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ARTICLE

Evaluation of the frost tolerance of Hungarian-bred walnut cultivars

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ABSTRACT At present very few walnut cultivars can be said to be cosmopolitan cultivars, grown widely in the walnut-producing countries of the world. Walnut (*Juglans regia* L.) has poor ecological adaptability, as its cultivation is greatly influenced by low temperatures during the winter dormancy period and in early spring. The breeding activities conducted in various countries are therefore of great significance. Choosing suitable locations for cultivation is of key importance if optimum yield stability is to be achieved. The introduction of foreign walnut cultivars regularly runs into problems if, despite their high yielding ability, they are unable to adapt to the Hungarian climate. In Hungary the most critical weather events for walnuts are the frequent frosts in early spring. Buds therefore need to burst late to avoid damage to the flowers. Many papers have dealt with the frost tolerance of stone fruit, but only limited information has been published on the frost tolerance of walnut. For the first time in Hungary, artificial freezing tests were performed in the present work to determine the frost tolerance of the cultivars available in Hungary. The results could be of service to Hungarian growers in choosing the most suitable cultivar. **Acta Biol Szeged 65(2):163-170 (2021)**

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Introduction

If walnut production is to be profitable, however, the growing site requirements must be fully met. As Hungary is situated close to the northern boundary of the walnut production area, walnuts are not cultivated for commercial purposes in more northerly countries. Winter cold and spring frosts are of great significance for cultivation in Hungary. The trees must receive satisfactory nutrition and the shoots must be fully matured if they are to survive frosts; this can be achieved by foliage protection in summer. It is characteristic of the Hungarian climate that several days of mild weather during January or February are frequently followed by sudden sharp temperature declines.

One of the most decisive factors in yield safety is the frequency of spring frosts, to which early blossoming cultivars are especially sensitive. Despite the considerable frost tolerance of walnut trees, early spring frosts may cause damage to young shoots (Hemery et al. 2010; Poirier et al. 2010). During the dormancy period the trees may endure temperatures as low as minus 25 or 28 °C with little damage (Szentiványi 1978), but studies made by Fady et al. (2003) indicated that early spring frosts may injure the apical buds, or in more severe cases, the ends of young shoots. A close correlation was detected between frost tolerance and origin (Guàrdia et al. 2013). Although differences in the frost tolerance of species and cultivars can be attributed to genetically inherited traits, frost tolerance is not a static phenomenon, but exhibits constant changes. Plants can protect themselves against frost in two ways: by avoidance or tolerance (Charrier et al. 2011). Avoidance means that the annual development cycle is synchronized with the critical environmental periods (Parmesan and Yohe 2003; Menzel et al. 2006), so that the overwintering organs are gradually cold hardened after the foliage has fallen. In autumn the dynamics of decreasing temperature and increasing frost tolerance can be observed for both deciduous and evergreen species (Greer et al. 2000; Luoranen et al. 2004). The dynamics of acclimatization is determined primarily by environ-

Table 1. Flowering dates of the female flowers of Hungarian walnut cultivars (Szentiványi 1980).

Flowering date of female flowers	Early	Mid-Early	Mid-Late	Late
Cultivar	None	Milotai 10	Alsószentiváni 117 Tiszacsécsi 83 Milotai bőtermő Milotai intenzív	Milotai kései Alsószentiváni kései

mental factors between September and January, while from January onwards significant genotype effects have been observed in the time of budburst in connection with both tolerance and avoidance mechanisms (Charrier et al. 2001). Frost damage is one of the factors causing the greatest losses in walnut production (Xin and Browse 2000; Sanghera et al. 2011). The frost tolerance of the cultivars exhibited a close correlation with their flowering traits. The flowering time of the female flowers of Hungarian-bred cultivars is shown in Table 1.

The leafless period is often thought to be inactive, but in fact the trees need to actively modify their metabolism through cold acclimatization processes if they are to cope with winter frost effects (Charrier et al. 2018). The level of frost damage differs for different plant organs (Mahmodzahed and Imani 2011), the flowers being the most sensitive (Charrier et al. 2013).

