

## Climatic factors controlling the eastern and altitudinal distribution boundary of *Digitalis purpurea* L. in Germany

HELGE BRUELHEIDE<sup>1\*</sup> & ANDREAS HEINEMEYER<sup>2</sup>

<sup>1</sup> Albrecht-von-Haller-Institute for Plant Sciences, Department of Ecology and Ecosystem Research, Untere Karspüle 2, D-37073 Göttingen, Germany

<sup>2</sup> Department of Biology, University of York, YO10 5DD, Heslington, Great Britain

\* e-mail corresponding author: e-mail: hbruelh@gwdg.de

Submitted: Apr 29, 2002 · Accepted: Aug 12, 2002

### Summary

*Digitalis purpurea* was studied as a representative of a plant species with subatlantic geographical distribution in Central Germany with the objective of identifying the underlying climatic causes of the species' eastern distribution boundary. A transplantation experiment with 1-year-old plants and seeds was performed along a ca. 100 km west-east transect across the regional distribution boundary in northwest Thuringia and along an altitudinal transect from 380 m to 1130 m a.s.l. in the Harz Mountains. Continuous measurements of air temperatures below rosette leaves confirmed a clear gradient of increasing mean temperatures with decreasing elevation and, with the exception of one field site, from west to east. Biometrical measurements of the transplants and the survival rate of seedlings generally matched the temperature gradients. Photosynthesis measurements along the elevational transect showed that differences in growth between altitudes were reflected in differences of temperature optima of net assimilation. Seedling mortality was highest in winter at the highest and at the easternmost plot. Although this should be a clear indication of frost sensitivity, there was no satisfactory correlation with absolute minimum temperatures below the rosettes, which exhibited a range between  $-4.1^{\circ}\text{C}$  and  $-9.2^{\circ}\text{C}$ . Frost tolerance experiments were carried out in a freeze chamber at the end of the winter. Significant damage to leaves was found at  $-12^{\circ}\text{C}$  or below; whereas the threshold for buds and roots was  $-15^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$ , respectively. Although frost events of this magnitude were not observed below the rosettes during the period of investigation, mainly because of snow cover, they occur regularly in the study area. In addition to the winter conditions, summer drought was found to have a strong influence on growth of adult plants at the eastern sites. This was confirmed by a manipulative experiment with additional watering. Transpiration experiments also showed a strong water deficit in unwatered plants in the east. The general conclusion from the study is that frost events in winter, which mainly affect the survival of seedlings, combined with summer drought periods, which mainly limit growth of adults, explain the eastern distribution boundary of *Digitalis purpurea*.

Key words: Biogeography, frost hardiness, germination rate, geographical range, growth analysis, microclimate, snow cover

### Introduction

Numerous approaches have been made to predict the geographical range of species from climatic data (DAHL 1951, 1998; JÄGER 1972, 1990, 1992; MEUSEL & JÄGER 1989; BOX et al. 1993; HOLTEN 1993; HUNTLEY et al. 1995; SHAO & HALPIN 1995; SÆTERSDAL & BIRKS 1997; HOFFMANN 2001). Overlaying species distribution maps and climatic maps is frequently used for this purpose but has the drawback of relying on correlation techniques. The resulting relationships provide hypotheses which may be tested in subsequent experiments

with the objective of transforming correlations into causal explanations (BRUELHEIDE 1999). Several attempts of quantifying climatic control mechanisms have shown that simple correlations may have a complex causal basis. For example, HUNTLEY et al. (1995) interpreted the northern range boundary of *Tilia cordata* as coinciding with the line of 1000 degree days above a threshold of  $5^{\circ}\text{C}$ , indicating the need for a minimum length of vegetation period. The underlying cause are probably low summer temperatures, which do not allow an adequate pollen tube growth rate, thus resulting in wilting of the flower before fertilization is achieved (PIGOTT &

HUNTLEY 1978, 1980, 1981). Consequently, the tree produces no fertile seeds at its northern distribution boundary (PIGOTT 1981). Climatic control can even be more complicated when interactions with other species are involved (DAVIS et al. 1998), as for example in *Arnica montana*, whose lower altitudinal distribution is probably limited by increased slug herbivory at low elevations (BRUELHEIDE & SCHEIDEL 1999, SCHEIDEL & BRUELHEIDE 1999, 2001).

Plants exhibiting very simple distributional relationships in Europe seem to be oceanic species, because eastern distribution boundaries are thought to be caused by frost damage. Good correlations with January isotherms have been reported for many oceanic species, e.g. *Erica vagans* (+1°C, HUNTLEY et al. 1995), *Ilex aquifolium* (0°C, IVERSEN 1944), *Digitalis purpurea* (-3°C, HOLMBOE 1925; HOLTEN 1993) and *Quercus petraea* (-4°C, DAHL 1998). Frost hardiness determined under laboratory conditions was shown to be within the range of minimum temperatures observed at the range boundary (LARCHER 1954; TILL 1956; KAPPEN 1964; POLWART 1970 cited after BANNISTER 1976; CALLAUCH 1986). However, such correspondence is still no guarantee for a causal relationship, because other interfering factors may be involved under field conditions. For example, SCHULZ & BRUELHEIDE (1999) demonstrated that frost damage to *Euphorbia amygdaloides* observed at the species' eastern limit in Central Germany was not related to the minimum air temperatures measured along a transect across the distribution boundary, but to the snow cover that decreased to the east.

In this study we have investigated another oceanic species, *Digitalis purpurea*, whose eastern distribution boundary runs through Germany. One problem with experimental work on geographical ranges is that the natural response of the species outside its natural range cannot be investigated in the field. Therefore, we set up a transplantation experiment (WOODWARD 1990; HOLTEN 1993; BRUELHEIDE 1999) across the eastern range boundary. Since the underlying macroclimatic gradient can be strongly modified by relief (GEIGER 1965), by vegetation structure (STOUTESDIJK & BARKMAN 1992) and – especially important in the context of frost damage – by snow cover (STURGES 1989; SCHULZ & BRUELHEIDE 1999), the microclimate experienced by the plant can differ considerably from the macroclimate. The microclimate may even differ between different parts of the plant (KÖRNER & LARCHER 1988). Our first objective, therefore, was to determine whether the microclimate experienced by the leaves on the ground conformed to a gradient. Although the main focus of the work was the west-east gradient, an additional altitudinal transect was established since many subatlantic species prefer increasingly montane elevations towards

their distribution boundaries (MEUSEL 1943). Furthermore, the proximity of the Harz Mountains offered supplemental experimental options with a much steeper climatic gradient than along the west-east transect.

The second objective was to determine whether age-specific response differences exist in *Digitalis purpurea*, as numerous papers have asserted that climate is only effective at a specific stage in the life cycle of a plant (WOODWARD & WILLIAMS 1987; GRACE 1987).

With respect to the assumed frost sensitivity, the third objective was to quantify the plant's frost hardiness both under laboratory conditions and by monitoring the plants along the field transects. Along the altitudinal transect the decrease in ground temperature with increasing elevation is distorted by an increase in snow cover. Consequently, a purely observational approach was considered to be insufficient. Therefore, we additionally included a manipulative field experiment by manually reducing the snow cover on a fraction of the test plants along the altitudinal transect.

Our fourth objective was to consider alternative parameters that could limit the geographic range, i.e. factors other than winter temperatures. In Central Germany, the gradient of increasing summer temperatures from west to east is combined with a gradient of decreasing precipitation. Therefore, it is equally possible that the climatic conditions in summer may contribute to the distribution limits of *Digitalis purpurea*, in which case the most probable factor would be summer drought. The drought impact was investigated by a further manipulative field experiment in which some transplanted individuals were watered regularly.

## Materials and methods

### Study species

*Digitalis purpurea* L. is a biennial plant with an overwintering rosette in the first year and an inflorescence in the following one. However, under unfavorable conditions plants may remain in the rosette stadium for two or three years before flowering. Similarly, plants may occasionally survive for one or two additional years after flowering. In general, the shoots die after flowering and winter-survival is a crucial factor for generative propagation. An average plant may produce more than 70000 seeds (SALISBURY 1942). Due to their low weight of 0.07 mg the seeds are dispersed by wind (GRIME et al. 1988). In seed scatter experiments dispersal distances exceeded 5.5 m when wind velocity was greater than 3.9 m s<sup>-1</sup> at a dispersal height of 1.5 m or greater than 7.5 m s<sup>-1</sup> at 1.0 m (unpublished observations). The species has a persistent seed bank (VAN BAALEN & PRINS 1983), which uncouples population development from the generative success in a specific year to some extent.

