Budburst in Detached Birch Shoots (*Betula pendula*) of Different Varieties Winter-Stored in Darkness at Three Different Temperatures

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Budburst timing and the relationship to storage temperature and duration were investigated in four varieties (entries) of 1–2 metre tall silver birch (*Betula pendula* Roth) trees. A total of 2160 shoots were sampled, and the material stored in darkness at 0, 3 or 6 °C from November 29, 1993. When the shoots were placed in storage they had been through a period of 29 days with temperatures below 0 °C (since October 15). By that time the autumn dormancy was assumed already broken, and the trees were expected to respond to increased temperature by bud development. On January 4, 1994, and four subsequent dates, January 19, February 1, March 4 and March 17, shoots were taken out of storage and set in growth chambers at 9, 12 or 15 °C. The time to budburst was recorded. Duration of storage, storage temperatures and varieties were all highly significant for budburst. The interaction terms were of less statistical importance. Based on the contrasts between the three different growth chamber environments, three different methods were used to calculate the threshold temperatures for each entry. In spite of the pre-selection of variable budburst performers, the threshold values, varying between 0 °C to –2 °C, could not be shown to be statistically different. According to the results, the time to budburst changes in accordance with both winter and spring temperatures, being extremely early after a mild winter and warm spring, given sufficient autumn chilling. The similarities in the threshold temperatures indicate that the ranking in earliness between varieties will most likely be the same from year to year without regard to climate change.

Keywords *Betula pendula*, genetics, buds, development, threshold temperatures, heat sums, climatic change.

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1 Introduction

Many studies on northern forest trees have shown that the characteristic "budburst" is highly heritable. Spring phenology varies between different provenances, progenies from different trees, different genotypes within the same category of trees, and even between individuals or clones within the same stand or family. By means of selection and tree breeding it is possible to select for, either very early or the very late budbursting varieties. Reports on genetics and budburst phenology are numerous, including Norway spruce (*Picea abies*) (Stern 1966) and the recent report on silver birch (*Betula pendula*) by Wang and Tigerstedt (1993). The timing of budburst is critical for spring frost-resistance of trees (Oksbjerg 1954, Langlet 1960), and could also be of importance for frequently observed late winter damage. The latter because early budbursting trees might respond faster upon warm winter weather by dehardening (Dietrichson 1992, 93). But environment also plays an important role in regulating budburst timing.

The longest running phenological study of budburst at the same location was carried out in England (Williams 1953). In a nearly 200 year observation series from 1751 to 1947, the leafing out of ash (Fraxinus excelsior) and oak (*Quercus robur*) varied as much as 63 and 54 days respectively. Such large differences between years can primarily be explained by thermal effects in the winter, and in the spring.

In the case of birch in Eastern Canada and U.S.A., Braathe (1957) postulated a connection between the birch dieback and the March thaw of 1936. According to newer studies of Braathe (pers. comm. 1995), and in progress for publication, thaw and freezing experiments in Norway since 1989 have reproduced the birch dieback symptoms from the middle thirties in Eastern Canada and Northeast U.S.A.

The postulated hypothesis, that the green-house effect in Northwest Europe might lead to +4 °C higher winter temperatures (IPCC 1990), is serious for species with a small winter chilling requirement. Such trees might more easily respond upon winter thaws, and get increased risks of spring frost damage.

Several researchers working with different species have studied both genetic and environmental components of budburst-phenology. (Nienstaedt 1966, Campbell and Sugano 1975, Campbell 1978, Cannell and Smith 1983, Worrall 1983, Cannell 1984, Murray et al. 1989, Heide 1993). Provenance trials with wide seed tranfers in forestry have good evidence for genotype–environment interaction for budburst timing. (Kriebel and Wang 1962, Hermann and Lavender 1968, Dietrichson 1969, Mc Gee 1974, Krutszch 1975).