Laboratory tests are required for the precise measurement of the frost tolerance of individual plant organs. The analysis of tissues after artificial freezing reveals the extent of damage caused by a given temperature. The LT_{50} index, or frost tolerance mean value, can then be calculated (Szalay 2001; Szalay et al. 2010, 2016, 2017). Frost tolerance must be tested on several occasions during the dormancy period in order to obtain a clear picture of the frost tolerance of each cultivar. Frost tolerance is an extremely complex phenomenon from both the physiological and genetic points of view, being the result of several independent processes. It is advisable to use a climate chamber to test winter hardiness. As the frost tolerance of the buds changes in the course of the winter, the temperature in the chamber must reflect that experienced in nature. Several temperatures should be applied at each testing date, at least three, if possible. The rate of chilling and the duration of the treatment are also important. The rate of temperature change should be 1-2 °C an hour, and experience has shown that the buds should be kept at the critical temperature for 3.5 - 4.0 hours (Szalay et al. 2016, 2017).

Materials and Methods

The experiments were performed between October and March in 2013–2014, 2014–2015 and 2015–2016. Ten to twelve one-year-old shoots measuring 50 cm in length were collected once a month from each cultivar in a large-scale non-irrigated plantation in its 20th year in Lengyeltóti, where the annual number of sunshine hours averaged 2098 hours, the annual mean temperature was 11.2 °C and the annual mean precipitation sum 550 mm. The soil was a chernozem (upper limit of plasticity, K_A = 38, pH 8, total lime content in the upper 60 cm soil layer 5%, humus content 1.9%). The height above sea level was 150–160 m, and the summer was long enough for the shoots to reach full maturity (Bujdosó et al. 2019).

The cultivars examined were the Hungarian-bred 'Alsószentiváni 117', 'Milotai 10' and 'Tiszacsécsi 83', all of which only produce nuts from terminal buds, and hybrids of the first two cultivars with 'Pedro': 'Milotai bőtermő' (high-yielding), 'Milotai kései' (late), 'Milotai intenzív' (intensive) and 'Alsószentiváni kései' (late). The Californian-bred 'Pedro' cultivar was used as the control. All the cultivars were grafted onto walnut sapling rootstocks.

The laboratory analyses were carried out in the Department of Pomology at Corvinus University of Budapest (now Fruit Production Department, Institute of Horticultural Sciences, Hungarian University of Agriculture and Life Sciences). The artificial freezing tests were carried out in Rumed 3301 climate chambers (Rubarth Apparate, Laatzen, Germany). At each testing date, three freezing temperatures were applied, chosen to represent the external temperatures. While the lowest temperature tested in October was -16 °C, in January a test temperature of -26 °C was applied to buds that had undergone hardening. The rate of cooling and warming was 2 °C an hour, and the shoots were maintained at the freezing temperature for 4 hours.

After the end of the treatment, the samples were kept at room temperature for 12 hours, after which the buds were cut open and the degree of frost damage was determined based on the discoloration of the tissues. Green tissues were regarded as healthy and brown tissues as frost damaged. The aim of the investigations was to determine the LT_{50} values, i.e. the temperature that caused 50% freezing damage to mixed buds of the given cultivar at the given time. The frost damage values recorded for the three

