermal analysis (Ashworth et al. 1983; Flinn, Ashworth 1999; Gu 1999; Tromp 2005). Natural frost damages can be scored (Faust 1989; Szabó et al. 1995). The process by which frost hardiness changes can be monitored precisely in artificial freezing tests (Faust 1989; Westwood 1993; Layne, Gadsby

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1995; Pedryc et al. 1999; Szalay 2001; Miranda et al. 2005). The effect of temperature on the rate of frost damage can be described by a sigmoid curve, on which the section between 20% and 80% frost damage can be regarded as linear (Gu 1999). The characteristic points of the sigmoid curve and the slope of the linear section provide a good description of the frost hardiness of genotype at the certain time. In general, the quantile of the sigmoid curve associated with 50% frost hardiness is used to characterise frost hardiness (Quamme 1974; Proebsting, Mills 1978; Kang et al. 1998; Pedryc et al. 1999). With the help of the Spearman-Kärber's method, Bittenbender and Howell (1974) compiled a mathematical formula to calculate the LT_{50} value from measured data. Other authors used various mathematical models to estimate the LT_{50} value, including linear regression (Burr et al. 1993), non-linear regression (Zhu, Liu 1987) and a logarithmic model (Lindén et al. 1996).

Flower buds are the most frost sensitive organs during dormancy, thus to determine the frost hardiness of genotype, the flower buds have to be studied as the weakest chain link (Smeeton 1964; Stushnoff 1972; Pénzes, Szalay 2003; Bartolini et al. 2006; Gunes 2006). Since 1994 artificial freezing tests have been performed regularly in the Department of Pomology of the Corvinus University of Budapest. The main objective was to describe the general character of frost hardiness of Hungarian apricot cultivars. Based on 11 years of analysis, changes in the flower bud frost hardiness in three apricot cultivars (Ceglédi bíborkajszi (CB), Gönci magyar kajszi (GÖ) and Rózsakajszi C. 1406 (RÓ)) were described using a mathematical model in the current study.

MATERIAL AND METHODS

Samples were collected from the orchard at the Soroksár Research Station of the Faculty of Horticultural Science of Corvinus University of Budapest. Six trees of each of the Ceglédi bíborkajszi (CB), Gönci magyar kajszi (GÖ) and Rózsakajszi C. 1406 (RÓ) apricot cultivars were used for this study. Rootstock is cv. Myrobalan, training system is compact vase with 5 m row distance and 3 m in row tree distance. Integrated plant protection was applied. Six one-year-old shoots (with 40–60 buds) were collected for testing at each freezing temperature. Artificial frost treatment was carried out in Conviron C-912 and Rumed 3301 (Rubarth Apparate GmbH, Laatzen, Germany) climatic chambers. The experiments took place over 11 years, and the flower buds were tested 2–3 times a month from September 1 until the trees blossomed.

Starting in the autumn of 1994, 11 dormancy periods were analysed. Since in some years severe natural frost damage occurred, there was not always appropriate number of flower buds for accurate observations. Therefore, the analysis was restricted to the following set of years with sufficient availability of flower buds: G = {1995, 1998, 1999, 2000, 2002, 2005, 2007, 2008, 2009, 2010, 2011}.

Three or four appropriate freezing temperatures were used at each testing date, according to the part of dormancy. Both cooling and warming were performed at a rate of 2°C/h, and shoots were kept at the given freezing temperature for four hours. After freezing, all the shoots were kept at room temperature until analysis. The flower buds were cut longitudinally and the frost damage was determined based on discolouration of tissues (green – unharmed, brown – frost damaged). The mean value of frost hardiness (LT_{50}) was calculated using a linear regression model, assuming that the section of the hardiness sigmoid curve between 20% and 80% could be regarded as linear (Gu 1999). Exploring the data characteristics revealed that LT_{50} values change from autumn to spring in three well detectable phases (Figs 1 and 2). The first phase shows a decreasing trend, in the second phase it decreases at first until it reaches its minimum point and then starts to increase, and in the third phase it continues to increase. The first phase ends with a so-called cut point where the decrease becomes suddenly very rapid. Supposed that the day of "cut point" depends every year on the min. temperatures of the days in the previous time interval (or on the min. temperatures below a base temperature that will be optimized later), a cut point model was developed. Then a non-linear regression model was fitted to the LT_{50} values for each of the three cultivars. Regression analysis was performed with the help of the IBM SPSS 20 statistical software (IBM Corp., Armonk, USA, 2011). Source of daily min. data was the automatic meteorology station in Soroksár.