Sarvas (1974) subdivided the annual cycle of forest trees into the following three parts:

1. The active growth through the summer.
2. The chilling period and autumn dormancy.
3. The winter dormancy.

The autumn dormancy is considered complete when the buds in the trees are able to respond by bud development and eventual budburst if the temperature rises above a certain threshold. The autumn dormancy might be very short. Sugar maple (*Acer saccharum*) in U.S.A. has been reported to complete autumn dormancy as early as the beginning of December (Kriebel and Wang 1962). Similar results have been shown in silver birch in Norway (Heide 1993). Based on the study of Murray et al. (1989) it is evident that trees in the early stage after autumn dormancy need a high number of degree days to reach budburst. But they found that as winter progresses, the total required temperature sum is reduced as a function of the increased number of chill days. However, exact temperature requirements for chilling remain unknown.

According to Worrall (1983, 93), the threshold values in forest trees show genetic variation. In Pacific silver fir (*Abies amabilis*), subalpine fir (*Abies lasiocarpa*) and subalpine larch (*Larix lyallii*), the lowest threshold values were found in the alpine provenances having early budburst. Worrall (1983) reworked the data of Oberarzbacher (1977). Here early flushing clones of Norway spruce had a threshold value of approximately 2.6 °C, and late flushing 5.7 °C. But since the material was collected along a transect from low to high elevation in Tirol the result might be masked with a provenance effect. Sarvas (1972) stated that progress of the active period of *Populus tremula* begins at temperatures below zero.

Table 1. Material used in the experiment located at "Skansgården", Norway. 60°12'N latitude, 12°04'E longitude, 170 m a.s.l.

Entry		Origin	Latitude	Longitude	m a.s.l.
421	Clone 802	Pälkäne, Finland	61°30'	24°20'	100
423	Clone 806	Pälkäne, Finland	61°30'	24°20'	100
538	Progeny no. 57	Ådal, Norway	60°30'	10°10'	150
541	Progeny no. 1	Ås, Norway	59°40'	10°80'	100

The following study was conducted in order to elucidate the importance of the winter ecology of forest trees, first for the tree breeding itself, and second for the threat of a climate change leading to a rise in temperatures.

2 Material and Methods

Four earlier genetically tested varieties of silver birch were used in these experiments, two clones from South Finland, and two openly pollinated families from South Norway. They were selected out of larger populations representing different budburst performers. The material covers a latitudinal range of two degrees north-south, and an altitudinal range of ±50 m.a.s.l. (Table 1). Latitudinally and altitudinally the ecotypes are very similar, though the east-west distance from central South-East Norway to South Finland is rather wide. The temperature climate for the two areas is, however, much the same (See also Dietrichson 1964).

The four entries were planted in early May 1991 in one tree plots randomly distributed within each of 20 blocks or 20 trees per source. By the end of the 1993 growing season the plant heights varied from 1 to 2 metres.

On November 25, 1993, after a cool autumn (Fig. 1), 12 trees from each entry were selected for sampling of shoots. The selection was made blockwise from Block I to XII. One tree of each one was sampled within each block. In the cases where a tree of a particular entry was too small to give 50 shoots, trees of the same source were selected from Blocks XIII to XIX.

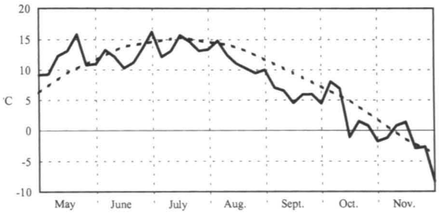

Fig. 1. Pentad means for some months in 1993: —— Normal mean -----.

The 50 shoots taken from each tree, 12–15 cm long, were put into plastic bags, marked and transported frozen to Ås. The following day, all open cuts on each shoot were sealed with wax, and the shoots were subdivided into three equal groups and placed into bags with a small portion of wet spaghnum moss. The fungicide "Bravo" was sprayed lightly inside each bag, and the material was stored at 0 °C until November 29. The three groups of material were then put into cool-storage, under complete darkness at 0, 3 or 6 °C.

On January 4, January 19, February 1, March 4 and March 17, 1994, material was taken out of storage and placed in growth chambers at three different temperatures: 9, 12 and 15 °C. For each source and treatment combination there were 12 replicates. The total number of shoots was 2160: 4 entries × 12 replications × 3 storage temperatures of each entry within the same growth chamber × 3 growth temperatures × 5 placement dates.

Before being placed in the growth chambers, the shoots were glued to tape in strips of 12 (Fig.