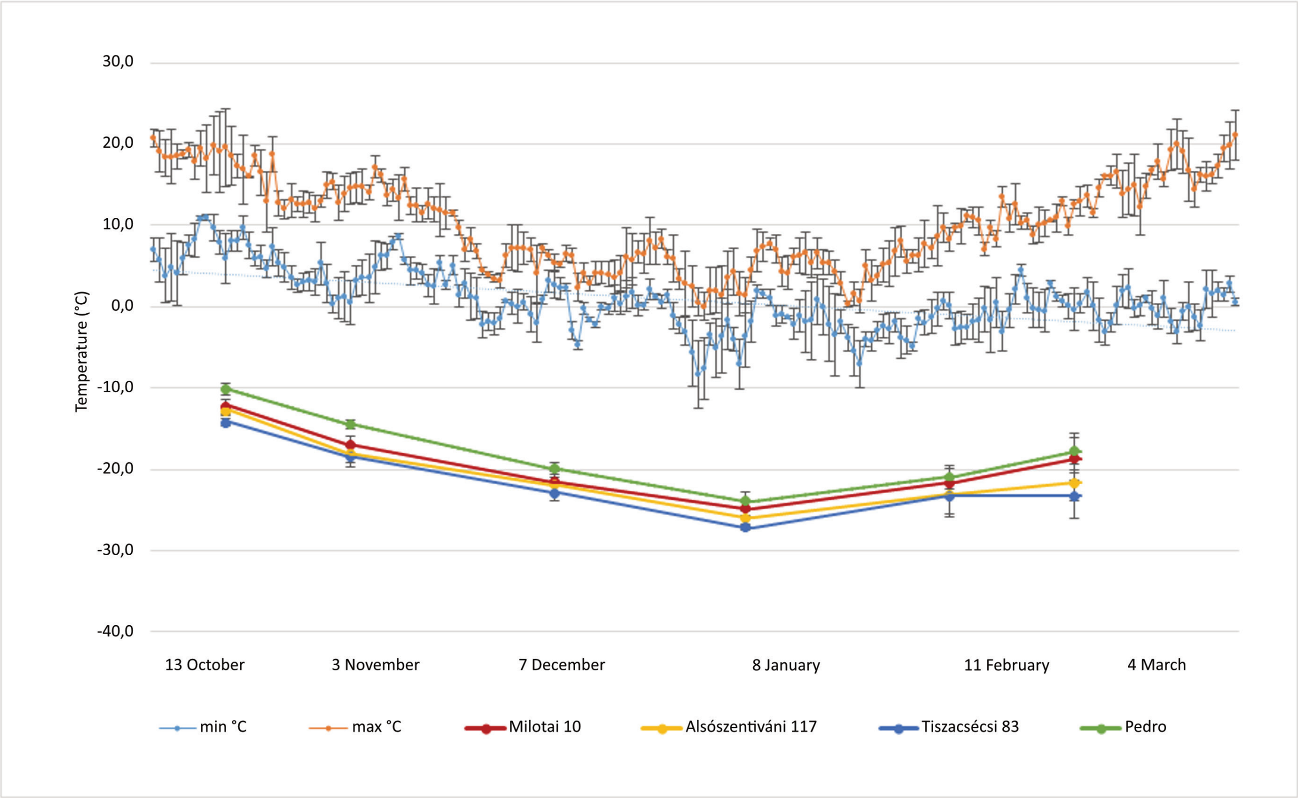

Figure 1. Frost tolerance mean values (mean and standard deviation) of the mixed buds of standard cultivars and the cultivar 'Pedro', and three-year means and standard deviations of daily maximum and minimum temperatures (upper part of the figure).

different freezing temperatures gave the frost tolerance profile of the given cultivar at the individual sampling dates, which could be approximated by a sigmoid graph where the section between 20 and 80% could be regarded as linear (Gu 1999). The LT_{50} values were then determined by linear regression from the results of artificial freezing. The IBM PASW Statistic 18 statistical program package was used for the statistical evaluation. The data were analyzed using the ANOVA model, while means were compared and significant differences determined with the help of Duncan's test at the 95% probability level. The SPSS 25.0 (Chicago, USA) program package was used for the statistical evaluation of the data.

Results

The results are illustrated in Figs. 1-3. As no significant differences were detected between the years, the results of the three years were averaged, and the mean values and standard deviation were used to determine the frost tolerance dynamics of the flower buds and differences between the cultivars. Fig. 4 demonstrates the homogeneous groups determined by means of analysis of variance, from which the frost sensitivity order was deduced. The frost tolerance of the generative organs of the walnut cultivars was characterized using the LT_{50} values obtained from the artificial freezing tests. The frost tolerance of the flower buds gradually developed in the first half of the winter. The hardening process had begun well before the first sampling date, as LT_{50} values of below -10 °C were generally recorded in mid-October. The initial stage of hardening occurs when the external temperature is still well above freezing point. By the time long-term frosts were experienced, the LT_{50} values of the flower buds approached -20 °C for some cultivars. The hardening process continued until January: the LT_{50} values were the lowest at the January sampling date in all the years. This was followed by the dehardening process, when the frost tolerance of the flower buds gradually declined, parallel with the gradual rise in the external temperature.

