

An experimental study on the impact of winter temperature on the distribution of *Euphorbia amygdaloides* L. in Central Germany

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Abstract

The general range of *Euphorbia amygdaloides* L. in Europe and Asia is characteristic of a subatlantic-submediterranean distribution type. The main factor limiting its eastern distribution may be the minimum winter temperature. Field experiments were conducted to show the direct effects of decreasing temperature in a west-east gradient on individual plants. *Euphorbia amygdaloides* plants were transplanted along a transect beyond the distribution boundary in NW-Thuringia, Germany. Air temperature and soil temperature were recorded with data loggers at eight experimental plots. Frost damage was analysed at the end of the winter of 1995/96 and during the winter of 1996/97. Frost tolerance experiments were conducted in a freeze chamber during the same periods. Furthermore, frost damage in plots with and without snow cover was analysed. Frost damage was quantified by determining rates of electrolyte leakage (Murray et al. 1989). Additional information on the species' population biology and growth patterns near the distribution boundary is given. Photosynthesis measurements in response to changes in temperature provide data on the autecology of *Euphorbia amygdaloides*.

Introduction

Climate can be considered as the most important factor limiting plant ranges (e.g. Woodward 1987, Grace 1987). Consequently, there are numerous approaches in plant geography to determine the relationship between the geographic range limits of species and climatic constraints. To show

coincidences, researchers have mainly employed a broad geographical scale with a low resolution of 50 km or more (Dahl 1951, Jäger 1968, 1972, 1990, 1992, 1995a, Meusel & Jäger 1989, Box et al. 1993, Huntley et al. 1995). Investigations on a local or regional scale are scarce although range boundaries on this scale can clearly be encountered in nature. Ultimate boundaries of plant ranges are often well-known to plant geographers and therefore offer good opportunities for research. Such local work has been conducted in northern England (Grime 1990) and in Norway (Holten 1993). We are not aware of any Central European studies.

Among all climatic parameters, winter temperature can be considered the most significant in explaining the northern and eastern range limit of plant species (Sakai & Larcher 1987:1, Begon et al. 1996:61). By causing frost damage and killing whole individuals or important reproductive organs, low temperatures directly influence plant distribution. This was recognised quite early by vegetation scientists. Grisebach (1880) for instance tried to compare range limits of plants in Northwest Germany with isotherms of the coldest month in 1847. Up to the present, climatic maps with isotherms of the mean temperature of January as the coldest month of the year have proven useful to reveal coincidences with plant ranges (Hjelmqvist 1940, Woodward & Williams 1987). Closer relationships between plant ranges and temperatures can be determined by directly using temperatures measured by official weather stations and noticing whether the species under consideration is present or absent near the station (Iversen 1944, Hintikka 1963, Huntley et al. 1989, Box et al. 1993). Another way to utilise station data directly is to interpolate them to grid cells of distribution maps, as was done by Huntley et al. (1995) with Atlas Flora Europaea maps.

Recently, plant range climate correlations have gained much importance by offering the opportunity to predict range shifts as a response to anthropogenic climatic changes (Holten 1993, Hendry 1993, Jäger 1995a,b, Huntley 1991, Huntley et al. 1995). Climatic warming may cause an expansion of plant ranges of ornamental species that have been introduced from other climatic regions, were kept in gardens and now find suitable conditions to regenerate and escape into the surrounding area (Klötzli et al. 1996). To some extent, it is even possible to predict the range extension of anthropochorous weeds (Jäger 1988). A problem with predicting the range of adventitious species is that they are most probably not in equilibrium with range limiting factors other than climate, e.g. dispersal agents, herbivores or parasites. In contrast, this equilibrium can be assumed for most indigenous species. Therefore, indigenous species may be better indicators for climatic shifts.

Despite the general consensus that frost is a key limiting factor, there is disagreement as to whether frost should be characterised by mean temperatures or by extremes. Jäger (1995a) argues that range boundaries reflect averages because they are the result of a long-term confrontation with the plants' abiotic

environment. This may be true for heat or radiation requirements but not for frost damage. As far back as 80 years ago Eichler et al. (1927:305) argued that damage is not caused by low monthly or annual averages but by single frost events. They were able to relate the absence of suboceanic-submediterranean species such as *Ilex aquifolium*, *Tamus communis* and *Buxus sempervirens* in some areas in Southwest Germany to the occurrence of absolute minimum below -23°C measured by weather stations. Even when using extremes, however, the plant may experience temperatures that differ considerably from those measured in the air two meters above ground, which is the standard for weather stations (Grace 1987). We do not know of any field studies that directly relate frost damage observed close to a range boundary to minimum temperatures measured in the field.

