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Preliminary Results on Seasonal Changes in Flower Bud Cold Hardiness of Sour Cherry

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Summary

Irregular yields of *Prunus cerasus* 'Stevnsbaer Birgitte', which is the preferred sour cherry cultivar in Denmark, have been proposed to be a result of freeze injury to floral tissues. This study determined floral mortality of *P. cerasus* 'Stevnsbaer Birgitte' and the supposedly more cold hardy *P. cerasus* 'Kelleriis 16' under natural conditions, and investigated seasonal changes in flower bud cold hardiness of 'Stevnsbaer Birgitte'. In a cold winter with unusual low temperatures in December, the injury rate of buds of 'Stevnsbaer Birgitte' was significantly higher than that of 'Kelleriis 16', confirming that buds of the latter cultivar are considerably more cold hardy than buds of 'Stevnsbaer Birgitte'. The majority of frost injuries in buds of 'Stevnsbaer Birgitte' occurred mid-winter, but dehardening appeared fast, indicating that the critical injury times of buds of 'Stevnsbaer Birgitte' include both the mid-winter and dehardening phases.

Key words. cold acclimation – deacclimation – dehardening – freezing tolerance – *Prunus cerasus* – winter injury

Introduction

Sour cherry (*Prunus cerasus* L.) is an important fruit crop in Denmark, with Denmark being the third biggest producer of sour cherry in the EU (Kosmala et al. 2009). Clones of the cultivar 'Stevnsbaer' have been recommended for cultivation for many years due to their superior juice quality and productivity (Vittrup-Christensen 1977). From 1992 onwards 'Stevnsbaer Birgitte' has been the preferred clone. The sour cherry production in Denmark is highly efficient due to mechanical harvesting and a rational orchard system, but during the last couple of decades large variations in yields and low yield efficiencies have been observed (Pedersen 2002). Freeze injury to floral tissues is believed to be a main cause of irregular fruit production in 'Stevnsbaer' (Kühn and Callesen 2001; Dencker and Toldam-Andersen 2005). According to Mathers (2004), autumn and spring are critical times for flower bud freezing injury in *P. cerasus*. Autumn injuries would occur when cold hardening is slow and/or delayed and sudden low temperatures occur without sufficient pre-exposure to freezing temperatures. Injuries in spring would occur when increased temperatures, inducing dehardening, are followed by freezing temperatures. Spring frost injuries are generally regarded as being more important than low winter temperatures in limiting crop production of deciduous fruit trees in temperate climates (Rodrigo 2000). In Denmark specifically, it has additionally been suggested that flower buds of 'Stevnsbaer Birgitte' suffer frost injuries during brief periods of freezing in otherwise mild winters (Dencker 2000, 2001). Another commonly cultivated Danish sour cherry cultivar is 'Kelleriis 16', which is also an important cultivar in Poland and Germany (Kosmala et al. 2009). The juice quality of 'Kelleriis 16' is less attractive than that of 'Stevnsbaer', but it is a stable-yielding cultivar, which seems less susceptible to frost injuries. Despite the key role of flower buds in 'Stevnsbaer Birgittes' commercial value, cold hardiness of 'Stevnsbaer Birgitte' buds have, to our knowledge, never been quantified.

The objectives of this study were; firstly, to investigate floral mortality of *P. cerasus* 'Stevnsbaer Birgitte' and the supposedly more cold hardy 'Kelleriis 16' under natural conditions. Secondly, to determine flower bud cold hardiness of 'Stevnsbaer Birgitte' during cold hardening in the autumn, midwinter and during spring dehardening and relate the obtained data to freeze injuries observed in the field. Improved knowledge on seasonal changes in 'Stevnsbaer Birgitte' bud cold hardiness is vital in order to be able to determine whether frost injuries is a main cause of irregular yields in the Danish sour cherry production and is valuable for assessing the cultivation potential of this cultivar under current and expected future climate conditions.