The frost tolerance of the three standard cultivars and 'Pedro' in the three years is illustrated in Fig. 1. Averaged over the three years, 'Pedro' was the most frost-sensitive and 'Tiszacsécsi 83' the most tolerant, the LT_{50} value of the latter being 4 °C lower on average in October than that of

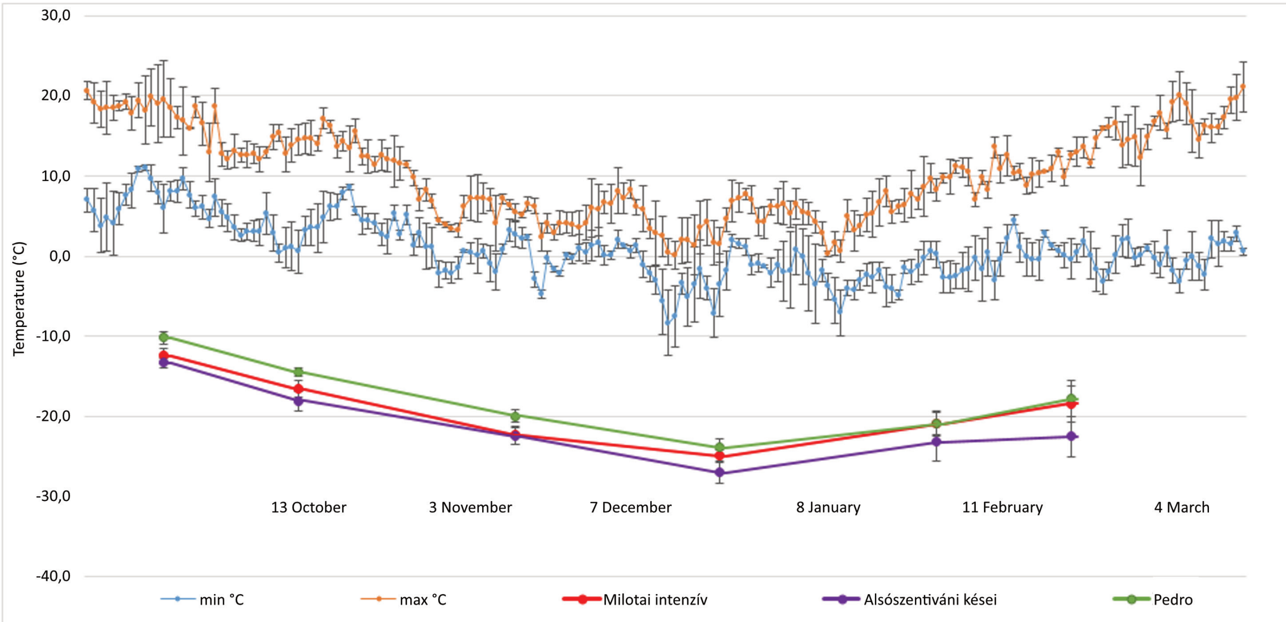

Figure 2. Frost tolerance mean values (mean and standard deviation) of the mixed buds of 'Alsószentiváni kései', 'Milotai intenzív' and 'Pedro', and three-year means and standard deviations of daily maximum and minimum temperatures (upper part of the figure).

'Pedro'. The average difference between the two cultivars was 3 °C in January and 5 °C in March. The maximum level of frost tolerance was recorded in January for all the cultivars, after which it gradually declined, with a continuous rise in the LT_{50} values. The frost tolerance of 'Milotai 10' was closer to that of 'Pedro', while that of 'Alsószentiváni 117' was almost the same as that of 'Tiszacsécsi 83'.