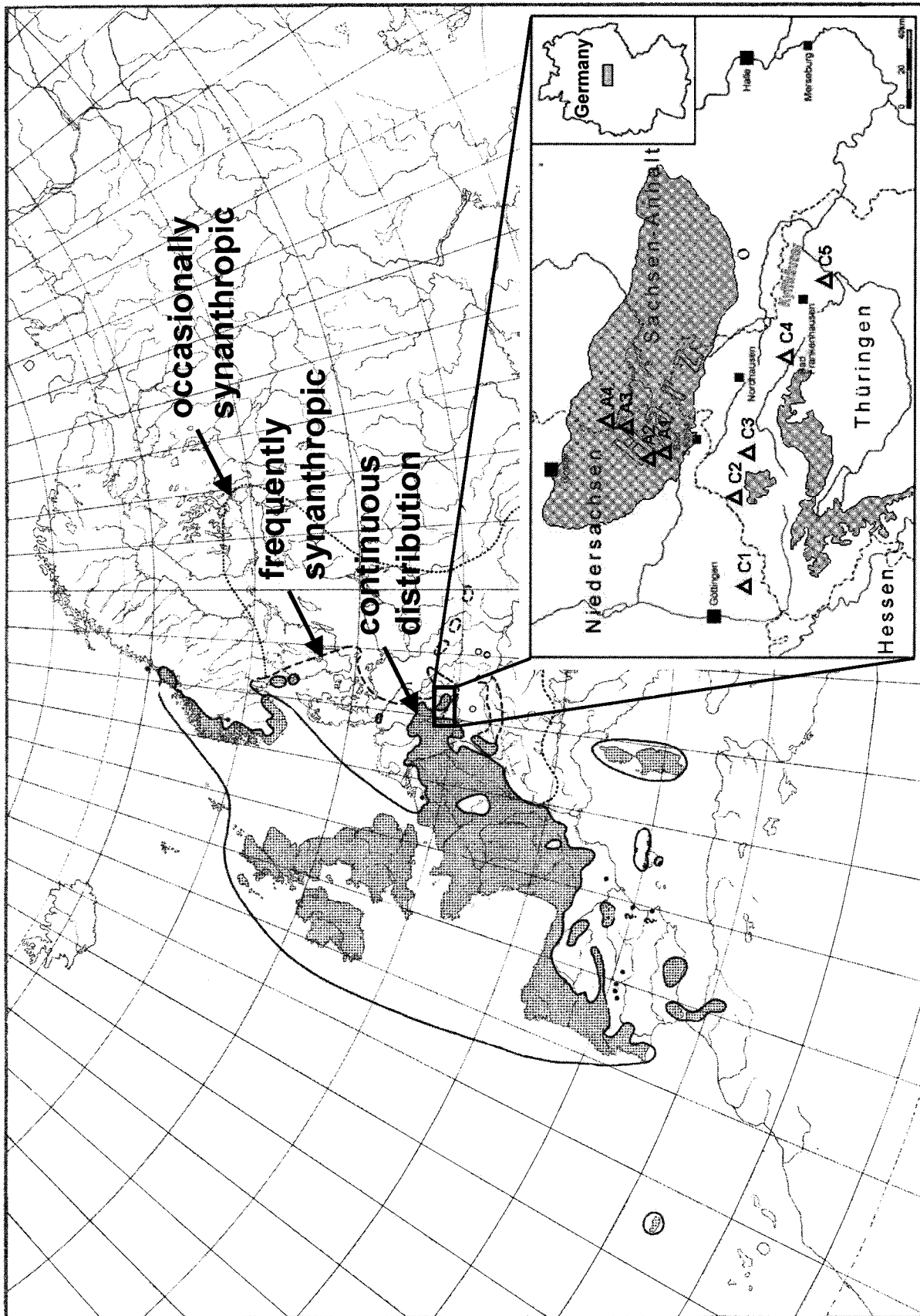


Fig. 1. Distribution range of *Digitalis purpurea* (modified after MEUSEL et al. 1978). The inset shows the location of the experimental plots (C1 to C5 in continental direction and A1 to A4 at ascending elevation); mountain areas are shaded in grey.

The species occurs primarily on acidic substrates of low pH with soil types ranging between well-developed cambisols to podzols. It is a characteristic floristic element of clear-cut forests in the subatlantic part of Europe and is absent in the continental parts (Fig. 1). The restriction of the species' range to areas in Europe with mild winters has been recognized early by HOLMBOE (HOLMBOE 1925, 1927, 1928; LAGERBERG & HOLMBOE 1937). *D. purpurea* extended its range into subcontinental areas over the last century (MEUSEL 1954). In Germany, despite suitable soil conditions and availability of clear-cuttings the species becomes increasingly less abundant from west to east (WERNER 1964). In the lowlands of eastern Germany *Digitalis purpurea* is considered to be only a synanthropic species (BENKERT et al. 1998) (Fig. 1). Because of unsteady occurrences in east Germany and Poland the species is considered a ephemerophyte. In the investigational area *Digitalis purpurea* is probably a neophyte (JÄGER personal communication). For example, the species was not mentioned by Johannes THAL (1588) in his famous floristic inventory of the Harz Mountains. The first record was by ROYER (1651).

## Field sites

A sandstone mountain ridge running from WNW to ESE parallel to, and north of, the Hainleite Mountains in southern Lower Saxony and Thuringia provided a suitable study area for setting up a transplantation experiment across the distribution boundary. The region is well-known for having distribution boundaries of subatlantic species such as *Digitalis purpurea*, *Euphorbia amygdaloides*, *Polygonatum verticillatum* and *Festuca altissima*. In continental direction from west to east, five field sites were established at 20 km intervals (C1 to C5, Fig. 1). The altitude along this transect varied only between 250 m and 305 m a.s.l. Furthermore, an altitudinal transect was set up in the western part of the Harz Mountains. Four field sites (A1 to A4 at ascending elevation) were located at 3 km intervals, differing in altitude by approximately 300 m each (Fig. 1). Altitude ranged from 380 m a.s.l. at Bad Lauterberg (A1) to 1130 m at Mt. Brocken (A4). In general, all sites exhibited a southern aspect and an inclination of between 2° and 5° and were established on clear-cuttings that were at least 2 years old, except for Mt. Brocken, which is covered by a natural grassland community above the treeline. Specimens of *D. purpurea* were found growing naturally at all sites, except for A4 and C4. The treeless sites (nearest trees at least 10 m distance from the plot) provided no shelter from frost damage. The geological substrate of the west-east transect was sandstone (Mittlerer Buntsandstein, sm). The soil type was a loamy dystric cambisol (pH<sub>KCl</sub> between 3.1–5.6 and 3.0–3.6 for A<sub>h</sub> and B<sub>v</sub>, respectively) with weak podzolization at C4 and C5. The soil type of A1 and A2 on the altitudinal transect was also a loamy dystric cambisol on greywacke; A3 and A4 were located on podzols on granite with a mor horizon of ca. 8 cm (pH<sub>KCl</sub> between 2.8–3.5 and 3.0–4.5 for O<sub>h</sub> and A<sub>eh</sub>/B<sub>v</sub>, respectively).

At each site an area of 2 m × 2 m was fenced and planted with 24 *D. purpurea* individuals. The plants were sown of seeds from one plant collected on 24/04/1996 at 450 m a.s.l. in the eastern part of the Harz Mountains. Plants were sown in

trays on 08/05/1996 in a sand/humus/peat mixture (pH<sub>KCl</sub> 5.0) and kept in the Botanical Garden Göttingen until transplanting on 26/09 and 09/10/1996 to the sites of the west-east and altitudinal transect, respectively. Planting was performed without pots and by mixing growth medium with the local topsoil.

Half of the planted individuals (12 per site) were experimentally treated with snow reduction during the winter and additional watering during the summer while the other half (12 per site) served as a control. For reduction of snow cover (only at A1 to A4 along the altitudinal transect), snow was brushed away after heavy snowfall events by sliding a shovel over some fixed wooden boards which limited snow cover to 8 cm. This was done on several occasions (A1: 23/01/1997; A2 and A3: 19/12/1996, 06/01/1997, 23/01, 27/02, 10/03; A4: 26/12/1996, 23/01/1997, 10/03, 21/04). Due to lack of snow cover along the west-east transect there was no such treatment at C1 to C5. Irrigation during the summer was applied at all field sites. Quantity of irrigation was always sufficient to raise the water content of the uppermost 10 cm of soil to field capacity. Watering dates and quantity (mm precipitation) were the same for A1 to A4 with 11/06 (8 mm), 03/07 (16 mm), 07/08 (24 mm), 19/08 (24 mm) and 02/09 (16 mm) and for C1, C2, C3 and C4 with 14/06 (48 mm), 26/06 (48 mm), 03/07 (48 mm), 08/08 (32 mm), 12/08 (32 mm), 19/08 (32 mm) and 02/09/1997 (32 mm) except for the quantity at C5: 64, 64, 64, 40, 40, 32 and 32 mm, respectively.

To test germination and seedling survival 10 pots (10 × 10 × 14 cm) were buried within the fenced area of the plot each containing 20 *D. purpurea* seeds in a sand/humus/peat mixture (pH<sub>KCl</sub> 5.0); only the uppermost centimeter of the pot edge extended groundlevel avoiding seeds being washed-out.

On 26/09 and 09/10/1996 in the west-east and the altitudinal transect, respectively, micrometeorological measurement devices were installed at each site. Precipitation measurements during the winter were performed with a resealable 10 l-bucket to collect the snow; the water volume was subsequently determined in the laboratory. Evaporation during this period was found to be negligible. During the summer, standard rain funnels were installed at a height of 1 m above ground level, they were connected to a 500 ml reservoir buried in the soil. Precipitation data for A4 were obtained from the weather station (DEUTSCHER WETTERDIENST 1996/1997) that was located at a distance of 50 m from the plot. Temperature was measured under the rosettes of one randomly chosen transplanted individual at each site with thermistor probes (Tinytag-Temp, Miniature Temperature Logger, Orion Components Ltd.). The sensors were covered with soil except for 1 cm of the tip that was shaded only by a leaf. Strictly speaking, the temperatures referred to air temperature between soil and rosettes. Measurements were taken every 72 min, resulting in 20 values a day.

## Biometric measurements

Biometric measurements were made at intervals of ca. 28 days. The growth of the transplanted individuals was recorded by counting the number of healthy, partially damaged and dead leaves and by measuring rosette height and diameter. The rela-

tive growth rate (RGR) and leaf number for subsequent dates  $t_n$ ,  $t_{n+1}$  was calculated according to HUNT (1989). During the investigation period no flowering of the transplanted individuals occurred on either the west-east or the altitudinal transect. In the seed experiment the germination rate of the seeds, seedling survival rate and growth rates were recorded.

## Photosynthesis measurements

Photosynthesis was measured once during the period from 26/09 to 01/10/1997 on four of the untreated transplants per site along the altitudinal transect (except for A4 where only 2 plants survived), using a compact minicuvette system (CMS 400, Walz). Individuals at each site were transplanted into pots (16×16×16 cm) and their assimilation rate was measured in the laboratory on the following day. Preliminary experiments were performed one week earlier using individuals taken from a natural population of 1-year-old plants adjacent to A2. These individuals showed a comparatively low light compensation  $I_k$  of 18–20  $\mu\text{E m}^{-2} \text{s}^{-1}$  and an extrapolated light saturation of 850  $\mu\text{E m}^{-2} \text{s}^{-1}$ . The highest net assimilation rates ( $A_{\text{max}}$ ) were observed on the fifth youngest leaf. Thus, the measurements on the field site plants for determination of the optimum temperature ( $T_{\text{opt}}$ ) and  $A_{\text{max}}$  were carried out with the fifth youngest leaf at approximately 70% light saturation at 580 ( $\mu\text{E m}^{-2} \text{s}^{-1}$ ) and at ambient  $\text{CO}_2$ -concentration.