Since different plant species show distinctly different responses to frost, autecological knowledge about the species is essential. This is especially true if climatic data are used to form a hypothesis on changes in the distribution pattern of a species (Holten 1993). Unfortunately, knowledge about the climatic factors limiting plant distribution is scarce and mainly available for species of commercial value (Holten 1993). Whereas in most cases detecting the autecological, climate dependent factor that limits a plant's range is a difficult and complex undertaking, (Pigott & Huntley 1981, Jäger 1989), the mechanisms of frost damage and frost hardiness are well understood (Sakai & Larcher 1987). Correlations between range limits and frost hardiness under controlled conditions in the laboratory were found by Till (1956), Kappen (1964), Polwart (1970 in Bannister 1976) and Callauch (1986). However, there is still no evidence (1) to show that minimum temperatures which cause damage in laboratory experiments also cause damage under field conditions and (2) that these temperatures differ inside and outside of the range of a given plant on a regional scale. The aim of this study is to fill this gap.

As a test species we chose *Euphorbia amygdaloides* because we considered it a suitable indicator of changing winter temperatures. The main question was whether the eastern range boundary of this species is determined by a minimum winter temperature.

We approached this question in various ways:

- by conducting a transplantation experiment along a transect beyond the distribution boundary
- by recording winter temperatures at sites with native and transplanted occurrences
- by quantifying frost damage in a controlled environment and in the field
- by performing biometrical investigations of native and transplanted individuals
- by determining the population pattern at the distribution boundary
- by measuring photosynthesis
- and by generally characterising site conditions (pH, light, soil moisture).

Study Object and Study Area

Euphorbia amygdaloides is characteristic of beech and oak-hornbeam forests with favourable nutrient supplies. The species mainly occurs on moist rendzinas on limestone with high pH and high base saturation. The plant is an evergreen perennial with a thick stock and tufted stems. During the winter it has shoots of about 30 cm in length which bear bunches of leaves at the top. The survival of shoots over the winter is essential for generative propagation. Only the previous year's shoots blossom at the end of April or beginning of May. Fruits ripen at the end of July. The generative shoots then die.

The general range of *Euphorbia amygdaloides* in Europe and Asia is characteristic of a subatlantic-submediterranean distribution type. According to Jäger (1970) *Euphorbia amygdaloides* belongs to the *Globularia*-type as a characteristic element of submeridional thermophilous deciduous forests. The species does not occur in the continental and northern parts of Europe but colonises the east up to the pannonical region and even into the colchic and hyrcanic provinces (Figure 1). The north-eastern distribution limit may be due to avoidance of low winter temperatures. The north-western limit indicates a distinct heat requirement (Meusel et al. 1978), since hard winter frosts can not be considered as a limiting distribution factor in Great Britain.

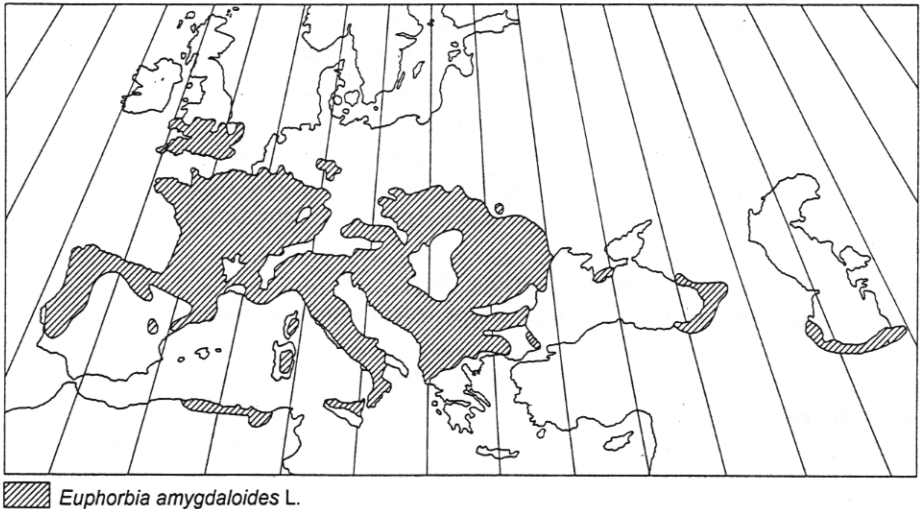


Figure 1: Distribution map of *Euphorbia amygdaloides* (modified after Meusel et al. 1978).