Materials and Methods

Location, climate and plant material

Two experiments including *Prunus cerasus* (L.) were carried out. In both experiments, studies were conducted on flower buds from detached shoots of the current season's growth collected in the experimental orchard at the Department of Food Science, Aarhus University in Årslev, Denmark (latitude 55° 18' N). Trees were 6-year old and grafted onto the rootstock Colt. In experiment one, severity of flower bud freezing injuries under natural conditions was investigated in *P. cerasus* cvs. 'Stevnsbaer Birgitte' and 'Kelleriis 16'. In experiment two, seasonal changes in flower bud cold hardiness of 'Stevnsbaer Birgitte' was determined at selected freezing temperatures. The experiments were carried out from August 2010 to April 2011 and local air temperature data were obtained from the Department's climate station, which is operated by the Danish Meteorological Institute. From August to December 2010 the daily mean temperature declined gradually by ca. 4 °C per month from on average 16.4 °C in August to 3.7 °C in November (Fig. 1). In October, the temperature dropped below the freezing point on one occasion, whereas November had 10 d with temperatures below 0 °C. December turned out to be unusual cold with an average daily mean temperature of −3.9 °C and an absolute minimum of −19.7 °C at 22 December. In January, February and March of 2011 the average daily mean temperatures were 0.5, −0.1 and 3.4 °C, respectively, and freezing temperatures occurred at approximately two thirds of the days in January and February and at one third of the days in March.

Evaluation of flower buds

In experiment one, buds of 'Stevnsbaer Birgitte' and 'Kelleriis 16' were evaluated for freezing injuries at 24 August, 15 September, 18 October, 1 November, 15 November, 30 November, 14 December, 11 January, 16 February, 1 March, 15 March and 30 March. At each sampling date 6 annual short shoots of 'Stevnsbaer Birgitte' and 6 extended annual shoots of 'Kelleriis 16' were randomly sampled from each of 6 trees per cultivar at a height of ca. 1.5 m above the ground. Samples were sealed in plastic bags and brought to the laboratory. Non-frozen buds were evaluated immediately upon return to the laboratory, whereas buds sampled on days with freezing temperatures were evaluated after 24 h of thawing at 4 °C. From each shoot the second lateral bud from the distal end was cut off with a short piece of wood, dissected under a dissecting microscope and assessed for injury. The bud scales were removed, and the individual flowers of each bud were classified visually as either dead (all brown) or alive (succulent and green or partly brown) (Fig. 2A–C). Succulent flowers were sliced lengthwise to examine pistils for damage, and the floral data were classified as alive or injured (Fig. 2D–E). A total of 6 buds per tree were evaluated, giving a total of 36 buds per cultivar and sampling date.

In experiment two, bud cold hardiness of 'Stevnsbaer Birgitte' was evaluated at four temperatures; one control (4 °C) and three subfreezing temperatures at August 24, October 18, November 15, December 14, January 11, February 16 and March 15. Temperatures chosen depended on the projected seasonal changes in bud freezing tolerance. 12 annual short shoots were randomly collected from each of six trees, sealed in plastic bags and brought to the laboratory. In the laboratory, the shoots were wrapped in moist paper towels to ensure ice nucleation and inserted into sealed plastic bags. Shoots were incubated in a prechilled controlled freezer on top of an aluminium grating. The samples were cooled at a rate of maximum 5 °C per hour to 0 °C and subsequently at 2 °C per hour until the selected temperature were reached. The selected tem-