## Frost tolerance

Frost sensitivity was investigated by using the electrolyte leakage method according to MURRAY et al. (1989) with increased conductivity being a measure of tissue damage. The experiment was carried out at the end of April on 1-year-old individuals taken from one population close to A2. The plants were put in pots and transferred to a freeze chamber after two days. Twelve plants each were randomly exposed either to a non-freezing control temperature (+4°C) or to -0.1, -3, -6, -9, -12, -15 and -18°C, respectively. Each temperature level lasted for two hours, with a cooling-down period of one hour between levels (n.b. -18°C was reached during the early morning hours).

Comparable plant material, separated into leaf, root and bud tissue on the day after treatment, was placed in test tubes with demineralized water and 3% propanol. The electrical conductivity of the solution was measured (LF 2000, WTW) at the beginning ( $C_0$ ) and then regularly over a period of about 4 days ( $C_t$ ). The solution's conductivity after being boiled for 20 minutes ( $C_{\text{boiled}}$ ) was used as reference. Each relative conductivity (RC) time series was fitted using an exponential function (1)  $\text{RC} = (C_t - C_0) / (C_{\text{boiled}} - C_0) = 1 - e^{-k \cdot t}$  (SAS 6.02: proc reg, SAS INSTITUTE 1987). The resulting parameter  $k$  increases with the rate of electrolyte leakage and can therefore be evaluated as a measure of frost damage. To control for insulating effects and the effect of temperature increase resulting from crystallization of soil water, the soil temperature was monitored in one of the pots.

## Transpiration measurements

Transpiration was measured for three transplanted control individuals and three irrigated individuals in two campaigns (07/07–11/07/1997 and 11/09–18/09/1997) at the sites along the west-east transect (except for C3), using a steady state-porometer (LI-COR, LI-1600). Transpiration of the sixth youngest leaf was measured at about 60 min intervals over an approximately seven hour period. PAR and leaf temperature were also recorded at each measurement. Identical leaves were used in the two measurement periods. For each period the transpiration data ( $\text{Tr}$ ) were transformed to transpiration per hour and a mean daily transpiration ( $\text{Tr}_D$ ) ( $\text{gH}_2\text{O cm}^{-2} \text{h}^{-1}$ ) was calculated by adding all transpiration values for one day and dividing them by seven hours.

## Statistical analysis

All statistics were performed using SAS 6.02. Tests of departure from the Gaussian distribution were made according to Shapiro Wilk (using proc univariate, SAS INSTITUTE 1988) at a significance level of  $\alpha = 0.05$ . The normally distributed parameters were tested by ANOVA with subsequent single comparisons after Scheffé (proc glm, SAS INSTITUTE 1987). For non-parametrical analysis, tests of sum of ranks were performed according to the Kruskal-Wallis method (proc npar1way, SAS INSTITUTE 1987) following manual calculation of single comparisons according to the Schaich-Hamerle test (after BORTZ et al. 1990). In the frost damage experiment the  $\alpha$ -values were corrected according to Bonferoni by dividing by the number of pairwise comparisons made in the analysis.

## Results

### Climatic conditions

Fig. 2 shows the monthly mean temperature measured directly below the leaf rosettes for the four field sites along the altitudinal transect together with the data from the official weather stations nearest to A3 (Braunlage, 3 km from A3) and A4 (Mt. Brocken, 30 m from A4). The rosette temperatures followed the altitudinal gradient and decreased from A1 at lowest to A4 at highest elevation. The daily mean temperatures were significantly different between at least two of the four field sites in almost all months (according to the Kruskal-Wallis test with subsequent Schaich-Hamerle test). A4 always showed the lowest values until February 1997. In March, no significant differences between sites were observed. From May onwards, A3 exhibited significantly the lowest daily mean temperatures, because of a failure of the data loggers at A4. The absolute minimum temperatures recorded during the whole observation period differed only slightly between field stations (Table 1). A clear gradient was neither encountered for

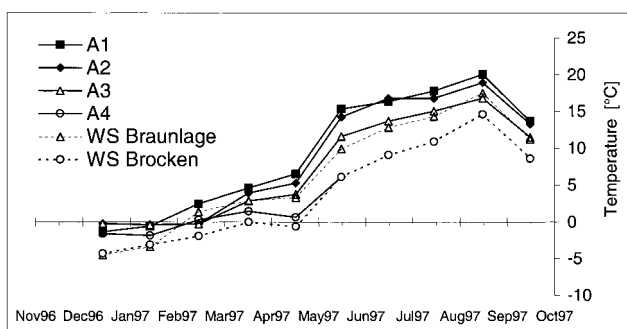


Fig. 2. Monthly mean temperature below *Digitalis* rosettes at the field sites along the altitudinal transect during the investigation period. The data for the weather station (WS) at Braunlage are included for comparison with A3. Data for A4 were continued by data from the WS at Mt. Brocken (dashed line).

cumulative temperatures below 0°C nor for the number of frost days (Table 1). The last frost event was observed on the same date (24/04) at all sites except A4. The only parameter related to altitude was the amount of precipitation (Table 1).

The snow reduction treatment from December 1996 to February 1997 resulted in significantly lower mean, minimum or maximum temperatures at A1, A2 and A4 (Table 2). Contrary to expectations, A3 showed the converse pattern with significantly higher temperatures when snow cover was reduced. This was probably due to the strong winds that resulted in uncontrolled reduction of snow cover also for the non-treated plants. From March to April, a period in which continuous measurements could be obtained only for A2 and A4, the treatment effect was even larger. In this period the reduction of the snow cover promoted snow melting at A2, indi-

cated by higher maximum temperatures below the treated rosettes (Table 2).

The air temperatures measured at the weather station in Braunlage had almost identical values to the rosette temperatures at A3 in this period, but were lower in winter. This was confirmed by regression analysis for A3 between the temperatures recorded at the leaf ( $T_{ros}$ ) and those recorded at 2 m above ground at the weather station in Braunlage ( $T_{WS}$ , DEUTSCHER WETTERDIENST 1996/1997) between April and October 1997 ( $T_{ros} = 0.8681 * T_{WS} + 1.8716$ ;  $r^2 = 0.883$ ,  $n = 184$ ,  $p < 0.001$ ). On average, the leaf temperature in this period was 0.34 K higher than the air temperature recorded at the weather station in Braunlage. Given the good match between rosette temperatures and leaf temperatures at A3, it seems justified to complement the missing summer data at A4 with data of the weather station at Mt. Brocken (Fig. 2). In contrast, the temperatures below the rosettes were almost unrelated to air temperatures at 2 m between December 1996 and March 1997 ( $T_{ros} = 0.1777 * T_{WS} + 0.9711$ ;  $r^2 = 0.224$ ,  $n = 107$ ,  $p < 0.001$ ), due to insulation of the rosette by snow for extended periods in winter. A continuous snow cover was recorded at A4 from December 1996 to 03/03/1997 and from 19/03/1997 to 14/04/1997, and at A3 from December 1996 to 21/02/1997 and from 19/03/1997 to 26/03/97. In those periods the rosette temperatures were always found to be just below 0°C. For example, the mean January temperatures differed only between -0.3°C (A3) to -2.0°C (A4).

The monthly mean temperatures for the west-east transect (from C1 in the west to C5 in the east) are given in Fig. 3. Göttingen and Artern were the nearest western and eastern weather stations to the transect's westernmost and easternmost ends with distances of 8 km and 12 km to C1 and C5, respectively. Between Göttingen

Table 1. Climatic parameters recorded at all field sites during the whole observation period (Dec. 1996 to Oct. 1997) along the altitudinal (A1–A4) and continental (C1–C5) transect: absolute minimum temperature [°C], cumulated temperature below 0°C [degree days], number of frost days, date of last frost event and precipitation [mm]. Climatic data of A4 were complemented according to DEUTSCHER WETTERDIENST (1996/1997).

Site	Absolute min. temperature	Cumulated temp. < 0°C	Number of frost days	Date of last frost event	Precipitation
A1	-5.3	-179.6	124	24/4/1997	1050
A2	-4.1	-121.6	113	24/4/1997	1100
A3	-5.8	-83.2	112	24/4/1997	1250
A4	-4.9	-244.3	136	19/6/1997	1570
C1	-9.2	-163.7	68	24/4/1997	600
C2	-5.4	-121.2	94	24/4/1997	650
C3	-4.1	-65.2	86	24/4/1997	700
C4	-4.1	-90.6	101	24/4/1997	500
C5	-5.8	-143.9	99	31/5/1997	550

Table 2. Daily mean, minimum and maximum temperatures [°C] measured below the leaf rosettes when snow cover was manually reduced compared to untreated control plants. Asterisks indicate statistically significant differences between treatment and control for each site according to ANOVA.

Considered Period	Site	n	Mean temperature		Minimum temperature		Maximum temperature	
			Control	Snow reduction	Control	Snow reduction	Control	Snow reduction
02/12/96–17/02/97	A1	78	-0.56	-0.87*	-1.42	-1.34	0.49	-0.34*
	A2	78	-0.29	-0.45*	-0.34	-0.82*	-0.25	0.11
	A3	78	-0.28	0.06*	-0.31	-0.04*	-0.23	0.18*
	A4	78	-1.48	-1.89*	-1.82	-2.32*	-1.18	-1.52*
	C1	78	1.17	1.13	0.48	0.19	3.95	3.35
	C5	78	0.42	0.80*	-0.90	-0.53*	3.00	3.34*
04/03/97–21/04/97	A2	49	4.33	4.47	0.05	-0.54*	13.97	16.19*
	A4	49	5.40	0.27*	3.80	-0.85*	8.39	2.46*
	C4	49	6.97	6.27	0.61	0.73	19.42	15.73*
	C5	49	7.21	6.80*	0.62	0.56	18.67	17.06*

and Artern the long-term annual mean temperature rose by 0.8°C and the annual precipitation declined by 170 mm. With the exception of C1, which had the highest temperatures from February to April, a microclimatic gradient was also confirmed for the west-east transect with significantly highest daily mean temperatures at C5 during the entire observation period compared to C2 or C3 with lowest values (significant according to the Schaich-Hamerle test). From May onwards the sites showed almost continuously increasing daily mean temperatures from west to east. The abnormal high daily mean temperatures of C1 in winter, which were accompanied by the lowest observed daily minimum temperature (-9.2°C on 28/12/1996, Table 1) were due to a die-back of rosette leaves at the end of December 1996. The visual assessment in January showed that most leaves had turned brown and thus no longer provided sufficient insulation protection for the sensors leaving them partly exposed to direct sunlight from February to March. Similar to the altitudinal transect, the absolute minimum

temperatures of the other field stations varied only moderately (Table 1). The temperature sums below 0°C did not reflect the west-east gradient, neither did the number of frost days or the amount of precipitation (Table 1). The easternmost site was characterized by exceptionally late frosts. Whereas the last frost for C1 to C4 was encountered on 24/04, several frosts were recorded at C5 in May (Table 1). The last frost that occurred on 31/05 at C5 with -2.8°C was comparatively severe.

