Low-temperature Tolerance of Blackcurrant Flowers

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Abstract. The low-temperature tolerance of flowers from three blackcurrant (*Ribes nigrum* L.) cultivars, 'Brödtorp', 'Ben Tirran', and 'Baldwin', was determined at two stages of floral development. The three cultivars together represent a large part of the available genetic base for this subgenus of *Ribes*. Plants were maintained either at 4 °C in a growth cabinet under a 16-hour photoperiod or outdoors in Scotland during Spring 1997. Observed genotypic differences in survival were not associated with differences in LT₅₀ of the flowers, and observations of freezing damage to flowers on intact plants suggest that the flowers can often survive by supercooling. This hypothesis is partly confirmed by the finding that detached flowers from all three cultivars have the capacity to supercool to at least –9 °C. Ice nucleation in stem tissue, however, was found to occur at or above –2 °C. That flowers on intact plants can apparently survive by supercooling, together with the finding that ice nucleation in stem tissue occurs at temperatures well above the LT₅₀ of flowers, indicate the presence of barriers to propagation of ice from stem tissue to raceme. Such barriers within individual racemes are also indicated by patterns of freezing damage to flowers on intact plants cooled to –5 °C.

Because blackcurrants flower early in spring, frost damage is a common and serious problem for commercial producers across the temperate regions of the globe. Tolerance to spring frost damage has been an objective of the blackcurrant breeding program at the Scottish Crop Research Institute (SCRI) for several years, and genotypic differences for this trait have been noted and exploited in commercially successful cultivars (Brennan, 1996).

Genotypic differences in tolerance to spring frost damage could be the result of several factors: 1) timing of flowering, i.e., the later flowers emerge, the less likely they are to experience a frost; 2) genotypic differences in

the freezing tolerance of the flowers themselves; 3) genotypic differences in the ability to supercool and thus avoid freezing; or 4) a combination of differences in floral phenology and inherent differences in freezing tolerance or avoidance associated with each phenological stage.

The timing of flowering is genetically controlled, and much of the breeding effort to date has concentrated on the use of delayed flowering as a means of frost avoidance rather than frost tolerance (Brennan, 1996). This strategy culminated with the cultivar Ben Tirran, the fruit of which is typically ready for harvest in early August. Inevitably, a point is reached where further delay of flowering will compromise fruit yield, and a search for physiological tolerance to frost in the floral tissues becomes necessary. Similarly, the development of early-fruited cultivars requires freezing tolerance in the flowers because of the correspondingly early flowering.

To ascertain genotypic variation in frost tolerance in flowers of blackcurrant, a range of germplasm, sourced mainly from northern latitudes, notably Scandinavia and Russia, was evaluated under controlled freezing conditions by Dale (1981, 1987) and Brennan (1991). They used intact plants in conditions of simulated frost down to –4 °C, and clear differences were reported between genotypes of northern and southern origins. Furthermore, sensitivity to freezing appears to increase as flowering progresses within many of the genotypes tested. The improved freezing tolerance in flowers of

some of the Scandinavian genotypes has been exploited in the SCRI breeding program (Dale, 1987), resulting in the release of commercially successful cultivars. Differences in cultivar response have also been reported by Gill and Waister (1980), Mather et al. (1980), and Keep et al. (1983), all using whole plants in controlled-temperature environments.

Genotypic variability in freezing tolerance of detached flowers was examined in the present study, and the results were related to the effects of freezing on whole plants (Brennan, 1991; Dale, 1987). To this end, the LT₅₀ (temperature at which 50% of the tissue under study is killed as measured by electrolyte leakage and visual observation) was determined for flowers of 'Brödtorp' (an early-flowering frost-hardy cultivar of Finnish origin), 'Baldwin' (a U.K.-derived cultivar of unknown origin, grown commercially for >100 years, which is intermediate in time of flowering and is recognized as being highly frost-sensitive in European field situations), and 'Ben Tirran' (a very late-flowering cultivar from the SCRI breeding program). Values of LT₅₀ as a function of flower development within a single genotype were also determined. In addition, the freezing tolerance of flowers from plants kept at 4 °C was observed to determine whether flower development at low temperature has a measurable effect on freezing tolerance.

Lastly, ice nucleation temperatures for stem tissue from several blackcurrant cultivars were determined, and the question of whether blackcurrant flowers have the capability to supercool was addressed. The goal of this study was to develop a better understanding of the freezing process in blackcurrant plants in flower. Such information will be important in formulating new breeding strategies and developing new frost-protection management practices.