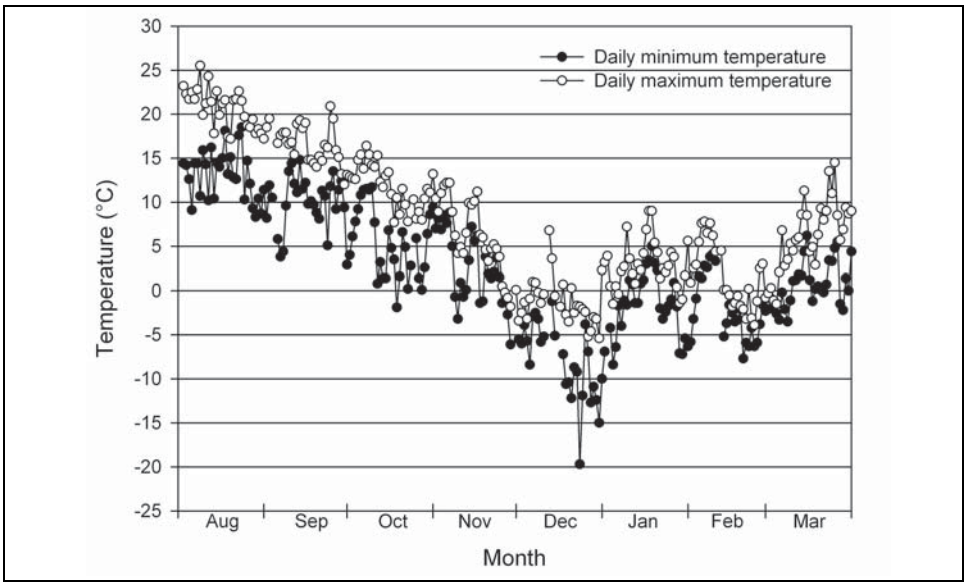


Fig. 1. Minimum and maximum daily air temperatures (°C) at the expermental site from August 2010 to April 2011.

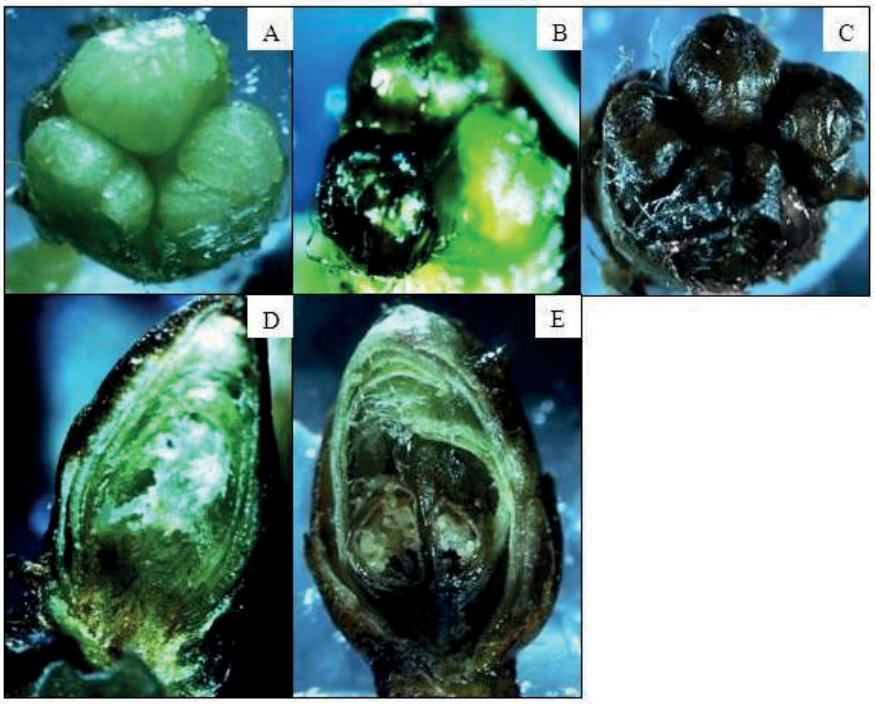


Fig. 2. Pictures of flower buds of *Prunus cerasus* 'Stevnsbaer Birgitte' assessed for frost injuries. After removal of bud scales, individual flowers of each bud were classified visually as either alive (succulent and green or partly brown, A–B) or dead (all brown, C). Succulent flowers were sliced lengthwise to examine pistils for damage, and the floral data were classified as alive (D) or injured (E).

peratures were 0, −2 and −5 °C at August 24, −5, −10 and −15 °C at October 18, November 15, December 14, January 11 and February 16 and −1, −5 and −10 °C at the last sampling date. The selected temperature was maintained for 2 h, thereafter the samples were withdrawn and thawed overnight on ice at 4 °C. After freezing and thawing, shoots were incubated at 20 °C for one to three days. All buds on each short shoot were then dissected and evaluated in the same way as in experiment one.

Results

Bud freezing injuries under natural conditions

Only few percentages of flower buds of annual shoots of *P. cerasus* 'Kelleriis 16' were classified as 'injured' at specific sampling dates and none were dead, indicating that buds of 'Kelleriis 16' did not suffer any freezing injuries in autumn, mid-winter or in spring (Table 1). In contrast, 20–28 % of the flower buds of short shoots of 'Stevnsbaer Birgitte' were freeze injured in late November and mid-December. In February, this number increased to 67 %. At the end of the experiment, approximately one third of the flower buds of 'Stevnsbaer Birgitte' were injured, while the remaining two thirds were classified as being alive.