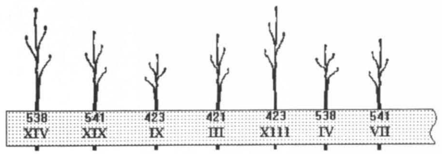

Fig. 2. The shoots were randomly glued on tape in strips of 12.

2), in a completely randomized design, and placed into 100 ml glasses half filled with distilled water. Four strips from each winter storage temperature were put together in each of the growth chambers, making the experimental set 144 shoots (48 shoots × 3 storage treatments) for each growth chamber on each placement date.

The growth chambers had eight hours of natural daylight suplemented with $125\ \mu mol\ m^{-2}\,s^{-1}$ (Lightline HPI 400 W). Sixteen hours of night light was also provided by incandescent lamps which gave approximately $5\ \mu mol\ m^{-2}\,s^{-1}$. All the chambers had a water vapour pressure deficit of 530 Pa.

Daily observation and recording of budburst was carried out from the time the first buds began to develop on the cuttings. Budburst was estimated to be the first day when the first bud on each shoot broke the budscale and the leaf edge became visible.

Calculations

The calculations, all concerning budburst, concentrated upon three important topics:

1. The effect of storage temperature and duration of storage on the number of days each variety needed to reach budburst at a given growth chamber temperature.
2. The effect of growth chamber temperatures in days, and the effect of temperature without regard to storage conditions, for calculation of the threshold values, defined as the lower limit for the temperature at which growth or other life processes take place in a tree.
3. Degree days to budburst once the threshold values are known.

Step 1: The analyses of variance have followed a three-factor model (Snedecor and Cocheran 1972). The mean values were set up in histograms.

Step 2: Threshold values can be calculated in several ways, and in this experiment we used three different methods:

A. The equation method

$$T = (t - x)\,n \qquad (1)$$

where
T = heat sum to reach budburst
t = growth chamber temperature
n = number of days in the growth chamber
x = threshold temperature

This equation assumes that the heat sum requirement is the same whether the variety is grown at a mean temperature of 9, 12 or 15 °C, but this is only true if the correct threshold temperature (x) is being used (Bliss 1967, 1970). By using the experimental data from the different growth chambers, a total of 39 equations for each of the four varieties was set up as follows:

$$(t_1 - x)\,n_1 = (t_2 - x)\,n_2 \qquad (2)$$

where
x = threshold temperature
t_1 = growth chamber temperature 1
t_2 = growth chamber temperature 2
n_1 = number of days in the growth chamber 1
n_2 = number of days in the growth chamber 2

The mean of the threshold values (x) found for each entry on the basis of each equation was calculated, and the variance tested. Because the budburst observations on each source were made only on 12 small cuttings in each chamber, and only once a day, the experimental errors of the n-values were high. To reduce the error of the threshold values for each entry the calculations were made on the basis of the total means of the budburst in the three different growth chamber environments, 9, 12 and 15 °C.

Complete linearity in the heat response up to 15 °C has been assumed, and might not be entirely true. However, development rates of Norway spruce shoots (Mork 1941) and pollen-catkins in birch (Sarvas 1972), fitted to temperature response equations, have been shown to be approximately linear up to temperatures of 12–15 °C.

B. The standard deviation method

According to Arnold (1959), threshold temperatures can also be calculated in other ways. His method to find the threshold temperature which gives the least standard deviation in days has also been used.

C. The slope of the equation method

The third method uses regression, testing the different values for the threshold temperatures in the equations. The value that gives a regression coefficient value equal to zero should also be the correct threshold temperature (Wittink 1988).

All three calculation methods were used and compared.

Step 3: The number of degree days the different varieties needed to reach budburst was calculated for each storage treatment, using threshold values calculated in Step 2.

3 Results

3.1 Effect of Storage Temperatures and Duration of Storage upon Budburst in the Growth Chambers

No significant difference was found in standard deviation between the two groupings of entries: the clones (421 and 423) and the families (538 and 541), in days to budburst. The data from the four sources were therefore combined into analyses of variance, to test if the means were significantly different and separate for the three growth chamber experiments in Table 2. The analyses show that all the main effects are highly significant, and the interactions are of minor importance.