Cut Point Model. Every year g, the daily minimum temperature $T^{g}_{\min\_i}$ of day i below a base temperature T_{base} is cumulated from i = 1 to j (j = 1, 2, …, 242 or 243; depending on whether it was

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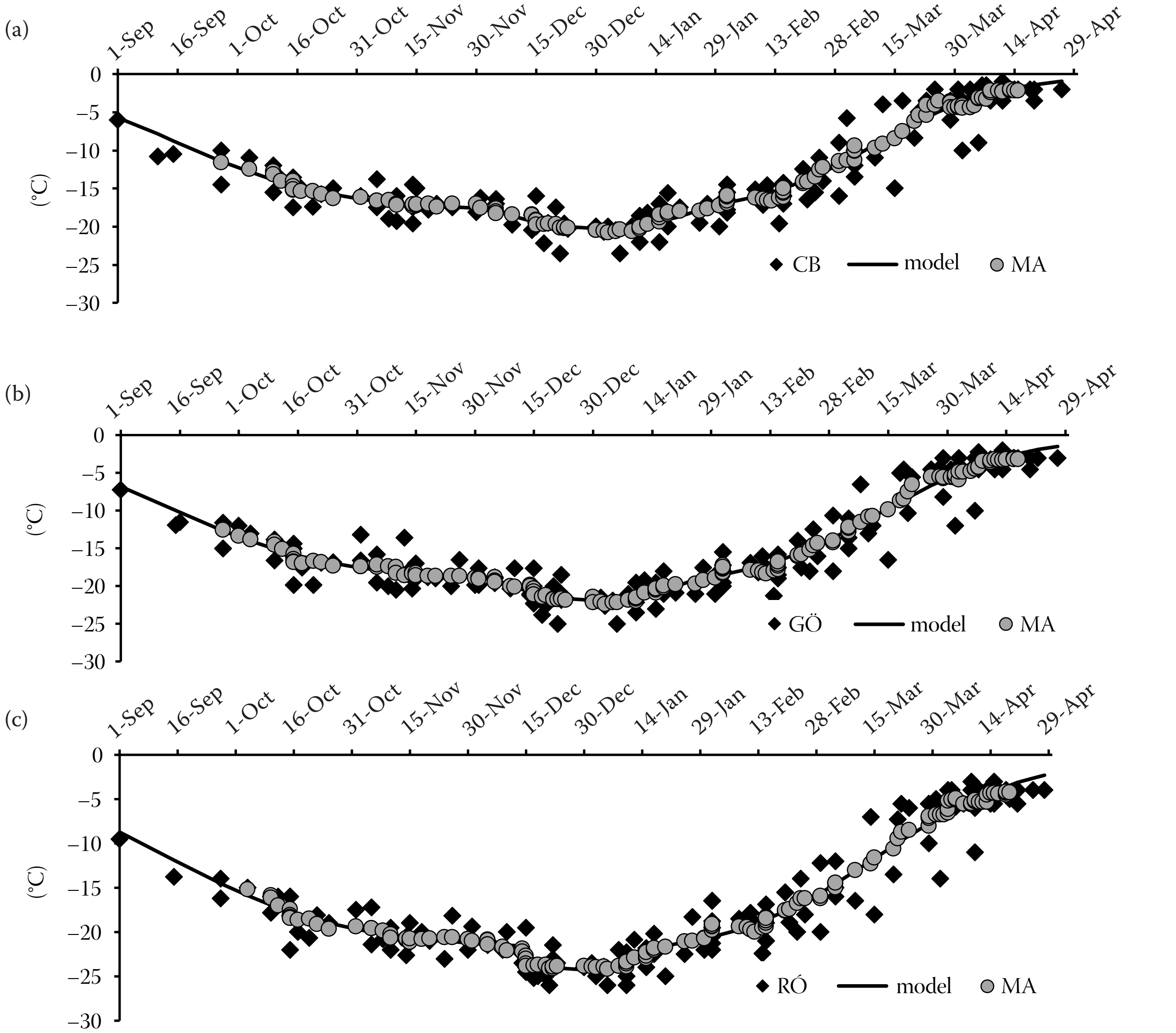