Bud cold hardiness of 'Stevnsbaer Birgitte'

As expected, in experiment two, the percentages of injured and dead flower buds of short shoots of 'Stevnsbaer Birgitte' increased as the treatment temperatures decreased (Table 2). However, from late August to mid-October the percentages of injured and dead buds of samples frozen to −5 °C decreased considerably, indicating that buds became increasingly cold hardy. Similarly, in mid-December relatively more flower buds survived and less became injured when frozen to −15 °C compared to in October and November. In January, this trend was slightly reversed, and in February freezing to −15 °C resulted in approximately the same percentages of alive, injured and dead buds as in October and November. Cold hardiness of flower buds decreased further in March, as no buds survived freezing to −10 °C and even at −5 °C the percentage of surviving buds was greatly reduced. In accordance with experiment one, some flower buds were freeze-injured in the field, implying that at some sampling dates even buds kept at 4 °C appeared injured in experiment two. This was the case in mid-December and particularly at the last two sampling dates

Discussion

Experiment one confirmed that flower buds of *P. cerasus* 'Kelleriis 16' was considerably more cold hardy than flower buds of 'Stevnsbaer Birgitte'. An evaluation of different *P. cerasus* genotypes has previously shown that floral bud hardiness varies considerably among genotypes and that some genotypes mid-winter have LT_{50} values lower than −20 °C (Mathers 2004). Since cold hardening and dehardening are controlled by environmental factors,

Table 1. The percentages of flower buds of *Prunus cerasus* 'Kelleriis 16' and *P. cerasus* 'Stevnsbaer Birgitte', grown in the field, being alive, injured or dead at the indicated sampling days (n = 36).

	'Kelleriis 16'			'Stevnsbaer Birgitte'		
Date	Alive (%)	Injured (%)	Dead (%)	Alive (%)	Injured (%)	Dead (%)
24 Aug	100.0	0.0	0.0	100.0	0.0	0.0
15 Sept	100.0	0.0	0.0	100.0	0.0	0.0
18 Oct	97.2	2.8	0.0	94.3	2.9	2.9
01 Nov	100.0	0.0	0.0	97.2	2.8	0.0
15 Nov	100.0	0.0	0.0	97.2	2.8	0.0
30 Nov	97.2	2.8	0.0	72.2	27.8	0.0
14 Dec	100.0	0.0	0.0	80.0	20.0	0.0
11 Jan	94.4	5.6	0.0	83.3	13.9	2.8
16 Feb	97.2	2.8	0.0	33.3	66.7	0.0
01 Mar	94.4	5.6	0.0	47.2	44.4	8.3
15 Mar	97.2	2.8	0.0	60.0	38.6	1.4
30 Mar	100.0	0.0	0.0	66.2	33.8	0.0

Table 2. The percentages of flower buds of *Prunus cerasus* 'Stevnsbaer Birgitte' being alive, injured or dead after exposure to 4 °C or freezing to the indicated temperatures (n = 30 – 95). Trees were grown in the field and flower buds were evaluated at 24 August, 18 October, 15 November, 14 December, 11 January, 16 February and 15 March.

		Test temperature					
Date		4 °C	0 °C	−2 °C	−5 °C	−10 °C	−15 °C
24 Aug	Alive (%)	100.0	100.0	100.0	33.3		
	Injured (%)	0.0	0.0	0.0	26.7		
	Dead (%)	0.0	0.0	0.0	40.0		
18 Oct	Alive (%)	100.0			100.0	97.8	1.1
	Injured (%)	0.0			0.0	2.2	81.9
	Dead (%)	0.0			0.0	0.0	17.0
15 Nov	Alive (%)	97.6			98.9	84.9	0.0
	Injured (%)	2.4			1.1	15.1	89.4
	Dead (%)	0.0			0.0	0.0	10.6
14 Dec	Alive (%)	79.4			72.0	73.3	43.6
	Injured (%)	20.6			25.6	25.7	33.3
	Dead (%)	0.0			2.4	1.0	23.1
11 Jan	Alive (%)	86.8			85.1	78.1	38.5
	Injured (%)	11.8			6.8	8.2	46.2
	Dead (%)	1.3			8.1	13.7	15.4
16 Feb	Alive (%)	33.3			35.7	13.6	0
	Injured (%)	66.7			63.1	85.2	97.6
	Dead (%)	0.0			1.2	1.2	2.4
15 Mar	Alive (%)	60.0		60.0	11.8	0	
	Injured (%)	38.6		36.5	84.2	68.1	
	Dead (%)	1.4		3.5	3.9	31.9	