Duration in storage was the most important component regulating time to budburst, but budburst was also affected by storage temperature and somewhat less by source. There were significant interactions between entry and storage temperature, and between storage temperature and duration of storage. Fig. 3 illustrates the mean effects and their mean errors of storage temperature and the three growth chamber temperatures for the different entries.

Fig. 4 shows that the difference in budburst between 0 and 3 °C storage temperature is slight in the beginning, but increases as duration of storage increases. The shoots stored at 6 °C had already started to leaf out by March 4, and were

Table 2. Analysis of variance of 3-factor birch experiment with a randomized block design. Data in days to budburst. The experiments started January 4, January 19, and February 1, 1994.

Source of variation	Degrees of freedom	MS, 9 °C	MS, 12 °C	MS, 15 °C
Replications (R)	11	8.76	2.91	4.17
Entry (V)	3	543.56***	405.64***	251.48***
Storage temperatures (ST)	2	982.15***	752.51***	717.52***
Duration of storage	2	1249.88***	1101.01***	802.71***
V × ST	6	9.81*	11.98*	5.05*
V × DS	6	8.07	8.68*	5.55*
ST × DS	4	9.88*	13.31**	2.37
V × ST × DS	12	3.62	3.62	4.38*
Error	385	3.97	3.05	2.01

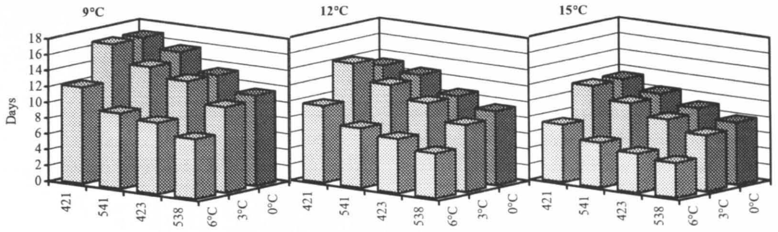

Fig. 3. The number of days the different entries (421, 541, 423, 538) needed to reach budburst in three different storage temperatures (0, 3 and 6 °C) and three different growth chamber temperatures (9, 12 and 15 °C). The material is the means of the experiments started January 4, January 19 and February 1, 1994. The three different analyses of variance as in Table 2 showed the following entry within treatment mean errors in days: 9 °C, (0.58), 12 °C (0.51) and 15 °C (0.41).

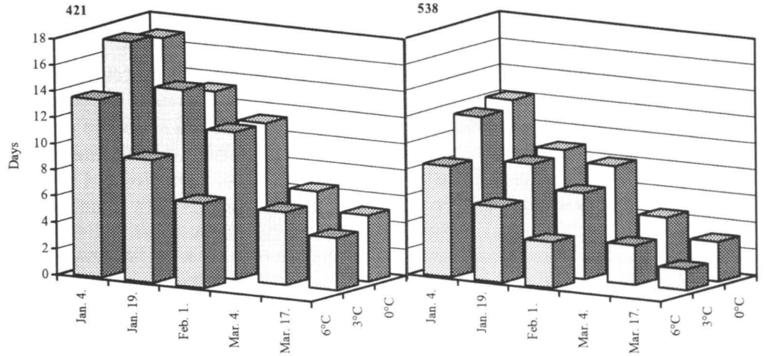

Fig. 4. The mean number of days the latest (421) and the earliest budbursting entry (538) needed to reach budburst with five different placement dates in the growth chambers at 9, 12 and 15 °C, after having been stored at 0, 3 or 6 °C. Material from the storage at 6 °C was excluded from March 4 because some of the shoots had started to leaf out.

therefore excluded from the data analysis from that point. The material stored at 0 and 3 °C until March 17 developed their buds in a few days, and approximately twice as fast in the 3 °C storage as in the 0 °C storage. If the material had been stored a few weeks longer, both storage temperatures would at least for the earliest performing source have led to full budburst in spite of the complete darkness. The results show that birch reacts to rather low winter temperatures, and that light is not a necessity for leafing out.