Materials and Methods

Two-year-old plants grown in pots in peat-based compost were maintained outdoors at SCRI (lat. 55°N). Plants were well-watered and free of disease. In early March, one set of plants was transferred from outside, where they had overwintered, to a temperature-programmable growth cabinet (deRoma, Liverpool, England) and held at a constant 4 °C with a 16-h photoperiod supplied by high-density mercury vapor lights (100 µmol·m⁻²·s⁻¹ at plant height). The plants that were allowed to deacclimate normally were maintained outside in a well-ventilated screen house. Flowers were assessed at the closed ("grape") and fully opened stages.

To facilitate the use of specialized equipment (described below), stem tissue for the measurement of ice nucleation temperature was harvested in Feb. 1998 from mature, field-grown 'Baldwin' plants at the U.S. Dept. of Agriculture–Agricultural Research Service (USDA–ARS) facilities in Beltsville, Md.

Assessment of freezing injury: visual observation. After a minimum of 48 h following the controlled cooling treatment, flowers were

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scored as either alive (green, healthy appearance, no fungal infection) or dead (brown or black, water soaked, saprophytic fungi present).

Assessment of freezing injury: electrolyte leakage. Flowers from each treatment were placed in three 50-mL plastic centrifuge tubes to which 12 mL of distilled water was added. After at least 24 h following the controlled cooling treatment, conductivity (in μS) was determined for each sample using a conductivity bridge (Jenway Model 4070; Jencons Scientific Ltd., Leighton Buzzard, Bedfordshire, England). All samples were then frozen to -80°C , thawed, and conductivity again measured. The percent leakage was determined from the ratio of these two values. Three replicates were used for each temperature treatment.

Determination of LT_{50} of detached flowers, peduncles, and pedicels. Open and grape-stage flowers were harvested, placed in wide-mouthed vials, 10 flowers per vial, and the vials placed in a temperature-programmable chamber (Tenney Benchmaster Versa Tenn II, Union, N.J.). Chamber temperature was held at $0.0 \pm 0.2^\circ\text{C}$ for 1 h. Flowers were floated on 0.5 to 1.0 mL of distilled water in each vial (so that all flowers in a vial were in contact with the same pool of water) and at the end of the 1-h period at 0°C , a small quantity of ice was added to each vial to provide ice nucleation. Thus, as the temperature dropped below 0°C , the water froze, ensuring that each flower was in contact with ice. The chamber temperature was lowered at $2^\circ\text{C}/\text{h}$ and maintained at each test temperature for 0.5 h prior to removal of samples. Since samples were routinely removed at -2 , -4 , -6 , -8 , and -10°C , the effective cooling rate was $1.3^\circ\text{C}/\text{h}$. Samples were placed in an ice bucket at 0°C after removal from the chamber until completion of the entire controlled cooling run, then placed in a 4°C chamber overnight. To allow the freezing damage to develop, they were left at ambient temperature for at least 24 h prior to conductivity measurements. This method was also used to determine LT_{50} values for peduncles and pedicels. Peduncles and pedicels were taken from racemes that were fully developed and that contained both open and grape-stage flowers.

Supercooling capacity. Flowers were subjected to the same cooling regime as described above but were not wetted and were not nucleated with ice. Injury resulting from this treatment was assessed both by visual observation and by electrolyte leakage, using the cultivars Baldwin and Ben Tirran.

Comparison of freezing effects on attached and detached flowers. To compare the effects of exposure to subzero temperatures on attached and detached flowers, whole 'Baldwin' and 'Ben Tirran' plants were cooled to -5°C . After holding at 0°C for 1 h, the chamber's temperature was lowered at $2^\circ\text{C}/\text{h}$ to a selected temperature, then raised to 0°C at $1^\circ\text{C}/\text{h}$, held at 0°C for 4 h, and raised to 4°C at $1^\circ\text{C}/\text{h}$. Visual observations of injury were made after at least 48 h at 4°C .

Determination of ice nucleation temperatures in stem tissue. Measurements of ice nucleation temperature were made at the

USDA-ARS facility in Kearneysville, W. Va., by infrared thermography using an Inframetric Model 760 Radiometer (Inframetrics, Billerica, Mass.), as described by Wisniewski et al. (1997). 'Baldwin' stem sections were harvested from plants in the collection at the USDA-ARS facility in Beltsville, Md., wrapped in moist paper towels, and shipped by overnight mail to Kearneysville. The infrared video camera was mounted vertically in a temperature-programmable cabinet (Tenney). Stem sections were held in an open cardboard box to minimize thermal fluctuations caused by air circulation patterns within the chamber. A cooling rate of $5^\circ\text{C}/\text{h}$ was used, based on previous work (Wisniewski et al., 1997) in which no differences in nucleation temperatures or ice propagation were found for cooling rates between 1 and $5^\circ\text{C}/\text{h}$. Images were analyzed in real time and recorded on videotape. A temperature span of 2°C was selected for the camera such that, on the color palette, the color on the extreme left of the scale is 2° lower than the color on the extreme right of the scale. The midpoint of this temperature range was periodically adjusted during an experiment such that freezing exotherms occurred within the set range. By replaying the videotape the position of a nucleation site could be precisely determined. The color of this site was then determined a few seconds prior to the nucleation event. By matching this color with the same color on the palette bar at the bottom of the screen, a nucleation temperature was determined by interpolation. Accuracy of temperature determination was $\pm 0.1^\circ\text{C}$.