In southern Lower Saxony and Thuringia the range features an outpost with Göttingen in its centre (Figure 2).

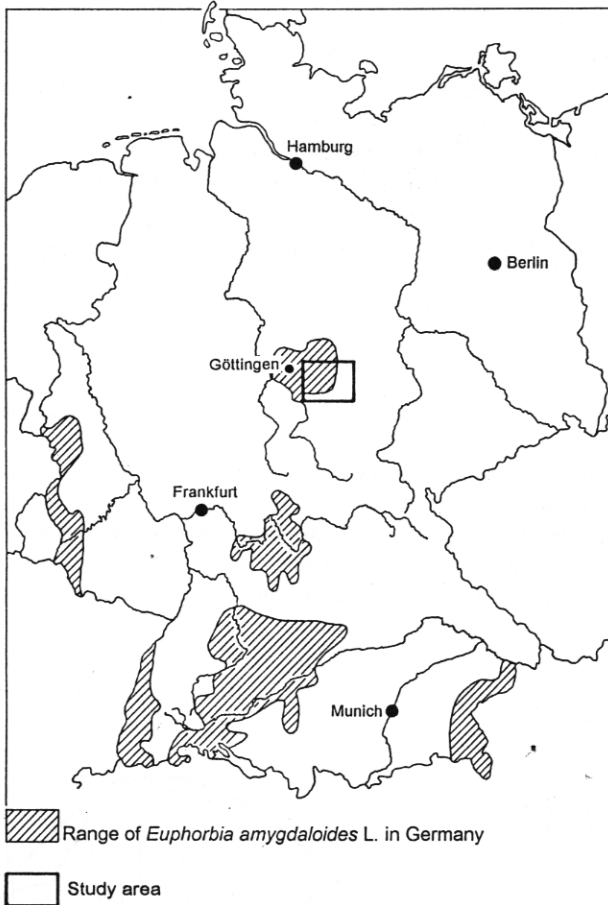


Figure 2: Distribution map of *Euphorbia amygdaloides* in Germany (modified after Haeupler & Schönfelder 1988 and Benkert et al. 1996).

There an exceptionally well-suited study area was found with a limestone mountain ridge called the Hainleite. A floristic inventory of this area was first carried out by Weinitschke (1959). He already pointed out the occurrence of distribution boundaries for several plant species along the mountain ridge. One of the plants he mentioned was *Euphorbia amygdaloides*, which occurs in the western Hainleite but not in the eastern part despite the existence of suitable soil conditions and vegetation types (Figure 3).