the timing and rate of hardening and dehardening and fluctuations in flower bud hardiness during midwinter may vary somewhat between years (Weiser 1970; Kalberer et al. 2006). However, the present data suggest that in the rather mild climate of Denmark, 'Kelleriis 16' develop greater flower bud hardiness than is usually required for successful overwintering, whereas insufficient cold hardiness may indeed limit fruit production of 'Stevnsbaer Birgitte'. The latter is in accordance with previous recordings, where large numbers of dead and injured flower buds were observed in 'Stevnsbaer' in spring (Kühn and Callesen 2001; Dencker and Toldam-Andersen 2005).

The lowest air temperatures occurred in the latter half of December and in early January; hence, it was surprising that in experiment one most flower buds of 'Stevnsbaer Birgitte' were classified as being alive at 11 January, whereas at 16 February, following a relatively milder period, the rate of injured buds increased substantially. Probably low temperatures between mid-December and 11 January delayed development of freezing injuries, as buds presumably were frozen most of the time. Thus, the large number of injured buds observed in mid-February was likely after effects of freezing stress occurring in late December and early January. In March, decreased and increased percentages of injured and alive buds, respectively, indicated that buds which were not irreversibly damaged during winter may have been able to recover somewhat. Similarly, signs of frost injuries to stems and buds of eight walnut cultivars and selections decreased considerably from February to April following a severe mid-winter freezing event (Aslamarz et al. 2010). Postthaw recovery of freezing injury in onion cells have been shown to depend on the functional activity of plasma membrane ATPases, which may actively pump leaked ions into the cell, leading to uptake of extracellular water and regain of turgor (Arora and Palta 1991).

Experiment two revealed that from late August to mid-October cold hardening increased flower bud hardiness of 'Stevnsbaer Birgitte' from less than −5 °C to between −10 °C to −15 °C. In mid-December 'Stevnsbaer Birgitte' flower bud hardiness had increased further compared to October and November. This increase in hardiness coincided with a decrease in air temperature, suggesting that development of maximum cold hardiness requires more than few mild freezing events. In three cultivars of *Prunus persica* Szalay et al. (2010) also observed two phases of cold hardening of flower buds. The greatest increase in hardiness developed in the first phase, which lasted until the external temperature dropped consistently below 0 °C. However, following a period of constant sub-zero air temperatures, bud cold hardiness increased further with about 5 °C. Maximum floral tissue hardiness of 'Stevnsbaer Birgitte' was limited to ca. −15 °C, supporting the suggestion that the frost injuries observed in experiment one were due to insufficient mid-winter hardiness.

Despite relative cool temperatures in February and March 2011, floral hardiness of 'Stevnsbaer Birgitte' decreased rapidly after January, indicating that flower buds respond sensitively to warming and that their frost resistance decreases rapidly in response to increasing temperature. This is in accordance with reports for *P. avium*, *P. cerasus* and *P. persica* (Kadir and Proebsting 1994; Mathers 2004; Szalay et al. 2010). It also suggests that the threshold temperature for onset of dehardening of fully cold hardy buds is rather low. The threshold temperature for onset of dehardening of fully cold hardy stems and bark tissues of other woody perennials has previously been suggested to be ca. 5 °C (Sauter et al. 1996; Pagter et al. 2011), but buds may be less resistant to dehardening and deharden faster than stems (Pagter and Williams 2011).

In summary, this study confirmed that buds of 'Kelleriis 16' are considerably more cold hardy than buds of 'Stevnsbaer Birgitte'. The critical injury times of buds of 'Stevnsbaer Birgitte' include both the mid-winter and dehardening phases, due to their modest maximum hardiness and fast dehardening in spring. To accurately determine the importance of low temperatures on fruit yield of 'Stevnsbaer Birgitte' further studies encompassing more years are required. However, our results indicate that selection of cultivars and/or clones with delayed dehardening and greater maximum bud cold hardiness than 'Stevnsbaer Birgitte' should be considered when planning new orchards.

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