Results

The LT_{50} values for open flowers ranged from -4.3 to -5.1°C , and for grape-stage flowers from -2.2 to -5.8°C (Table 1). Open flowers from field-grown plants and from plants held at 4°C during flower development had an average LT_{50} of -4.8°C across cultivars. Peduncle and pedicel LT_{50} , determined for 'Ben Tirran' plants, were both -5.0°C . Values for grape-stage flowers from plants held at 4°C during flower development averaged -5.1°C across cultivars, not significantly different from open flowers. 'Ben Tirran' was the only cultivar for which an LT_{50} value was measured for grape-stage flowers from field-grown plants; the reason that this material was

so much less tolerant to freezing than all other flowers examined is not clear from the data. It is possible, but unlikely, that, even though these flowers were in contact with ice at between 0 and -1°C , ice did not propagate into them and they supercooled to around -2°C before freezing. Apart from this value, the mean of all other LT_{50} values was -5.0°C , with SE of no more than 1.0°C . For open flowers, then, there was no variation in LT_{50} with cultivar or with temperature of flower development (4°C or field temperatures). For grape-stage flowers that developed at 4°C there was no variation in LT_{50} with cultivar. No statement can be made concerning cultivar differences in LT_{50} for grape-stage flowers that developed under field conditions because experimental constraints did not allow the completion of this data set.

In the nonwetted treatments, designed to test the capacity of flowers to supercool, all flowers were excised from plants and placed in vials containing no water; further, ice was not added to these vials between 0 and -1°C . Thus, the flowers were not forced to freeze. For 'Baldwin' (grape-stage flowers from 4°C -grown plants, and open and grape-stage flowers from field-grown plants), the lowest temperature to which flowers were exposed was -9°C , and they suffered no injury as determined by visual observation. Open flowers of 'Baldwin', and both open and grape-stage 'Ben Tirran' flowers, from plants grown at 4°C showed no injury after exposure to -10°C as determined by electrolyte leakage. Similar results were obtained for open and grape-stage flowers from field-grown 'Ben Tirran' plants. Thus, open and grape-stage flowers excised from 'Baldwin' and 'Ben Tirran' plants grown either at 4°C or under field conditions were able to supercool to at least -9°C in the laboratory.

In the examination of flowers on whole plants cooled to -5°C , many racemes were found on which all flowers were alive, and many others on which all flowers were dead (data not shown). Racemes were also found on which some flowers were dead and others were alive (Fig. 1a). Occasionally racemes were found in which the pedicel of a dead flower was alive (Fig. 1b), and, more commonly, in which the pedicel of a dead flower was also dead, but the peduncle was alive (Fig. 1c). Also noted was a raceme in which the

Table 1. Effects of stage of development and holding conditions on LT_{50} of blackcurrant flowers, peduncles, and pedicels as determined by electrolyte leakage. Flowers, peduncles, and pedicels were detached from plants, placed in small vials containing enough water to wet the flowers, and cooled at $1.33^\circ\text{C}/\text{h}$. Ice formation was ensured by adding ice crystals to each vial after treatment temperature reached -2°C .

Stage of development of flowers	Plants grown in:	Cultivar and tissue				
		Brödtorp flower	Baldwin flower	Ben Tirran		
				Flower	Peduncle ^z	Pedicel ^z
Grape	Cabinet ^y	-5.5 ± 0.2^x	4.0 ± 0.4	-5.8 ± 1.2	-5.0 ± 1.0^w	-5.0 ± 1.0^w
	Field	---	---	-2.2 ± 0.4		
Open	Cabinet	-5.1 ± 0.1	-5.0 ± 1.0^w	-4.3 ± 0.5		
	Field	-4.5 ± 1.0^w	$-5.0 \pm 1.0^{w,y}$	-4.8 ± 0.3		

^zPeduncles and pedicels were taken from racemes that were fully developed.

^yHeld at 4°C during flower development.