### Germination and seedling survival

Germination in the germination experiment occurred almost exclusively in October and November 1996. The germination rate until November 1996 was around 50% with no difference between altitudes (Fig. 4). Only single germination events were observed in spring 1997. The seedling survival along the altitudinal transect was

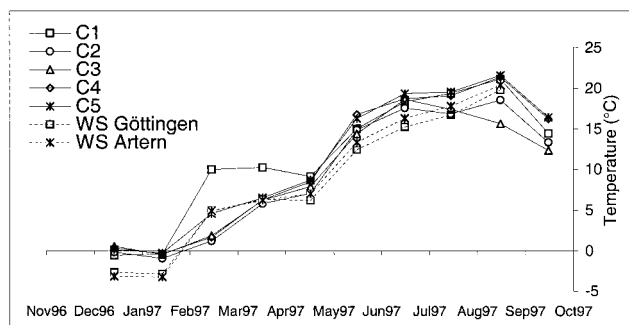


Fig. 3. Monthly mean temperature below *Digitalis* rosettes at the field sites along the west-east transect during the investigational period. The data for the westernmost and easternmost weather stations (WS) at Göttingen and Artern are included for comparison with C1 and C5, respectively.

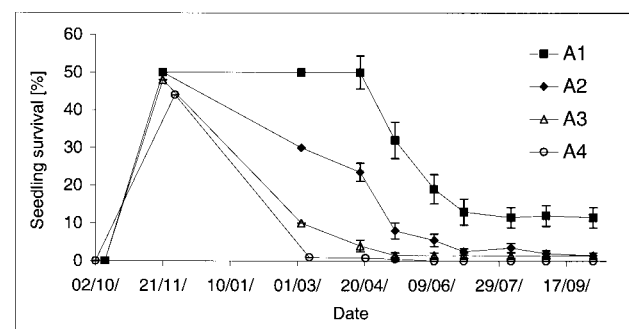


Fig. 4. Germination and seedling survival along the altitudinal transect as per cent of the total 200 sown seeds at each field site during the investigational period. Values are means ( $\pm$  SD) for  $n = 10$  pots, each containing 20 seeds. For the first three dates where some pots could not be counted due to snow cover, no SD is given.

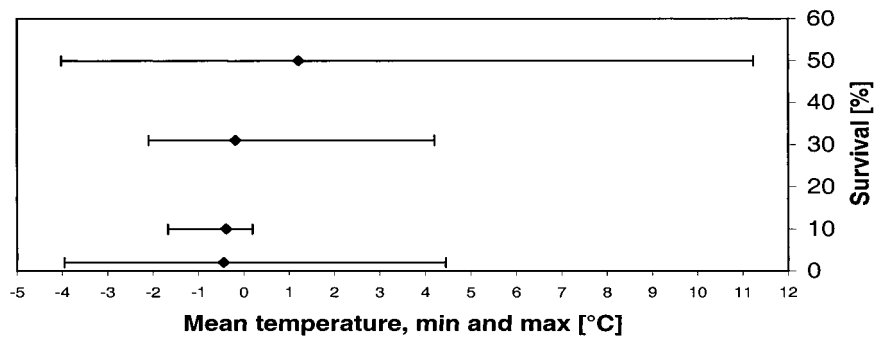


Fig. 5. Seedling survival along the altitudinal transect on 06/03/1997 as per cent ( $\pm$  SD) of the total 200 sown seeds at each field site against mean temperature in February 1997. Survival values are averages ( $\pm$  SD) for  $n = 10$  pots (each containing 20 seeds). Temperature values are averages of daily means ( $n = 28$ ); the bars indicate the absolute minimum and maximum temperature in February 1997.

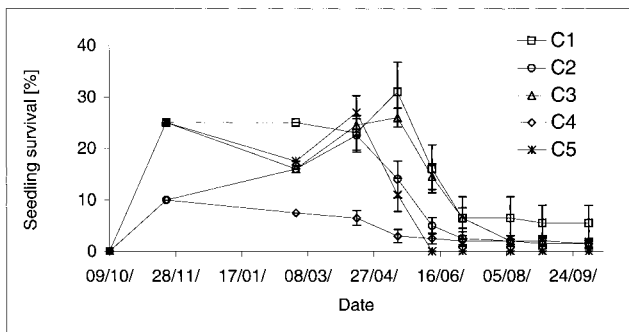


Fig. 6. Germination and seedling survival along the west-east transect as per cent of the total 200 sown seeds at each field site during the investigational period. Values are means ( $\pm$  SD) for  $n = 10$  pots, each containing 20 seeds. For the first three dates where some pots could not be counted due to snow cover, no SD is given.

characterized by a distinct mortality directly after the winter on the first observation date (4/03/1997 for A1 to A3, 10/03/1997 for A4). The survival rate after the winter corresponded to the elevational gradient, exemplified in Fig. 5, where the survival rate in March is plotted against February mean temperatures below the rosettes, the month preceding the sampling date. However, neither absolute minimum nor absolute maximum temperatures reflected the gradient in survival rate (Fig. 5). Nor was seedling survival related to other temperature derivatives, such as number of frost days, date of last frost or degree days below  $0^{\circ}\text{C}$ . On 11/06/1997 no seedling was left at A4 (Fig. 4). On the last recording date A2 and A3 had a survival rate of 1.5% and A1 of 11.5%. A pattern similar to that of the survival rates was also encountered for the growth rates of the surviving seedlings. On 07/10/1997 the average

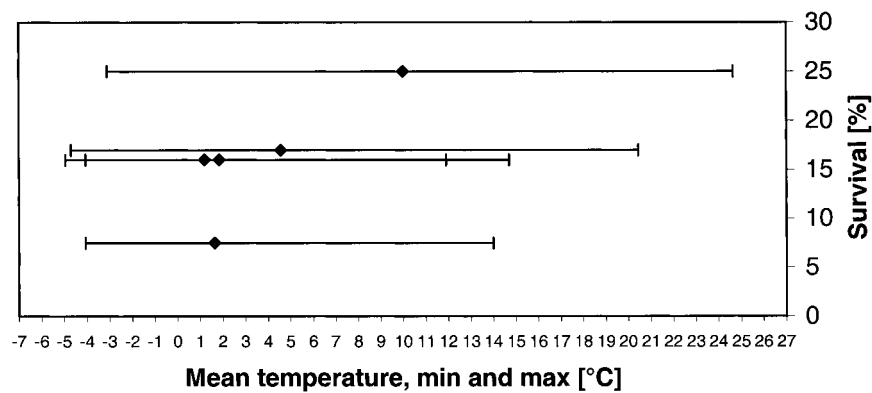


Fig. 7. Seedling survival along the west-east transect on 06/03/1997 as per cent ( $\pm$  SD) of the total 200 sown seeds at each field site against mean temperature in February 1997. Survival values are averages ( $\pm$  SD) for  $n = 10$  pots (each containing 20 seeds). Temperature values are averages of daily means ( $n = 28$ ); the bars indicate the absolute minimum and maximum temperature in February 1997.



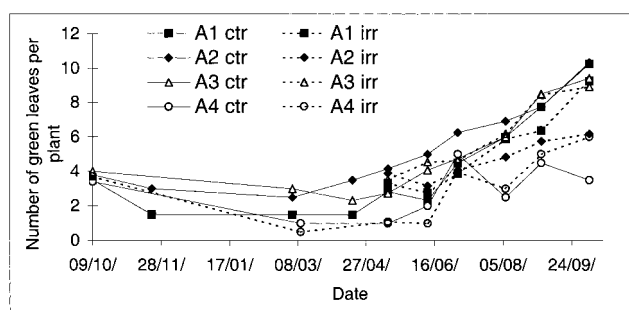


Fig. 8. Number of green leaves per plant along the altitudinal transect. Shown are data for the untreated (ctr) and watered (irr) field sites. The irrigation treatment started on 11/06/97.

leaf number at A1 was 16.1 with a rosette diameter of 35.3 cm ( $n = 23$ ); whereas at A2 and A3 the leaf number were 8.7 and 6.3 ( $n = 3$ ) and the diameters 6.2 cm and 9.5 cm ( $n = 3$ ), respectively.

Along the west-east transect most seeds also germinated by the end of November 1996 (Fig. 6). However, contrary to findings of the altitudinal transect, some seeds also germinated in April and May 1997. The seedlings started to die in April and the survival rate reflected the west-east gradient with lowest survival in the east and highest survival in the west. Even though survival rates in March showed again a relationship to the mean temperature in February, but not to the absolute minimum or maximum temperatures in February (Fig. 7), in late spring there was no relation to either temperatures in March or April nor to the accumulated temperatures below  $0^{\circ}\text{C}$  or to the number of frost days. On the last observation date the survival rates were 5.5% at C1 and 1.5% at C2, C3 and C4; no seedlings were found at C5. The sequence of sites for survival corresponded to the number and size of leaves of the surviving seedlings. On 16/10/1997 the average leaf number was 11.0 (C1,  $n = 11$ ), 5.4 (C2,  $n = 3$ ), 5.2 (C3,  $n = 3$ ) and 6.3 (C4,  $n = 3$ ). The rosette diameters were 19.5 cm, 5.0 cm, 5.2 cm and 3.3 cm, respectively.