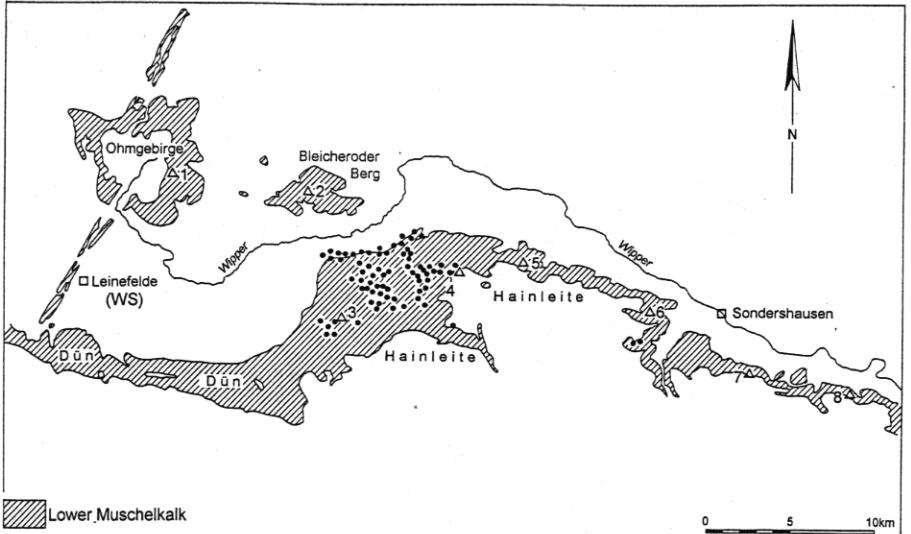


Figure 3: Study area (according to the geological map 1:200,000):
 WS: Weather station at Leinefelde
 Δ: experimental plots. Plots 1 to 4 are within the range of *Euphorbia amygdaloides*, plots 5 to 8 are outside.
 The black spots (•) mark populations mapped by Weinitschke (1959) in the Hainleite area at its distribution limit.

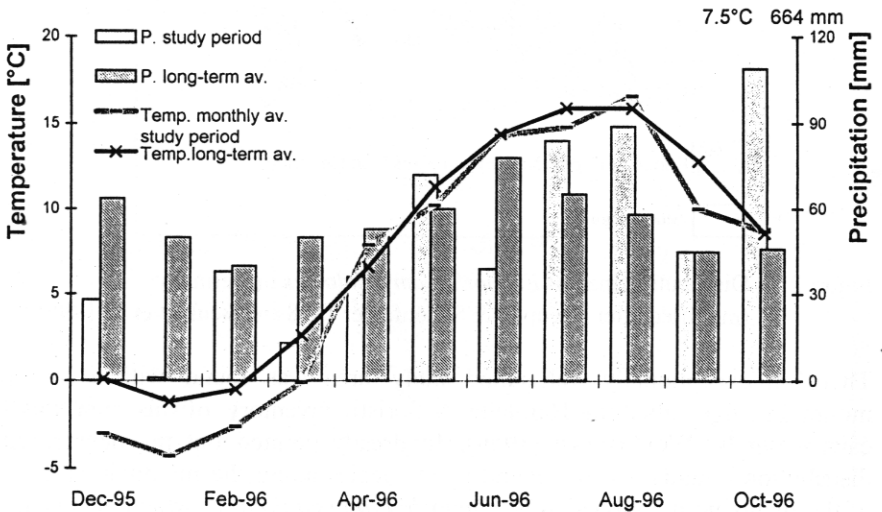


Figure 4: Monthly mean temperatures and precipitation for Leinefelde, 365m above sea level, for the investigation period from December 1995 to October 1996 and for the long-term average (1961-1990).

Figure 4 shows the climatic conditions for Leinefelde, the weather station closest to the study area, both for the investigation period and for the long-term average. The annual mean temperature is 7.5°C and average sum of annual precipitation is about 660 mm (Deutscher Wetterdienst 1995/1996). The precipitation in 1996 differed greatly from the long-term average due to a drier winter and a wetter summer. The winter of 1996 was exceptionally cold and therefore offered excellent conditions for investigating frost damage. The mean temperature recorded for January 1996 at the weather station was -4.3°C, which is 3.1°C colder than the long-term average (1961-1990). The mean temperature in February 1996 was -2.6°C, compared with a long-term average of -0.5°C. The average in March 1996 was still below the freezing point (the March average is normally about 2.6°C). The lowest temperature recorded by the weather station at the standard height of 2 m was -15.5 °C on the 7th of February 1996. The lowest temperature recorded near soil level was -19.1°C on February the 9th.

Methods

Transect

A transect was set up from WNW to ESE across the distribution boundary along the Hainleite mountain ridge (Figure 3). In December 1995 experimental plots were established about every six km along this transect. Plots 1 to 4 were placed within the *Euphorbia amygdaloides* range, and plots 5 to 8 were placed outside.

All plot sites chosen were comparable and fulfilled the requirements for *Euphorbia amygdaloides* habitats. The criteria were:

- *Fagus sylvatica* forests
- Plateau locations
- Limestone sites
- The plots within the *Euphorbia amygdaloides* range had to contain native *Euphorbia amygdaloides* populations
- Easily reached by car

Since the mountain dips gently to the east, the plots' elevation could not be kept completely constant (Table 1). The highest was plot 3 at 474 m above sea level. This plot was located within the *Euphorbia amygdaloides* population, second nearest to the distribution boundary. The plot with the lowest elevation was plot 8, located furthest towards the east, at 365 m above sea level.