^xStandard error determined by nonlinear regression analysis unless otherwise noted.

^wEstimated SE. Shape of regression did not allow calculation of SE.

^yDetermined by visual observation, rather than by electrolyte leakage.

Fig. 1. Different manifestations of freezing injury in blackcurrant racemes, resulting from slowly cooling whole plants to -5°C . (a) Raceme from a 'Baldwin' plant in which one flower and its pedicel show freezing injury but a nearby flower and its pedicel are unharmed. (b) Freeze-injured 'Ben Tirran' flower attached to undamaged pedicel. (c) 'Ben Tirran' flower with attached pedicel both showing freeze injury, attached to undamaged peduncle. (d) 'Ben Tirran' flower with attached pedicel, neither showing freeze damage, attached to freeze-injured peduncle.

peduncle showed freezing damage, but attached to it was a live flower with a healthy pedicel (Fig. 1d). Of the many pedicels and peduncles examined, none showed partial damage.

The freezing event occurring in a stem section ≈ 20 cm long was visualized by infrared thermography (Fig. 2). Ice initially formed close to the left end of the stem (Fig. 2a) at a temperature of -1.2°C , and propagated to the other end in ≈ 63 s (Fig. 2b and subsequent images not shown; at the time Fig. 2b was recorded, propagation was not complete). The three areas indicated by the arrows in Fig. 2b are buds, into which ice had not propagated. Ice did not propagate into any of the buds on any of the six stems during this experiment, which was terminated, after the last stem had frozen, at -4.2°C . The mean nucleation temperature for all stems in this experiment was -2.7°C .

Discussion

One of the objectives of this study was to determine the maximum freezing tolerance of blackcurrant flowers. Because the freezing tolerance of plant tissue can be reduced if it supercools prior to freezing (Lindstrom et al., 1983, 1992; Rajashekar et al., 1983), super-

cooling must be minimized in determining maximum freezing tolerance. The approach taken here was similar to that used in many other examinations of freezing tolerance: the plant tissue to be tested was floated on water, and, after thermal equilibrium at 0°C was attained, ice was added to each sample vial. When cooling resumed, the water froze, ensuring that each flower was in physical contact with ice from a temperature very close to 0°C to the lowest temperature reached during each experiment. If this treatment was effective at minimizing supercooling, then the LT_{50} of blackcurrant flowers is about -5°C . An alternative hypothesis is that the flowers supercooled even though in contact with ice, then froze, and died at the temperature at which they froze. Although the LT_{50} values in Table 1 do not rule out this alternative hypothesis, they argue against it; with one exception they are quite close to -5°C , whereas if the flowers had supercooled before freezing they would likely have exhibited a broader range of killing temperatures. Blackcurrant flowers thus appear to possess a surprising degree of freezing tolerance; if supercooling is minimized, they can withstand being frozen to about -5°C .

A blackcurrant flower can respond to a spring frost in three ways. It can freeze in isolation from the rest of the plant if it is in

contact with an effective ice nucleator. Thus, the flower could be the initial, or an independent, site of ice formation in the plant. (It would appear that the dead flower in Fig. 1a could have been frozen in this way.) Whether it survives this freeze will depend on the temperature at which ice forms in it and the minimum temperature it reaches.

The flower can also supercool and then freeze when it is nucleated by an ice front propagating from a distant part of the plant that had previously frozen. In this case, whether it survives depends on the temperature of the flower when the ice front reaches it and the minimum temperature it reaches thereafter.

Lastly, the flower can remain in a supercooled state for the duration of the frost and suffer no damage. *Solanum acaule* Bitt. plants exposed to -4°C for several hours were not injured if they remained supercooled, but if they froze after supercooling to -4°C they were severely injured (Lindstrom et al., 1992). The present study shows that excised 'Baldwin' and 'Ben Tirran' flowers, and presumably flowers of other blackcurrant cultivars, have the capacity to supercool to at least -9°C with no damage. While finding flowers that supercooled to -9°C on whole plants would be surprising, observations of whole plants cooled to -5°C containing racemes bearing only live

flowers (data not shown), only dead flowers (data not shown), and both live and dead flowers (Fig. 1a) make relevant the question of how much supercooling occurs before ice is initiated and propagated within blackcurrant racemes.