Fig. 2 gives a comparison of the frost tolerance of 'Milotai intenzív', 'Alsószentiváni kései' and 'Pedro', the first two of which are hybrids originating from a cross with 'Pedro' as pollinator. The results showed that the frost tolerance of 'Milotai intenzív' tended to be like that of 'Pedro', with lower values than that of the female parent 'Milotai 10'. Considerable differences were exhibited in the case of 'Alsószentiváni kései', particularly in January, February and March. In January the mixed buds of this genotype survived freezing at a temperature 2-3 °C lower than those of the other two genotypes on average, while in February and March the difference increased to 4-5 °C. The slower rise in LT_{50} values can be explained by the later budburst of this genotype.

Based on knowledge of the female flowering dates of the genotypes, a comparison was made of those in the medium late flowering group. Most of the genotypes included in the study belonged to this group, namely 'Milotai bőtermő', 'Milotai intenzív', Alsószentiváni 117', 'Tiszacsécsi 83' and 'Pedro' (Fig. 3). Genotypes in the same flowering groups could be expected to have similar levels of frost tolerance, which should be most evident at the March sampling date, shortly before budburst. Nevertheless, the results for individual genotypes could be clearly distinguished from each other, indicating that genetic traits other than the budburst date were also responsible for their winter hardiness. Even in January, the coldest month, 'Pedro' had the highest LT_{50} values, and the frost tolerance of 'Milotai intenzív' was closest to that of this cultivar, while 'Milotai bőtermő' was able to survive lower temperatures during the dormancy period than either of these genotypes. On the other hand, 'Alsószentiváni 117' and 'Tiszacsécsi 83' were both more frost-tolerant than the hybrids.

The flower buds of the genotypes were the most frost tolerant in January in all three dormancy periods. As the LT_{50} values of the individual genotypes did not differ significantly over the years, it was concluded that they had reached the maximum frost tolerance of which they were genetically capable, though tests need to be performed in further years to give convincing proof of this conclusion. The differences between the genotypes as regards the winter frost tolerance of the flower buds was analyzed for the January data. It can be seen in Fig. 4 that three homogeneous groups could be distinguished: 'Pedro' was frost-sensitive, 'Milotai kései', 'Milotai 10', 'Milotai bőtermő' and 'Mikotai intenzív' had medium frost tolerance, and 'Alsószentiváni 117', 'Alsószentiváni kései' and 'Tiszacsécsi 83' were frost-tolerant.