Fig. 1. Flower bud frost hardiness (LT_{50}) values (°C) (taken over the years of experiment) of (a) cv. Ceglédi bíborkajszi (CB), (b) cv. Gönci magyar kajszi (GÖ) and (c) cv. Rózsakajszi C. 1406 (RÓ) a with the moving average of window size 9 (MA) and the LT_{50} model fitted to the moving averages (model)

a leap year or not) which refers to the days of the time interval from the September 1 (as $i = 1$) to the April 30, weighted by i/k ($k \in R^+$) and referred to as the cumulated value (temperature sum) up to day j of year g:

$$C_j^g = \sum_{i=1}^{j} \frac{i}{k} \max\left[\left(T_{\text{base}} - T^g_{\text{min}\_i}\right); 0\right]$$

where: $g \in G$ = {1995, 1998, 2000, 2002, 2005, 2007, 2008, 2009, 2010, 2011}, i.e. G – set of years in which the relevant data were obtained to detect the cut point; k – appropriate weight that will be optimized later.

Let the observed cumulated value C^g_{obs} be the cumulated value up to the observed cut point j^g_{obs} of year g.

The critical value C_{crit} is then defined as the 10% trimmed average (Aver_{10}) of the observed critical values over all the years in G:

$$C_{\text{crit}} = \underset{g\in G}{\text{Aver}_{10}}(C^g_{\text{obs}})$$

The cut point of a year g is assigned by the model when C^g_j first exceeds the critical value, so for $g \in G$ the estimated cut point D^g_{est} is defined as:

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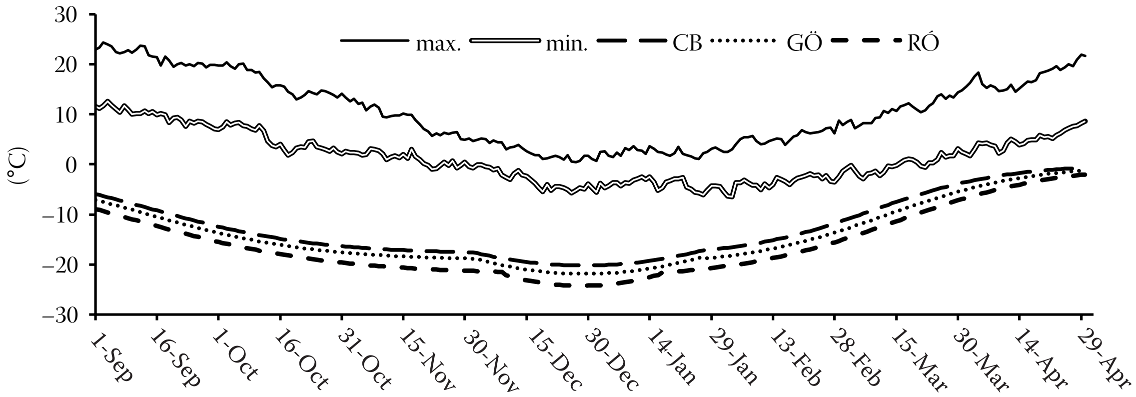

Fig. 2. LT_{50} models (°C) for the three apricot cultivars examined (CB – cv. Ceglédi bíborkajszi; GÖ – cv. Gönci magyar kajszi; RÓ – cv. Rózsakajszi C. 1406), maximum (max) minimum (min) daily temperatures (°C) at the experimental location, averaged over the years of experiment

$$D_{\text{est}}^{g} = \min\left(j : C_j^g > C_{\text{crit}}\right)$$

The root mean square error (*RMSE*) of the estimated cut points is calculated as:

$$RMSE = \sqrt{\frac{1}{|G|}\sum_{y}\left(D_{\text{est}}^{g} - D_{\text{obs}}^{g}\right)^2}$$