## Survival and growth of transplants along the altitudinal transect

Although survival rates of the 1-year-old transplants after winter was lowest at A4 (17%) for the individuals without snow reduction there was no altitudinal trend. Survival rates were 100% at A2 and A3 but only 50% at A1. None of the recorded growth parameters of 1-year-old plants along the altitudinal gradient revealed significant differences before April 1997 (Fig. 8). On 13/05/1997 leaf number of untreated plants varied significantly between 1.0 at A4 and 4.2 at A2 and by the end of the observation period on 07/10/1997 had decreased with increasing altitude and was 10.3, 10.3, 9.4 and 3.5 for untreated plants at A1, A2, A3 and A4, respectively.

The RGR showed similar patterns as the absolute number of leaves with decreasing values with increasing altitude for most observation intervals (data not shown) and corresponded to results of photosynthesis measurements (Table 3). The maximum net assimilation rate ( $A_{\text{max}}$ ) at maximum available artificial PAR ( $580 \mu\text{E m}^{-2} \text{s}^{-1}$ ) and ambient  $\text{CO}_2$  concentration ( $350 \mu\text{l l}^{-1}$ ) declined with increasing elevation. At A4, leaves attained only 53% of  $A_{\text{max}}$  at A1. The temperature optimum of net assimilation ( $T_{\text{opt}}$ ) displayed a less clear pattern with decreasing values from A2 to A4 but unexpectedly with the lowest value at A1. Whereas  $A_{\text{max}}$  was related both to the mean and the absolute maximum temperature in September 1997, which was the month preceding the date of photosynthesis measurement,  $T_{\text{opt}}$  was related to the minimum temperatures (Table 3). The minima at all plots were encountered on the same date (20/09/1997), one week before the measurements started.

Although the treatment of snow reduction along the altitudinal transect had caused lower temperatures that were experienced by the 1-year-old transplants (compare Table 2), no effects on survival or growth were found. At A1 with a survival rate of 75% for treated plants, the survival was even higher than for untreated plants (50%). Similarly, the treatment of additional irri-

Table 3. Photosynthesis measurements of transplanted *Digitalis purpurea* individuals between 26/09 and 01/10/1997.  $A_{\text{max}}$  = maximum net photosynthesis,  $T_{\text{opt}}$  = temperature optimum of photosynthesis. Values are averages ( $n = 4$  for each site)  $\pm$  SD. Different letters indicate statistically significant differences between sites according to ANOVA with subsequent Scheffé test. The temperature values refer to measurements made below the leaf rosettes, with the exception of A4 (WS) where the values refer to air temperature measured at the nearby weather station at 2 m above ground.

Site	$A_{\text{max}}$ [ $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ]	$T_{\text{opt}}$ [ $^{\circ}\text{C}$ ]	Mean temperature below rosettes [ $^{\circ}\text{C}$ ]	Min. temperature below rosettes [ $^{\circ}\text{C}$ ]	Max. temperature below rosettes [ $^{\circ}\text{C}$ ]
A1	$12.76^{\text{A}} \pm 0.23$	$21.05^{\text{B}} \pm 0.39$	13.9	1.1	42.4
A2	$7.89^{\text{B}} \pm 0.03$	$25.16^{\text{A}} \pm 0.65$	13.5	2.5	38.5
A3	$8.55^{\text{B}} \pm 0.09$	$24.26^{\text{A}} \pm 0.65$	11.5	0.6	29.2
A4	$6.75^{\text{B}} \pm 0.65$	$22.78^{\text{AB}} \pm 0.48$	$8.6^{\text{WS}}$	$-0.1^{\text{WS}}$	$22.7^{\text{WS}}$

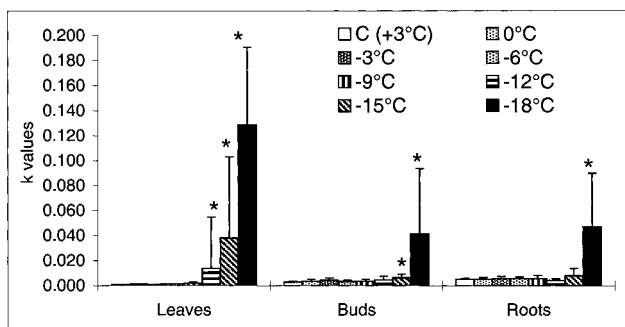


Fig. 9. Frost damage as measured by electrolyte leakage. Shown are average  $k$  values ( $\times 1000$ ) ( $\pm$  SD) ( $n = 12$ ). \* indicates significant differences between a certain treatment compared to the control ( $+3^\circ\text{C}$ ) according to the Kruskal-Wallis with Bonferoni correction.

gation of plants in summer had no significant effect on plant growth along the altitudinal transect. Instead, watering reduced growth significantly at A2 from June onwards. On 07/10/1997 the untreated plants at A2 had 10.3 leaves; whereas the irrigated plants had only 6.2 leaves (Fig. 8).

The lack of effect of snow reduction is in accordance with the frost tolerance experiment in April (Fig. 9). In comparison to the control, leaves were the most frost sensitive tissues with significant frost damage occurring at  $-12^\circ\text{C}$  or below, whereas the buds were not damaged above  $-15^\circ\text{C}$  and roots showed the highest frost resistance with significant damage only at  $-18^\circ\text{C}$ . The insulating effect of the surrounding soil in the pots and the increase in soil temperature due to crystallization energy of soil water was only apparent for the levels  $-3^\circ\text{C}$  and  $-6^\circ\text{C}$ . At  $-9^\circ\text{C}$  or below, soil water was frozen and thus chamber temperatures were fully experienced by the roots.

### Survival and growth of transplants along the continental transect

The survival rate of the 1-year-old plants was 100% at all sites along the west-east transect until 10/06/1997. Nevertheless, in the middle of March a considerable physiognomic frost damage was observed for the plants at all sites along the west-east transect. Most of the transplants survived and at the end of the observation period the survival rates were 92% at C1, 60% at C2, 100% at C3 and C4, 75% at C5. The growth at C2 was exceptionally poor (Fig. 10), due to a thunderstorm with heavy rains during early June. Water running downhill through the plot uprooted plants and subsequently damaged their roots. Therefore, the results of C2 will not be considered below. The growth parameters of 1-year-

old plants exhibited only a tentative gradient (Fig. 10). After the winter on 15/05/1997 the leaf number at C3 (5.9 leaves) was significantly highest in comparison to C1, C4 and C5 with 4.2, 4.0 and 3.6 leaves, respectively (significant according to the Schaich-Hamerle test). The RGR for the preceding 4 weeks (14/04–15/05) was lowest at C5, which was related to the lowest observed absolute minimum temperature ( $-5.6^\circ\text{C}$ ) along the west-east transect in this interval (Fig. 11a). At the end of the observation period on 06/10/1997, C5 and C4 with 6.4 and 4.6 leaves differed significantly from C1 with 12.7 leaves. In contrast to the spring period, the RGR in summer was about the same for all sites (approximately  $0.01\text{ d}^{-1}$ ) with a much smaller standard deviation, except for C2 with a negative RGR for the reasons mentioned above (Fig. 11b).

The plants that had received additional water in summer showed a significantly higher leaf number at all sites except for C1 (Fig. 10). The treatment effect was significant for all other sites from the beginning of July onwards for the absolute number of leaves as well as for the RGR (significant according to the Schaich-Hamerle test).

The results of the transpiration measurements in summer 1997 are summarized in Table 4. The average amount of transpired water by non-irrigated plants over the daily measuring time of 7 h was significantly highest at C5 for both recording campaigns (run 1 and 2) in comparison to all other sites; they were almost three times higher than at C2 in the first campaign and 60% higher in the second one. However, the low values for C2 are probably due to the thunderstorm mentioned above. With additional irrigation, the transpiration rates were higher but the differences between sites remained unchanged. The comparison of watered and untreated plants within one plot showed a significant treatment effect only for the easternmost C5. Averaged over the two campaigns, irrigation enhanced transpiration by about 50%.

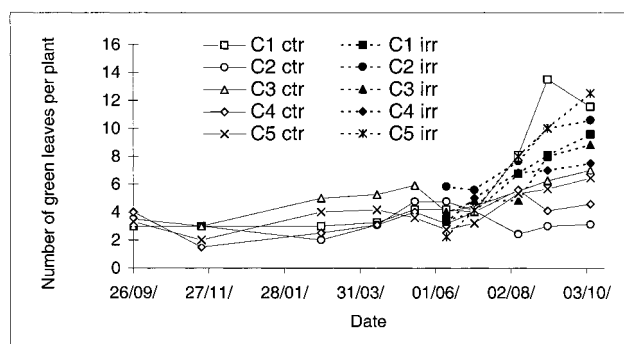


Fig. 10. Number of green leaves per plant along the west-east transect. Shown are data for the untreated (ctr) and watered (irr) field sites. The irrigation treatment started on 14/06/97.

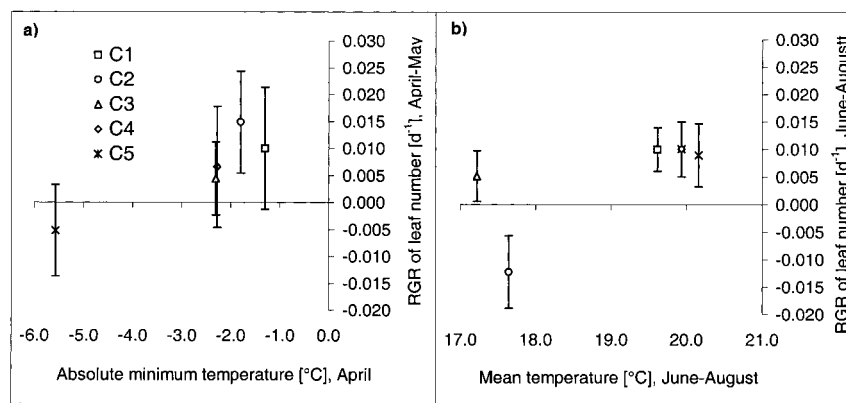


Fig. 11. Relative growth rate of the number of green leaves from 14/04 to 15/05 along the west-east transect against the absolute minimum temperature in April (a), and from 10/06 to 08/08 against the mean temperature from June to August (b). RGR values are averages ( $\pm$  SD) for  $n = 12$  plants per site.