Measurements of ice nucleation temperatures in blackcurrant stem tissue by infrared thermography demonstrate that ice will form in stems of intact plants at temperatures around -2°C , or even higher (Fig. 2), during winter when plants are dormant. Of the six stem sections in Fig. 2, the first one to freeze nucleated at -1.2°C , and it took 63 s for ice to move from the site of initiation, near the left end, to the right end, a distance of ≈ 20 cm. The last one to freeze nucleated at -4.2°C at a site close to the left end, and ice reached the other end 7 s later, also covering a distance of ≈ 20 cm. The mean nucleation temperature for these six stem sections was -2.7°C . In a separate experiment carried out on 30 Apr. 1998, while plants were in flower, the mean nucleation temperature of seven stem sections was -2.8°C (data not shown). This observation demonstrates that stem ice-nucleation characteristics do not change as plants exit eco-dormancy and enter reproductive phase. Stems of intact plants also may nucleate at a higher temperature than do cut stems, such as those used in these observations. Thus, when flowering blackcurrants are exposed to -5°C , ice will be present in stem tissue.

The presence of a barrier to propagation of ice into dormant blackcurrant buds has been indirectly demonstrated by the fact that certain parts of these buds exhibit deep supercooling (Stone et al., 1993; Takeda et al., 1993). Thus, it was not surprising to observe ice forming in stems and propagating past, but not into, dormant buds (Fig. 2). That many racemes, and parts of racemes, are not damaged by exposure to -5°C suggests that this barrier to ice propagation is not completely removed during the period of active growth leading up to flowering. Many racemes on the plants cooled to -5°C are completely killed, however, which can be explained in terms of this barrier being absent or penetrated when the ice front reaches the raceme. The dependence of propagation rate on degree of supercooling has been noted previously (Wisniewski et al., 1997). In addition to the increase in rate of ice propagation, the excess free energy of freezing increases as the degree of supercooling increases (Lindstrom and Carter, 1983), meaning the potential for injury related to the freezing event increases with the degree of supercooling. Thus, the barrier to propagation of ice from stem to raceme may not be broken by a gentle freeze in a stem at temperatures above -2°C , while a stem that happens to supercool significantly before freezing may have this barrier broken when the ice front reaches it.

If ice reached a dead flower on an otherwise healthy raceme by traveling through the peduncle and then the flower's pedicel, these two structures would have to be more freezing-tolerant than the flower. However, the freezing tolerances for peduncles and pedicels of 'Ben Tirran' racemes are -5°C (Table 1),

Fig. 2. Ice nucleation and propagation through stem sections of blackcurrant 'Baldwin'. (a) Six stem sections are within the camera's field of view. Not all are easily visible because only when stem temperature is significantly different from background can stems be seen. White, oval objects associated with stem sections are dormant buds. Site of nucleation in stem #4, the first to freeze, is noted by **arrow** at bottom left. Temperature at nucleation site when ice initially forms was -1.2°C . Outline of stem #6 can be seen in upper left, indicated by arrows. Ice nucleation in this stem, the last of the six to freeze, occurred at -4.2°C . (b) Propagation of ice through stem #4. Forty-six seconds after initiation, ice front has almost reached the other end of the section. Ice did not propagate into dormant buds on stem #4 (**arrowheads**), or into buds on any of the stems.

very close to the LT_{50} of open flowers. Thus, if ice initiates in a stem and moves into a raceme and the temperature then drops to the LT_{50} , the entire raceme—peduncle, pedicels, and flowers—would show freezing damage. Patterns of freezing damage as shown in Fig. 1 suggest the existence of multiple barriers to ice propagation within a raceme. The alternative hypothesis—that all tissues in all racemes are frozen but damage occurs in some, but not others—although not disproven by our results, seems unlikely, as it would require a

broader variation in freezing tolerance among individual flowers, peduncles, and pedicels than we observed.

The genotypic variation in frost tolerance of detached blackcurrant flowers appears to be low, in direct contrast with data on whole plants frozen to -4°C (Brennan 1991; Dale 1981, 1987), and also with the situation in the field. Commercial plantations of 'Baldwin' are frequently devastated by frost damage, while plantations of more recently introduced cultivars, at a similar stage of flowering, show

significantly less damage. This reflects the introgression of germplasm, especially from Scandinavia, such as 'Brödtorp', with increased tolerance of spring frosts as well as delayed flowering. Further study to compare the range of genotypic variability in whole plants vs. detached flowers is clearly needed to assess and develop future breeding strategies.

To reduce the frost risk to blackcurrant flowers, further studies are necessary to identify, characterize, and locate more precisely the putative barriers to ice propagation into and within flower racemes. If, as our results suggest, these barriers become less effective during growth in the spring, genotypic variation may occur in the rate of loss of barrier strength. Such variation may allow the development of targeted breeding and selection for slower breakdown of these barriers. Retarding the breakdown, such that even a few more degrees of supercooling are necessary before ice can enter or move within a raceme, could produce new blackcurrant cultivars with superior tolerance to spring frosts.

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