where: $|G|$ – cardinality of the set G

RMSE was minimized, while the base temperature (T_{base}) and weight (k) were optimized. The optimization process was executed using the Palisade's Risk Optimizer (www.Palisade.com) using the genetic algorithm (Davis 1991), which is a stochastic searching technique combined with the Latin Hypercube sampling (LHS) method. LHS involves a stratification of the input distribution without replacement (Iman et al. 1980), i.e. the cumulative curve is divided into intervals of equal probability. As the simulation progresses, each of the intervals is sampled once. LH sampling has the advantage of generating a set of samples that reflects the shape of a sampled distribution more precisely than pure random (Monte Carlo) samples. The explained variance R^2 was tested using the Fisher's test. Normality of the residuals was verified by the Shapiro-Wilk's test ($\chi^2(17) = 0.28$; $P > 0.9$). The independence of the residuals from the estimated dates of the cut points was proved ($R^2 = 0.24$; $P = 0.12$). The regression models were tested by their *F*-values and their significance levels. Finally, the rate of variance explained by the model (R^2) were evaluated.

LT_{50} Model. Despite scarce yearly data, pooled data for all years were suitable for model development. Therefore, our aim was to create a cultivar-specific model which can be considered as general for the years of experiment. All the LT_{50} values with their dates were pooled separately for each cultivar (Ceglédi bíborkajszi (CB), Gönci Magyar kajszi (GÖ) and Rózsakajszi C. 1406 (RÓ)). Thus, three datasets were obtained with LT_{50} values together with the date of observation (expressed by day j, $j \in \{1, 2, \ldots, 242, 243\}$). Moving averages of LT_{50} values (MA_t) with a window size 9 were calculated. $t \in \{5, 6, \ldots, 238, 239\}$, and the moving slope with a window size 5, *MS*, at a time point t was defined as:

$$MS_t = \frac{MA_{t+4} - MA_t}{i_{t+4} - i_t}$$

where: i_t – denotes the time elapsed from the September 1 belonging to MA_t. The overall cut points were defined as the day when the moving slope (MS_t) has a value three times higher than in the previous step, i.e. when $MS_t/MS_{t1} > 3$.

Therefore, the overall cut points were given as the points of a sudden decrease specifically for the three varieties and generally for the years of the experiment. The time interval between the September 1 and the cut point is referred to as Phase 1. Phase 2 is defined as the time interval beginning with the cut point and ending with the day when, after having decreased up to their minimum values and increased again, the LT_{50} values reach again the level recorded on the cut point day. Phase 3 begins immediately when Phase 2 ends and ends on the 30th April. The LT_{50} Model consists of three mod-

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els, one for each phase. Note that hardening process starts with Phase 1 and ends at the minimum point of Phase 2 while dehardening process starts at the minimum point of Phase 2 and ends with the end of Phase 3.

Phase 1 is modelled by a decreasing logistic model of the form:

$$Y(i) = p_1/[1 + \exp(p_2 \times (i - p_3))] + \varepsilon$$

where: i – time elapsed from the 1st September (day) as an independent variable; $p_1 < 0$, $p_2 > 0$; $p_1 = \lim_{i \to +\infty} Y(i)$; $\lim_{i \to -\infty} Y(i) = 0$; the model has an inflexion point at $i = p_3$ with a slope value of $p_1p_2/4$. ε – normally distributed error term with zero expectation

Phase 2 is modelled by a quadratic model of the form:

$$Y(i) = p_0 + p_1 \times i^2 + \varepsilon$$

where: $p_n \in R$ – coefficients of i^n (n = 1, 2, 3) and $p_2 > 0$

Phase 3 is modelled by an increasing logistic model with zero saturation value of the form:

$$Y(i) = -p_1/[1 + \exp(p_2 \times (i - p_3))] + \varepsilon$$

where: $p_1 < 0$, $p_2 > 0$; $-p_1 = \lim_{i \to -\infty} Y(i)$ and the model has an inflexion point at $i = p_3$ with a slope value of $p_1p_2/4$

The estimated model parameters with their significance levels, the F values of the Fisher's tests for the models with their significance levels and the explained variances (R^2) with their significance levels were calculated. The normality of the residuals was verified by d'Agostino's test ($\chi^2(2)$ = 4.86 with P = 0.09; $\chi^2(2)$ = 4.19 with P = 0.12; $\chi^2(2)$ = 5.63 with P = 0.06 for varieties CB, GÖ, RÓ, respectively) (D'Agostino et al. 1990). The independence of the residuals from the time elapsed, measured in days, was proved (R^2 = 0.004 with P = 0.47; R^2 = 0.005 with P = 0.37; R^2 = 0.005 with P = 0.38 with for varieties CB, GÖ and RÓ, respectively).