Table 4. Transpiration averaged over a 7-hr period at four field sites along the west-east transect obtained during two different periods (run 1 = 07/07/97–11/07/97; run 2 = 11/09/97–18/09/97). Values are averages  $\pm$  SD for leaves of  $n = 3$  different plants (except C2 in run 1,  $n = 2$ ). Different letters indicate statistically significant differences between sites according to ANOVA with subsequent Scheffé test. For the evaluation of the treatment effect the two runs have been pooled ( $n = 6$ ; except for C2,  $n = 5$ ). Asterisks indicate statistically significant differences between treatments according to ANOVA.

Run	Transpiration over a 7h period ( $\text{gH}_2\text{O cm}^{-2} \text{h}^{-1}$ )				ANOVA $p =$
	Control		Irrigation		
1	C1 ctr	0.034 <sup>B</sup> $\pm$ 0.001	C1 irr	0.029 <sup>B</sup> $\pm$ 0.009	
	C2 ctr	0.016 <sup>C</sup> $\pm$ 0.001	C2 irr	0.026 <sup>B</sup> $\pm$ 0.006	
	C4 ctr	0.033 <sup>B</sup> $\pm$ 0.005	C4 irr	0.053 <sup>A</sup> $\pm$ 0.003	
	C5 ctr	0.047 <sup>A</sup> $\pm$ 0.003	C5 irr	0.072 <sup>A</sup> $\pm$ 0.009	
	ANOVA	$p < 0.0001$	ANOVA	$p = 0.002$	
2	C1 ctr	0.034 <sup>AB</sup> $\pm$ 0.005	C1 irr	0.037 <sup>B</sup> $\pm$ 0.004	
	C2 ctr	0.025 <sup>B</sup> $\pm$ 0.004	C2 irr	0.032 <sup>C</sup> $\pm$ 0.002	
	C4 ctr	0.024 <sup>B</sup> $\pm$ 0.003	C4 irr	0.024 <sup>BC</sup> $\pm$ 0.002	
	C5 ctr	0.040 <sup>A</sup> $\pm$ 0.006	C5 irr	0.056 <sup>A</sup> $\pm$ 0.006	
	ANOVA	$p = 0.0093$	ANOVA	$p < 0.0001$	
1 and 2 pooled	C1 ctr	0.034 $\pm$ 0.003	C1 irr	0.033 $\pm$ 0.008	0.769
	C2 ctr	0.021 $\pm$ 0.006	C2 irr	0.029 $\pm$ 0.005	0.06
	C4 ctr	0.029 $\pm$ 0.006	C4 irr	0.038 $\pm$ 0.016	0.208
	C5 ctr	0.043 $\pm$ 0.006	C5 irr	0.064 $\pm$ 0.011	*0.0022

## Discussion

The first assumption that the leaf temperatures would follow both the altitudinal and the west-east gradient proved to be valid. This clearly indicates that the topological features of the selected sites and the measurement conditions were so uniform that they did not interfere with the relationship between macro- and microclimate; the only exception were sensors that were insuff-

ciently insulated because their leaf cover had died back. As expected, the microclimate below the rosette leaves was much less extreme than at a height of 2 m because of greater energy flows at ground level (GEIGER 1965; STOUTESDIJK & BARKMAN 1992). In comparison to other studies, the small deviation of 0.34 K between microclimate and macroclimate is remarkable. For example, SCHULZ & BRUELHEIDE (1999) found the minimum air temperature at growth sites of *Euphorbia*

*amygdaloides* (measured at a height of 0.3 m above the forest floor) to differ by 3.3 K compared to the nearest official weather station (measured at a height of 2 m above a lawn). Along a 100 km transect in calcareous grasslands running parallel to our west-east transect, JANDT (1998) found the air and soil temperatures of uniformly southern sloped sites to be more dependent on vegetation structure than on the macroclimatic gradient.

Growth of transplanted individuals, and even more clearly, the survival of seedlings also displayed a clear altitudinal and a west-east gradient. Seedling death along the altitudinal transect was mainly observed in winter. Such low winter survival of *Digitalis purpurea* has already been observed in other studies (WOODWARD & JONES 1984). Although this should be a clear indication of frost sensitivity, no satisfying correlation with absolute minimum temperatures was found. Consequently, no single factor was responsible for low survival but parameters other than winter temperature likely influenced seedling survival. Since the experimental design was identical in all plots, the following factors, which are well-known for their strong influence, can be ruled out as unimportant: neighbour effects (GRUBB 1977; RYSER 1993), vegetation structure (SCHENKEVELD & VERKAAR 1984; DE JONG & KLINKHAMER 1988; FISCHER & MATTHIES 1998) or size of microsites (OOMES & ELBERSE 1976). Another parameter to be considered on the altitudinal transect was wind. Mt. Brocken is known to have the highest mean wind velocity measured in Germany (DEUTSCHER WETTERDIENST 1996/1997). In a horizontal profile wind speed decreases exponentially towards the ground surface (GRACE 1977; STOUTEDIJK & BARKMAN 1992). Although strong wind will probably not inflict direct mechanical damage to seedlings it may change the microclimatic conditions considerably. Apart from removing the snow cover and thus exposing plants, wind increases transpiration and enhances the risk of frost drought (WILSON 1959; LARCHER 1963; SAKAI 1983). Since the summer observations along the west-east transect have revealed a drought sensitivity of *Digitalis* seedlings, they should be equally susceptible to desiccation conditions in winter, when the soil is frozen but the plant suffers from water loss by transpiration. However, this interpretation is speculative because the seedlings were not monitored daily. In the following vegetation period, the growth of transplanted 1-year-old individuals was related to the temperatures along the altitudinal transect and, to a minor degree, along the west-east transect. Hence, the negative RGR from April to May at the easternmost site coincided with a low minimum temperature. In general, satisfactory correlations of growth rates with temperatures are rarely found in field studies because many factors may interfere with temperature (BRUELHEIDE 1999), or because growth rates of certain species vary

only moderately with temperature, as was shown for the montane species *Meum athamanticum* (BRUELHEIDE & LIEBERUM 2000) and *Sedum rosea* (WOODWARD 1975). Although *Digitalis purpurea* exhibited a clear growth-temperature relationship, this response was not very pronounced in comparison to other species. For example, WOODWARD & PIGOTT (1975) demonstrated for *Sedum telephium* that a difference in mean temperature of 2.3 K between sites resulted in a two times higher dry weight at 150 m compared to 490 m a.s.l. over a period of 43 days in June/July. In comparison, the leaf number of *Digitalis purpurea* on 03/07/1997 was only 32% higher at A2 (630 m) compared to A3 (900 m) with a difference of 3.2 K in June mean temperature.

Growth differences between altitudes were reflected in differences between the temperature optima of net assimilation ( $T_{opt}$ ). Consequently, decreasing growth rates with decreasing temperatures are not only explained by a generally diminished metabolic rate but also partly due to acclimation. The shift of  $T_{opt}$  in direction of the prevailing temperatures is a well-known phenomenon (BILLINGS & MOONEY 1968; SMITH & HADLEY 1974; GRAVES & TAYLOR 1988; FRIEND & WOODWARD 1992). However, pure acclimation does not explain the low  $T_{opt}$  at A1. As indicated by the relation between  $T_{opt}$  and observed minimum temperatures, frosts or even low temperatures above 0°C might additionally have affected photosynthesis (LARCHER & BAUER 1981). Damage to the photosynthetic system would also explain the decreasing maximum net assimilation rate ( $A_{max}$ ) with increasing altitude. The opposite was expected (e.g. MÄCHLER & NÖSBERGER 1977), because leaf expansion is less reduced by low temperatures than the production of photosynthetic system; this results in a higher ratio of mesophyll to leaf surface area (FRIEND & WOODWARD 1992). There are also studies in population biology that emphasize the detrimental effects of low temperatures. For example, HUNT et al. (1985) found that cold nights, even with values well above 0°C, drastically diminished the growth of *Pyrola rotundifolia* in a British dune area.

With respect to the second objective of the study, seedlings proved to be much more sensitive to the environmental conditions than adult plants. The survival and growth rates of seedlings was more clearly related to environmental gradients than the ones of the 1-year-old plants. With regard to winter survival, averaged over all field sites, the seedling stage is about four times more at risk than the adult stage. The greater sensitivity of seedlings is completely in accordance with other studies (LARCHER & BAUER 1981; WOODWARD & WILLIAMS 1987; GRACE 1987). Besides survival rates, growth parameters of the 1-year-old plants were almost unresponsive to the winter conditions, apart from frost impacts in April at the easternmost site. In contrast, the growth of 1-year-old plants responded clearly to the irri-

gation along the west-east transect. This is an indication that climate acted differently on the two investigated age stages.

The third objective, the determination of frost hardiness in the laboratory and the comparison to observed survival after the winter in the field, gave consistent results. Although the leaves, buds and roots displayed clear differences in frost hardiness, only leaf values were in accordance with TILL (1956). Since the frost hardiness experiments were carried out in early spring, a possible dehardening of the plants cannot be ruled out (TILL 1956; KAPPEN 1964; LARCHER & BAUER 1981). The observed frost tolerance of the leaves of  $-9^{\circ}\text{C}$  was sufficient to survive the lowest temperatures of  $-5.8^{\circ}\text{C}$  that were measured at the rosettes of the 1-year-old plants (except for C1 with an absolute minimum of  $-9.2^{\circ}\text{C}$  due to failing sensor cover resulting from lacking leaves). The comparably high temperatures below the rosettes are in accordance with the data from weather stations. For example, air temperatures at a height of 2 m exceeding  $-9^{\circ}\text{C}$  were measured in the investigational period from December 1996 until September 1997 at the weather stations of Göttingen, Artern and Braunlage on 20, 19 and 22 days, respectively (DEUTSCHER WETTERDIENST 1996/1997). However, all those events coincided with a snow cover of more than 0.5 cm recorded for that specific day at the weather station (DEUTSCHER WETTERDIENST 1996/1997). This is another confirmation for the importance of snow cover for explaining range boundaries. Snow cover of more than 20 cm prevents soil surface temperatures from dropping below  $-5^{\circ}\text{C}$  (SAKAI & LARCHER 1987) and a similar insulating effect was also confirmed in this study. Consequently, the measurements taken below the rosettes have been much more relevant for the plants than air temperature values recorded at weather stations at standard heights. The importance of snow in the context of range boundaries was also emphasized by WOODWARD (1997), who found that young *Verbena officinalis* survived better in the field than expected from frost chamber experiments because of snow cover. In a study on the eastern distribution limit of *Euphorbia amygdaloides*, the leaves of individuals protected by a shallow snow cover suffered only a third of the frost damage experienced by unprotected leaves (SCHULZ & BRUELHEIDE 1999).