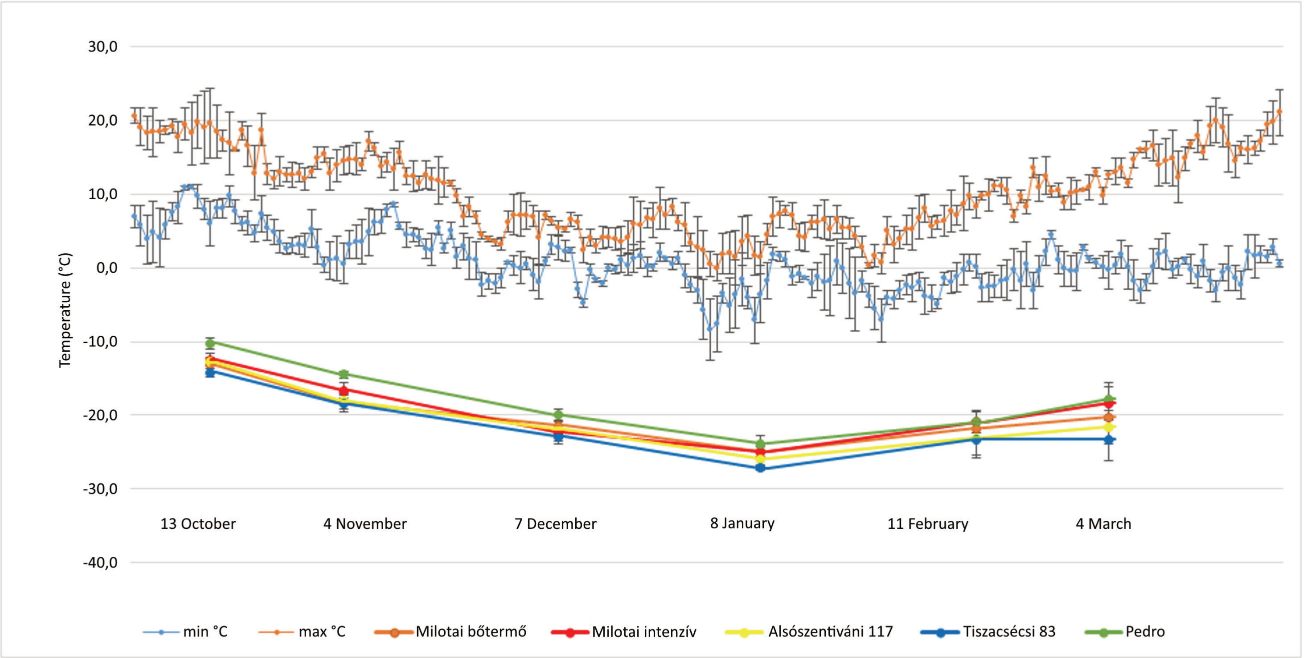

Figure 3. Frost tolerance mean values (mean and standard deviation) of the mixed buds of cultivars in the same flowering date groups, and three-year means and standard deviations of daily maximum and minimum temperatures (upper part of the figure).

Discussion

Frost damage in winter and spring often cause substantial yield losses in fruit and nut production. Among the overwintering organs of deciduous trees in the temperate zone, the generative organs (flower buds and mixed buds) are the most frost-sensitive during the dormancy period and suffer damage most frequently. The main emphasis has therefore always been placed on these organs in studies on the frost tolerance of different genotypes (Proebsting and Mills 1978; Faust 1989; Tromp 2005; Bartolini et al. 2006; Szalay et al. 2010; Salazar-Gutiérrez 2014). Under the same thermal conditions (including a constant mild temperature) walnut (*Juglans regia* L.) trees may exhibit diverse levels of frost tolerance (Charrier et al. 2013). In addition to genetic traits, frost tolerance is also influenced to a considerable extent by environmental factors, so the frost tolerance of individual genotypes may change as a function of the growing site and year (Pénzes and Szalay 2003; Szentiványi and Kállayné 2006). The general condition of the trees is a further influencing factor (Poirier et al. 2010). The most precise method for determining the frost tolerance of overwintering organs is the artificial freezing test (Pedryc 1999; Miranda et al. 2005; Szalay et al. 2010). In most cases LT_{50} values are used to determine frost tolerance (Lindén and Palonen 2000; Szalay et al. 2010; Ferguson et al. 2011; Salazar-Gutiérrez et al. 2014).

Walnut is not one of the most frost-sensitive fruit species in the temperate zone, as the overwintering organs are able to survive temperatures as low as -28-29 °C in December and January (Westwood 1993; Szentiványi and Kállayné 2006; Kállayné 2014). By comparison, the critical temperature is -25 °C for sweet cherry and -20 °C for peach during the winter dormancy period (Szewczuk et al. 2007). In Central Europe, including the Carpathian

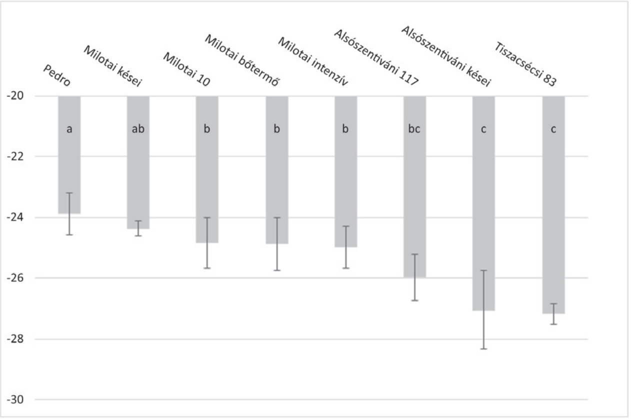

Figure 4. LT_{50} values of the flower buds of the tested cultivars averaged over three years, based on the data of freezing experiments in January. Different letters represent significant differences between cultivars at the LT_{50} value.