RESULTS

Cut point model

The optimized parameters are T_{base} = 3.3°C and k = 60. The explained variance was high (R^2 = 0.97; P > 0.9) with the Fisher's test $F(1;11)$ = 313.04 (P < 0.001). The minimized $RMSE$ is 15.38 days while the mean absolute error is 11.1 days. However, it was stressed that the time intervals between two observations are quite long, in the early experimental years they were about 30 days, in the last 5 years, around 14 days. It means that the errors, together with the $RMSE$ and the average absolute error are in most cases lower than the observation measure range. The difference between the average observed cut points and the average estimated cut points is 0.7 days.

LT_{50} Model

The estimated model parameters with their significance levels, the F values of the Fisher's tests for the models with their significance levels and the explained variances are presented (Table 1). At the beginning of the dormancy period the frost hardiness of the overwintering organs develops slowly. In the course of the hardening process the temperatures resulting in 50% damage to the flower buds of the three apricot cultivars (LT_{50} values) gradually dropped. The rate and extent of hardening was greatly influenced by the temperature, so substantial differences were observed in the individual years. The level of frost hardiness increased rapidly at first, thereafter the rate gradually slowed. A cut point was observed in the hardening process for all three cultivars, indicating that the development of frost hardiness in the flower buds takes place in two phases. By the end of the first phase, i.e. at the cut point, the flower buds achieved a certain level of frost hardiness. The model estimated values were at this point –17.9°C for cv. Ceglédi bíborkajszi, –19.0°C for cv. Gönci magyar kajszi and –21.5°C for cv. Rózsakajszi C. 1406 which can be considered as a mean value regarding the experimental years. In years when the hardening conditions were less favourable than usual, with great fluctuations in the temperature, the observed level of frost hardiness was 2.0–2.5°C higher than this mean. In years with favourable hardening conditions, i.e. the temperature decreased at an even rate during the autumn, observed frost hardiness levels by 1.5–2.0°C lower than the long-term mean were achieved in the flower buds by the end of the first hardening period. According to the model, the second phase of hardening began in early December. The cut point model indicates that the end of the first phase is

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Table 1. The estimated model parameters, with their Student's *t* values, the *F* values of the Fisher's tests for the models and the explained variance (R^2)

Cultivar	Phase	Estimated parameters		t	F	R^2
CB	1	p_1	−17.94	−78.83***	14,048.92***	0.97***
		p_2	0.05	15.40***		
		p_3	15.90	13.66***		
	2	p_0	32.70	8.50***	93.90***	0.91***
		p_1	−0.87	−13.61***		
		p_2	0.004	13.69***		
	3	p_1	−18.48	−42.49***	5,671.77***	0.99***
		p_2	0.06	21.00***		
		p_3	191.06	170.14***		
GÖ	1	p_1	−19.239	−95.88***	17,761.26***	0.97***
		p_2	0.05	15.70***		
		p_3	13.230	10.82***		
	2	p_0	40.80	8.43***	83.02***	0.90***
		p_1	−1.03	−12.84***		
		p_2	0.004	12.88***		
	3	p_1	−20.37	−56.58***	1,516.60	0.893
		p_2	0.06	28.50***		
		p_3	194.39	208.12***		
RÓ	1	p_1	−21.981	−95.51***	20,427.25***	0.97***
		p_2	0.04	15.68***		
		p_3	11.127	7.68***		
	2	p_0	65.94	6.34***	43.102***	0.87***
		p_1	−1.50	−8.70***		
		p_2	0.006	8.80***		
	3	p_1	−22.83	−58.68***	11,497.75***	0.99***
		p_2	0.05	25.50***		
		p_3	196.94	193.08***		

***significant at the $P < 0.001$ level; CB – cv. Ceglédi bíborkajszi; GÖ – cv. Gönci magyar kajszi; RÓ – cv. Rózsakajszi C. 1406; p_0–p_3 – as explained in the chapter Material and Methods