The moderate minimum temperatures below the rosettes do not imply that *Digitalis* plants are not at risk to frost in general. On the one hand, although the frost temperatures recorded below the rosettes might always have been above the lethal threshold in the investigational period, they probably had a cumulative effect in weakening the plant (which could not be simulated in the frost chambers) and finally resulted in the observed reduced growth and survival of seedlings of *Digitalis*

*purpurea*. On the other hand, a damage threshold of  $-9^{\circ}\text{C}$  might be exceeded in some years when exceptionally cold winters coincide with a lacking snow cover. However, singular effects like those of a dry freeze without snow protection are only rarely directly observed in field experiments (HOLTEN 1993). Nevertheless, the principal importance of such frost events is unquestionable. For example, EICHLER et al. (1927) were able to relate the absence of suboceanic-submediterranean species such as *Ilex aquifolium*, *Tamus communis* and *Buxus sempervirens* in Southwest Germany to the occurrence of exceptionally low absolute minima of air temperatures below  $-23^{\circ}\text{C}$ .

Our fourth objective was to test summer drought conditions as an alternative factor to low temperature in damaging *Digitalis* plants. Despite highest temperature at the two easternmost sites C4 and C5 growth of the 1-year-old plants was lowest at these sites. This can be explained by an increasing vapor pressure deficit (vpd) with temperature. Obviously, *Digitalis purpurea* did not respond to the high vpd in the east with a reduction in transpiration because the highest transpiration rates of non-irrigated plants were measured in the east. Transpiration seems to have been excessive, since it was inversely related to growth of the transplants. A growth limitation due to water shortage along the continental gradient was also confirmed by the manipulative irrigation treatment. There was a clear response to watering, with a pronounced effect for the easternmost station outside the geographical range, but no correlation was observed along the altitudinal transect, where precipitation was abundant.

Remarkably, an increase in summer drought towards the east was neither confirmed by our own monthly precipitation measurement at the field stations (not reported here), nor by precipitation data of the nearest official weather station (DEUTSCHER WETTERDIENST 1996/1997). In contrast, the summer rains in 1997 showed increasing values from west to east. Despite the higher amount of precipitation, the plants at the eastern sites probably suffered from more severe water stress because rainfall occurred infrequently in localized, heavy thunderstorms; whereas at the western sites the precipitation events were more scattered.

## Conclusion

Neither winter temperatures nor summer drought alone can be used to explain the distribution boundary of *Digitalis purpurea* in this study. The most probable cause for the eastern distribution boundary is a combination of low survival rate of seedlings in winter together with reduced growth of 1-year-old plants during summer. Against the background of global climatic change with

a predicted increase in Central European winter temperatures but also with increased droughts in summer (BENISTON & TOL 1998), the possible effects of expected climatic changes on *Digitalis purpurea* cannot be unequivocally predicted. From the results of this study we expect that with increasing summer drought *Digitalis purpurea* will secede from its eastern distribution boundary. However, such scenarios should be discussed with caution because of great uncertainties in climatic predictions (BENISTON & TOL 1998), unknown effects of biotic interactions (e.g. FOX et al. 1999) and a differential response of different genotypes (FISCHER et al. 1997).

## Acknowledgements

We are very grateful to Prof. M. Runge for providing all his laboratory equipment for our studies. We thank the staff of the New Botanical Garden Göttingen for helping to cultivate our *Digitalis* plants. The forest administrations of Oldisleben, Sondershausen, Groß-Bodungen, Bad Lauterberg and Braunlage owe our thanks for providing the access to field sites. We were very much supported by the administration of the Hochharz National Park in Saxony-Anhalt, especially by Dr. G. Karste, in our researches in the botanical garden on Mt. Brocken. We express our warm thanks to Dr. U. Jandt, Dr. P. Ineson and Dr. D. F. Whybrew for their comments on the manuscript. Suggestions of Prof. Dr. E. Jäger, Prof. Dr. R. Löscher and another anonymous reviewer are greatly acknowledged.

## References

- BANNISTER, P. (1976): Introduction to physiological plant ecology. – Blackwell, Oxford.
- BENISTON, M. & TOL, R. S. J. (1998): Europe. In: WATSON, R. T.; ZINYOWERA, M. C. & MOSS, R. H. (eds.): The regional impacts of climate change. A special report of IPCC Working Group II, 149–185. – Cambridge Univ. Press, Cambridge.
- BENKERT, D.; FUKAREK, F. & KORSCH, H. (1998): Verbreitungsatlas der Farn- und Blütenpflanzen Ostdeutschlands. – Fischer, Jena.
- BILLINGS, W. D. & MOONEY, H. A. (1968): The ecology of arctic and alpine plants. – Biol. Rev. **43**: 481–529.
- BORTZ, J.; LIENERT, G. A. & BOEHNKE, K. (1990): Verteilungsfreie Methoden in der Biostatistik. – Springer, Berlin.
- BOX, E. O.; CRUMPACKER, D. W. & HARDIN, D. (1993): A climatic model for location of plant species in Florida, U.S.A. – J. Biogeogr. **20**: 629–644.
- BRUELHEIDE, H. (1999): Experiments as a tool to investigate plant range boundaries. – Verh. Ges. Ökol. **29**: 19–26.
- BRUELHEIDE, H. & LIEBERUM, K. (2000): Investigations of the altitudinal distribution of *Meum athamanticum* Jacq. in the Harz Mountains. – Flora **196**: 227–241.
- BRUELHEIDE, H. & SCHEIDEL, U. (1999): Slug herbivory as a limiting factor for the geographical range of *Arnica montana*. – J. Ecol. **87**: 839–848.
- CALLAUCH, R. (1986): Die Frosthärte der Stechpalme (*Ilex aquifolium*). – Verh. Ges. Ökol. **14**: 523–527.
- DAHL, E. (1951): On the relation between summer temperature and the distribution of alpine vascular plants in the lowlands of Fennoscandia. – Oikos **3**: 22–52.
- DAHL, E. (1998): The phytogeography of northern Europe (British Isles, Fennoscandia and adjacent areas). – Cambridge Univ. Press, Cambridge.
- DAVIS, A. J.; JENKINSON, L. S.; LAWTON, J. H.; SHORROCKS, B. & WOOD, S. (1998): Making mistakes when predicting shifts in species range in response to global warming. – Nature **391**: 783–786.
- DE JONG, T. J. & KLINKHAMER, P. G. L. (1988): Population ecology of the biennials *Cirsium vulgare* and *Cynoglossum officinale* in a coastal sand-dune area. – J. Ecol. **76**: 366–382.
- DEUTSCHER WETTERDIENST (1996/1997): Monatlicher Witterungsbericht **44** (9–13), **45** (1–10). – Deutscher Wetterdienst, Offenbach.
- EICHLER, J.; GRADMANN, R. & MEIGEN, W. (1927): Ergebnisse der pflanzengeographischen Durchforschung von Württemberg, Baden und Hohenzollern. – Verein f. vaterl. Naturk. Württemberg, Grüniger, Stuttgart.
- FISCHER, M. & MATTHIES, D. (1998): Experimental demography of the rare *Gentianella germanica*: seed bank formation and microsite effects on seedling establishment. – Ecography **21**: 269–278.
- FISCHER, M.; MATTHIES, D. & SCHMID, B. (1997): Responses of rare calcareous grassland plants to elevated CO<sub>2</sub>: a field experiment with *Gentianella germanica* and *Gentiana cruciata*. – J. Ecol. **85**: 681–691.
- FRIEND, A. D. & WOODWARD, F. I. (1992): Evolutionary and ecophysiological responses of mountain plants to the growing season environment. – Adv. Ecol. Res. **20**: 60–124.
- FOX, L. R.; RIBEIRO, S. P.; BROWN, V. K.; MASTERS, G. F. & CLARKE, I. P. (1999): Direct and indirect effects of climate change on St. John's wort *Hypericum perforatum* L. (Hypericaceae). – Oecologia **120**: 113–122.
- GEIGER, R. (1965): The climate near the ground. – Harvard University Press, Cambridge.
- GRACE, J. (1977): Plant response to wind. – Academic Press, London.
- GRACE, J. (1987): Climatic tolerance and the distribution of plants. – New Phytol. **106** (suppl.): 113–130.
- GRAVES, J. D. & TAYLOR, K. (1988): A comparative study of *Geum rivale* L. and *G. urbanum* L. to determine those factors controlling their altitudinal distribution. II. Photosynthesis and respiration. – New Phytol. **108**: 297–304.
- GRIME, J. P.; HODGSON, J. G. & HUNT, R. (1988): Comparative plant ecology. – Unwin Hyman, London.
- GRUBB, P. J. (1977): The maintenance of species-richness in plant communities: the importance of the regeneration niche. – Biol. Rev. **52**: 107–145.
- HOFFMANN, M. H. (2001): The distribution of *Senecio vulgaris*: capacity of climatic range models for predicting adventitious ranges. – Flora **196**: 395–403.