3.2 Threshold Temperatures

Results from the three calculation methods are presented in Table 3. The three methods led to approximately the same results. A test of the separate threshold values showed that threshold temperatures were not affected by either storage temperature or duration of storage (Skuterud 1994). The threshold temperatures were not found to be significantly different between the four entries.

Table 3. Results from the three calculation methods. Threshold temperatures in °C.

Entry	Method A	Method B	Method C
421	–2.08	–2.15	–2.13
423	–0.89	–0.96	–0.94
538	–1.75	–1.59	–1.63
541	–1.42	–1.57	–1.52

3.3 Degree Days and Budburst for the Four Different Entries

Based on the estimated mean values of the thresholds of the different entries, the degree days needed to reach budburst have been calculated for each one, and set up as a function of storage duration and temperatures (Fig. 5). The requirement for degree days to reach budburst is drastically reduced by increased winter temperature and its duration.

4 Discussion

The budburst-timing in silver birch is highly influenced both by genetics and winter temperatures. Within the same provenance, different families have large heritable differences in budburst timing. (Wang and Tigerstedt 1993). The largest variance component for budburst, if the latitudinal range of the material is not very large, is nearly always found for the families within the same ecotype.

In spite of the large differences in budburst performance, the different entries respond in much the same linear way to duration of storage, storage temperature and different growth chamber temperatures. In this study, the early performers were always the earliest, regardless of treatment. The geneticist selecting early or late performing trees, with the goal to change the population mean in a certain direction should be able to do so without risks of future interactions, if the climate should change in either a cooler or a warmer direction. This is good news for the tree breeder.

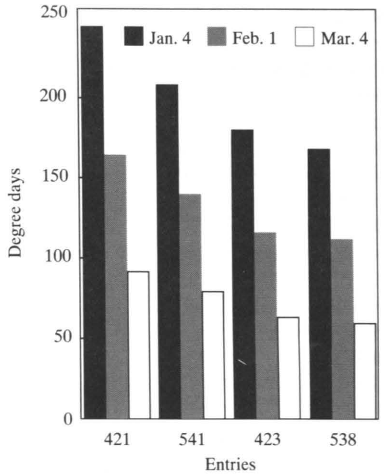

Fig. 5. The mean number of degree days the different entries stored at 0 and 3 °C needed to reach budburst by different placement dates in growth chambers, January 4, February 1 and March 4, 1994. The calculation is based on estimated threshold values for each entry. The means of the methods A, B and C in Table 3.

In this study the threshold temperatures were very low compared to the thresholds for other tree species, as mentioned in the introductory review. The values varied from approximately –2 to 0 °C, and could not be found to be significantly different in spite of the rather large differences in budburst between entries. The silver birch used in this study is of continental origin, adapted to relatively warm summers and cool winters. This might explain the low threshold values. If the winters become warmer, or if these birch varieties are grown in coastal areas, then the low threshold values might become a problem. If winter temperatures are higher after these varieties have passed autumn dormancy (in this material only 29 days with temperatures just below 0 °C, see Fig. 1), the trees might respond by early budburst, and the earliest performers be subject to late winter or spring frost. This in turn may lead to additional problems, such as the earlier mentioned birch dieback observed in the

U.S. and Canada (Braathe 1957, 1995 pers. comm.).

This study also raises many other questions which need answers. If the chilling effect is efficient only when temperatures are below the threshold values, no chilling differences should have occurred in this study. Other reports, however, indicate that chilling also might occur effectively by above zero temperatures and is a gradual process (Repo et al. 1991, Hänninen et al. 1993). The small interactions found in this experiment indicate that chilling has been almost complete before the onset of the experimental period. New studies, starting earlier in the autumn and using different storage temperatures and timing, might throw further light upon the importance of chilling. Different ecotypes, from south to the north, or from low to high altitudes need additional study for a basic understanding of the adaptation problem. But if threshold temperatures within ecotypes are more stable than between ecotypes, it is likely that tree breeders will make the most progress in their selection goals by within ecotype selection.

For nursery growers of silver birch the low threshold values must be taken into account. In order to avoid plant damage, storage temperatures must be kept below the threshold. In this way the physiological fitness of the seedlings will be in condition to meet the environmental stresses in the new plantations.

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