Basin, temperatures cold enough to damage walnut trees are rarely encountered in winter. In spring, however, after the buds burst, the young shoots and flower organs often sustain damage in these regions due to repeated spells of intense cold. For this reason, it is important for breeders to select cultivars with late budburst (Szentiványi and Kállayné 2006; Kállayné 2014). In order to achieve long-term yield safety, however, it is important to obtain knowledge on the frost tolerance of cultivated walnut genotypes during the winter dormancy period. For this reason, detailed investigations were begun on the winter frost tolerance of the cultivars selected or bred in Hungary that are grown most frequently in this country. The Californian-bred cultivar 'Pedro' was used as the control. A further incentive for this research was the fact that no detailed data are to be found either in the Hungarian or international literature on how the frost tolerance of the overwintering generative organs of walnut cultivars changes during the winter.

The LT_{50} values of the cultivars were determined on all the sampling dates during the dormancy period in all three years. Very similar results were obtained each year, so no significant year effect could be detected. The greatest frost tolerance was recorded for the cultivar 'Tiszacsécsi 83' during the years tested, while the most sensitive cultivar was the control genotype, 'Pedro'. The frost tolerance data for 'Alsószentiváni kései' were similar to those of 'Tiszacsécsi 83', while those of 'Milotai 10' were almost the same as those of 'Pedro'. Differences were observed between the hybrids, 'Milotai intenzív' being frost-sensitive, 'Milotai bőtermő' and 'Milotai kései' moderately frost-tolerant and 'Alsószentiváni 117' frost-tolerant. The flower buds were the most frost-tolerant in January. The values recorded in January were probably equivalent to the genetically programmed optimum frost tolerance levels, but this needs to be confirmed in further research. Charrier et al. (2011) observed a genotype effect between January and budburst, and during this period this played a greater role than the environmental effect.

The weather in the experimental years was characterized by slightly lower minimum temperatures in the first week of October, after which the temperature was like the long-term mean. The next substantial cold spell occurred in mid-December, which was followed by the second stage of hardening in the case of mixed buds. There was a further sudden intensive drop in temperature in mid-January, but by this time the mixed buds of walnut have reached maximum hardening, so frost tolerance means of -23.8 °C were measured on average even for the most frost-sensitive genotype. The gradual warming from January onwards was interrupted by very cold temperatures at dawn on one or two occasions, but as warming did not proceed at a rapid rate, these cold snaps were not a problem and the mixed buds retained their frost tolerance. By March the continuous rise in temperature led to higher LT_{50} values, but as the winter weather was similar at the experimental location in all three years, no significant year effect was detected. If the experiments were continued over a longer period, differences would probably be observed, as the effect of climatic factors on the frost tolerance of overwintering organs has been reported for a number of temperate fruit species (Faust 1989; Tromp 2005; Szalay et al. 2010). However, no relevant data on walnut are to be found in the literature.

It is difficult to compare the present results with earlier data, as those available were obtained at different locations for different cultivars. Based on the results obtained with various methods, however, it can be concluded that the frost tolerance of the overwintering organs constantly changes and that both genotypic and environmental factors play a role in hardening and dehardening processes, resulting in considerable differences between the cultivars (Aslamarz et al. 2010a; Aslamarz et al. 2010b; Charrier et al. 2013; Guàrdia et al. 2013; Charrier et al. 2018). The data recorded by Aslamarz et al. (2010a) in plantations in the neighborhood of Teheran showed 'Pedro' to be the most frost-tolerant of the cultivars, indicating that, under diverse climatic conditions, the same cultivar may give quite different results. This was confirmed in the present work, which was designed to provide information on the winter frost tolerance of walnut cultivars of importance for production in Hungary. Knowledge on the winter frost tolerance of cultivars is important for the determination of their suitability for cultivation at various growing sites, and is likely to become increasingly necessary in the light of climate change predictions, as alterations in climatic conditions will influence the developmental processes of cultivated plants and their levels of tolerance (Parmesan and Yohe 2003; Menzel et al. 2006).

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