- HOLMBOE, J. (1925): Einige Grundzüge von der Pflanzengeographie Norwegens. – Bergens Museums Aarbok **1924–1925**: 3–54.
- HOLMBOE, J. (1927): Nogen problemer i Vestlandets plantegeografi. – Naturen **51**: 211–229.
- HOLMBOE, J. (1928): Rævebjelden (*Digitalis purpurea* L.) og dens rolle i norsk natur og folkeliv. – Nyt Magazin for Naturvidenskaberne **66**: 193–248.
- HOLTEN, J. I. (1993): Potential effects of climatic change on distribution of plant species, with emphasis on Norway. In: HOLTEN, J. I.; PAULSEN, G. & OECHEL, W. C. (eds.): Impacts of climatic change on natural ecosystems, with emphasis on boreal and arctic/alpine areas, 84–104. – Norwegian Institute for Nature Research (NINA), Trondheim.
- HUNT, R. (1989): Basic growth analysis. – Unwin Hyman, London.
- HUNT, R.; HOPE-SIMPSON, J. F. & SNAPE, J. B. (1985): Growth of the dune wintergreen (*Pyrola rotundifolia* ssp. *maritima*) at Braunton Burrows in relation to weather factors. – Int. J. Biometeor. **29**: 323–334.
- HUNTLEY, B.; BERRY, P. M.; CRAMER, W. & McDONALD, A. P. (1995): Modelling present and potential future ranges of some European higher plants using climate response surfaces. – J. Biogeogr. **22**: 967–1001.
- IVERSEN, J. (1944): *Viscum*, *Hedera* and *Ilex* as climate indicators. – Geol. Fören. Förhandl. **66** (3): 463–483.
- JÄGER, E. J. (1972): Comments on the history and ecology of continental European plants. In: VALENTIN, D. H. (ed.): Taxonomy, phytogeography and evolution, 349–362. – Academic Press, London.
- JÄGER, E. J. (1990): Pflanzenarealgrenzen als Leistungsgrenzen. – Biol. Rundsch. **28**: 295–306.
- JÄGER, E. J. (1992): Die Verbreitung von *Frankenia* in der Mongolei, in Westurasien und im Weltmaßstab. – Flora **186**: 177–186.
- JANDT, U. (1998): Vegetation der Kalkmagerrasen des Südharzes und des Kyffhäusers. – NNA Berichte **2/98**: 120–124.
- KAPPEN, L. (1964): Untersuchungen über den Jahreslauf der Frost-, Hitze- und Austrocknungsresistenz von Sporophyten einheimischer Polypodiaceae (Filicinae). – Flora **155**: 123–166.
- KÖRNER, C. & LARCHER, W. (1988): Plant life in cold climates. In: LONG, S. P. & WOODWARD, F. I. (eds.): Plants and temperature, 25–57. – Soc. Exp. Botany, Cambridge.
- LAGERBERG, T. & HOLMBOE, J. (1937): Vare ville planter. Vol. 6. – Tanum, Oslo.
- Larcher, W. (1954): Die Kälteresistenz mediterraner Immergrüner und ihre Beeinflussbarkeit. – Planta **44**: 605–635.
- LARCHER, W. (1963): Zur spätwinterlichen Erschwerung der Wasserbilanz von Holzpflanzen an der Waldgrenze. – Ber. Naturwiss.-Med. Ver. Innsbruck **53**: 125–137.
- LARCHER, W. & BAUER, H. (1981): Ecological significance of resistance to low temperature. In: LANGE, O. L.; NOBEL, P. S.; OSMOND, C. B. & ZIEGLER, H. (eds.): Encyclopedia of plant physiology. Vol. 12A. Physiological Plant Ecology I, 403–437. – Springer, Berlin.
- MÄCHLER, F. & NÖSBERGER, J. (1977): Effect of light intensity and temperature on apparent photosynthesis of altitudinal ecotypes of *Trifolium repens* L. – Oecologia **31**: 73–78.
- MEUSEL, H. (1943): Vergleichende Arealkunde. Band 1. Borntraeger, Berlin-Zehlendorf.
- MEUSEL, H. (ed.) (1954): Verbreitungskarten mitteldeutscher Leitpflanzen. 7. – Math.-Nat. Reihe, Martin Luther Univ. Halle-Wittenberg, **3** (1): 10–17.
- MEUSEL, H. & JÄGER, E. J. (1989): Ecogeographical differentiation of the submediterranean deciduous forest flora. – Pl. Syst. Evol. **162**: 315–329.
- MEUSEL, H.; JÄGER, E. J.; RAUSCHERT, S. & WEINERT, E. (1978): Vergleichende Chorologie der zentraluropäischen Flora, Vol. 2, Kartenteil. – Gustav Fischer, Jena.
- MURRAY, M. B.; CAPE, J. N. & FOWLER, D. (1989): Quantification of frost damage in plant tissues by rates of electrolyte leakage. – New Phytol. **113**: 307–311.
- OOMES, M. J. M. & ELBERSE, W. T. (1976): Germination of six grassland herbs in microsites with different water contents. – J. Ecol. **64**: 745–755.
- PIGOTT, C. D. (1981): Nature of seed sterility and natural regeneration of *Tilia cordata* near its northern limit in Finland. – Ann. Bot. Fennici **18**: 255–263.
- PIGOTT, C. D. & HUNTLEY, J. P. (1978): Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. I. Distribution in north-west England. – New Phytol. **81**: 429–441.
- PIGOTT, C. D. & HUNTLEY, J. P. (1980): Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. II. History in north-west England. – New Phytol. **84**: 145–164.
- PIGOTT, C. D. & HUNTLEY, J. P. (1981): Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. III. Nature and causes of seed sterility. – New Phytol. **87**: 817–839.
- ROYER, J. (1651): Beschreibung des ganzen Fürstlich Braunschweigischen Gartens zu Hessen. – Andreas Denicher, Braunschweig.
- RYSER, P. (1993): Influences of neighbouring plants on seedling establishment in limestone grassland. – J. Veg. Sci. **4**: 195–202.
- SÆTERSDAL, M. & BIRKS, H. J. B. (1997): A comparative ecological study of Norwegian mountain plants in relation to possible future climatic change. – J. Biogeogr. **24**: 127–152.
- SAKAI, A. (1983): Comparative studies on freezing resistance of conifers with special reference to cold adaption and its evolutive aspects. – Can. J. Bot. **61**: 2323–2332.
- SAKAI, A. & LARCHER, W. (1987): Frost survival of plants. Responses and adaption to freezing stress. – Springer, Berlin.
- SALISBURY, E. J. (1942): The reproductive capacity of plants. – George Bell, London.
- SAS INSTITUTE (1987): SAS/STAT guide for personal computers. Vers 6. – Cary, NC.
- SAS INSTITUTE (1988): SAS Procedures guide. Release 6.03. – Cary, NC.
- SCHEIDEL, U. & BRUELHEIDE, H. (1999): Selective slug grazing on montane meadow plants. – J. Ecol. **87**: 828–838.
- SCHEIDEL, U. & BRUELHEIDE, H. (2001): Altitudinal differences in herbivory on montane Compositae species. – Oecologia **129**: 75–86.

- SCHENKEVELD, A. J. & VERKAAR, H. J. (1984): The ecology of short-lived forbs in chalk grasslands: distribution of germinative seeds and its significance for seedling emergence. – *J. Biogeogr.* **11**: 251–260.
- SCHULZ, C. & BRUELHEIDE, H. (1999): An experimental study on the impact of winter temperature on the distribution of *Euphorbia amygdaloides* L. in Central Germany. In: KLÖTZLI, F. & WALTHER, G. R. (eds.): Recent shifts in vegetation boundaries of deciduous forests, especially due to general global warming, 121–150. – Birkhäuser, Basel.
- SHAO, G. & HALPIN, P. N. (1995): Climatic controls of eastern North American coastal tree and shrub distributions. – *J. Biogeogr.* **22**: 1083–1089.
- SMITH, E. M. & HADLEY, E. B. (1974): Photosynthetic and respiratory acclimation to temperature in *Ledum groenlandicum* populations. – *Arctic Alpine Res.* **6**: 13–27.
- STOUTJESDIJK, P. & BARKMAN, J. J. (1992): Microclimate, Vegetation and Fauna. – Opulus Press, Knivsta.
- STURGES, D. L. (1989): Response of mountain big sagebrush to induced snow accumulation. – *J. Appl. Ecol.* **26**: 1035–1041.
- THAL, J. (1588): Sylva Hercynia, sive Catalogus plantarum sponte nascentium in montibus et locis vicinis Hercyniae. Reprinted 1977 (ed. by S. RAUSCHERT), Frankfurt/Main. 283 pp.
- TILL, O. (1956): Über die Frosthärte von Pflanzen sommergrüner Laubwälder. – *Flora* **143**: 499–542.
- VAN BAALEN, J. & PRINS, E. G. M. (1983): Growth and reproduction of *Digitalis purpurea* in different stages of succession. – *Oecologia* **58**: 84–91.
- WERNER, K. (1964): Die Verbreitung der *Digitalis* Arten. – *Math.-Nat. Reihe, Martin Luther Univ. Halle-Wittenberg*, **6**: 453–486.
- WILSON, J. W. (1959): Notes on wind and its effects in arctic-alpine vegetation. – *J. Ecol.* **47**: 415–427.
- WOODWARD, F. I. (1975): The climatic control of the altitudinal distribution of *Sedum rosea* (L.) Scop. and *S. telephium* L. II. The analysis of plant growth in controlled environments. – *New Phytol.* **74**: 335–348.
- WOODWARD, F. I. (1990): The impact of low temperatures in controlling the geographical distribution of plants. – *Phil. Trans. R. Soc. Lond. B* **326**: 585–593.
- WOODWARD, F. I. (1997): Life at the edge: A 14-year study of a *Verbena officinalis* population's interactions with climate. – *J. Ecol.* **85**: 899–906.
- WOODWARD, F. I. & JONES, N. (1984): Growth studies of selected plant species with well-defined European distributions. I. Field observations and computer simulations on plant life cycles at two altitudes. – *J. Ecol.* **72**: 1019–1030.
- WOODWARD, F. I. & PIGOTT, C. D. (1975): The climatic control of the altitudinal distribution of *Sedum rosea* L. Scop. and *S. telephium* L. I. Field observations. – *New Phytol.* **74**: 323–334.
- WOODWARD, F. I. & WILLIAMS, B. G. (1987): Climate and plant distribution at global and local scales. – *Vegetatio* **69**: 189–197.