Models – Phase 1: $Y(i) = p_1/[1 + \exp(p_2 \times (i - p_3))] + \varepsilon$; Phase 2: $Y(i) = p_0 + p_1 i + p_2 i^2 + \varepsilon$; Phase 3: $Y(i) = p_1/[1 + \exp(p_2 \times (i - p_3))] + \varepsilon$

regulated by an accumulated cold effect with positive but low daily minimum air temperature (below 3.3°C). During the second phase of hardening there is a further improvement in the level of frost hardiness, the rate of which was sudden and rapid at first, but then gradually slowed. Our data show that mild or fluctuating temperatures have detrimental effect on the hardening process. Averaged over the years, the highest values of frost hardiness were achieved in the flower buds of all three cultivars at the end of December, after which they gradually declined. According to the model estimations, the lowest LT_{50} values of the cultivars was detected on December 30, when the values were −20.2°C for cv. Ceglédi bíborkajszi, −21.9°C for cv. Gönci magyar kajszi and −24.3°C for cv. Rózsakajszi C. 1406.

During the dehardening period, the reduction in the level of frost hardiness first proceeded in a rapidly accelerating way and then at a gradually decreasing rate. Variability over the years were again considerable in this phase. In years when the external temperature rose slowly, without fluctuations, the flower buds lost their frost hardiness at a slower rate than average. In years when the temperature was mild or fluctuating frost hardiness declining was fast.

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DISCUSSION

Frost hardiness of apricot flower buds gradually improved in the first half of the winter (hardening), and gradually lost during the second half of the winter (dehardening), as reported earlier (Hatch and Walker 1969; Hewett 1976; Proebsting, Mills 1978). The 11-year data series revealed a strong year effect for all three cultivars, as the daily temperatures differed greatly from one year to the next. Nevertheless, this paper introduced a general, meanwhile cultivar-specific mathematical model which describes the main characteristic of the hardening/dehardening process. Frost hardiness develops in two distinguishable periods in the overwintering organs of deciduous trees in the temperate zone (Tromp 2005). This was confirmed for apricot flower buds by the present work. It was demonstrated that the hardening period could be divided into two distinct phases (Phase 1 and Phase 2 up to its minimum point). During the first phase the frost hardiness process of the flower buds first increased rapidly, and then at a slower rate, eventually reaching a level characteristic of the given cultivar and modelled as cut point. This cut point is reached when the daily minimum temperature is permanently below a certain point (estimated by model parameter optimization as 3.3°C) during several days and the cumulated weighted low temperature reaches an appropriate amount. The estimated 'cut points' were the November 30 for cv. Ceglédi bíborkajszi (with LT_{50} = 17.5°C), the December 5 for cv. Gönci magyar kajszi (with LT_{50} = –18.9°C) and the December 14 for cv. Rózsakajszi C. 1406 (with LT_{50} = –21.6°C). In the second phase of hardening, under continuous cold conditions and with a steep decrease of LT_{50} values, apricot flower buds can achieve the level of hardiness characteristic of the genotype estimated by the model as –20.2°C for cv. Ceglédi bíborkajszi, –21.9°C for cv. Gönci magyar kajszi and –24.3°C for cv. Rózsakajszi C. 1406. The dehardening period starts at this minimum point of Phase 2 with a rapid increase until the level of LT_{50} values at cut point is reached again. Dehardening proceeds then with the modelled Phase 3 which can be characterised first by a slow, then an accelerating increase and finally ends with a gentle decline. It was previously proved that the second phase of hardening did not take place in the bark tissues of apple until the temperature had dropped below 4.5°C (Howell, Weiser 1970). Cv. Redhaven peach flower buds need freezing temperatures for the second phase of hardening (Szalay et al. 2010). Based on the 11-year data, characteristic features of frost hardiness of three apricot cultivars could be described accurately. Tendencies in the changing of frost hardiness of the studied cultivars in the dormancy were similar, nevertheless the differences detected between them were pointed at. These data are essential for describing the expected frost hardiness levels of the studied cultivars in different parts of dormancy period, and they help to select and classify further suitable cultivars and orchard sites meeting the requirements of the plants.

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