The light conditions ranged between 2.8% and 4.6% with the exception of plot 2, which showed a significantly higher (8.8%) relative light intensity than the others (Table 1).

The pH at all sites was higher than 5.5 except for plot 7, which had a pH of only 4.6 due to loess influence. This plot was also the driest at the end of summer (Table 1).

All plots were equally installed in December 1995 (Figure 5).

	Inside range of <i>Euphorbia amygdaloides</i>				Outside range of <i>Euphorbia amygdaloides</i>			
	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8
Elevation [m a.s.l.]	465	457	474	422	415	410	390	365
Rel. intensity of light [%]	3,1	8,8	4,3	3,4	2,8	4,6	3,1	3,0
pH (H ₂ O)	6,1	5,5	7,2	6,5	6,3	6,1	4,6	5,4
Soil humidity [vol. %]								
21-May-96	37.5	35.0	34.4	36.7	38.3	36.3	33.2	36.9
09-Aug-96	22.9	22.8	25.3	22.9	23.0	24.3	13.5	21.4

Table 1: General characterisation of the eight experimental field plots.

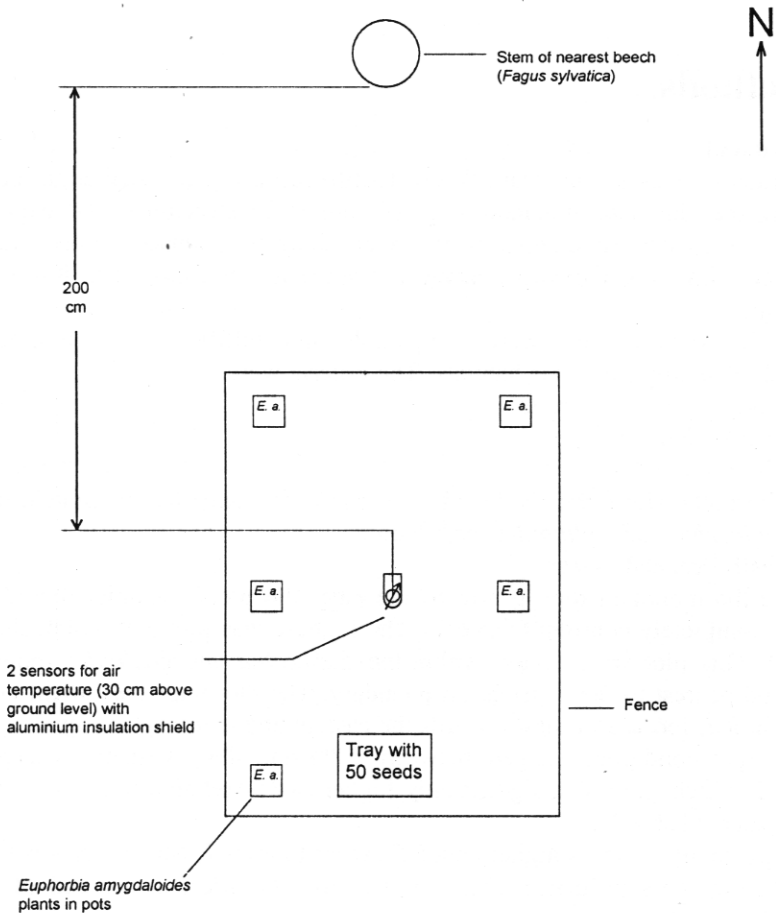


Figure 5: Schematic plan of the experimental plots.

Euphorbia amygdaloides plants which had previously been taken from a large population close to plot 1 and planted into pots were randomly transplanted throughout the eight plots along the transect. Each plot received five plants. Furthermore, each plot was provided with a tray filled with soil and 50 *Euphorbia amygdaloides* seeds in order to observe the climatic influence on germination.

Air temperature was recorded at 30 cm above ground level, which corresponds to the average height of *Euphorbia amygdaloides*, instead of at the standard height of 2 m, which probably would have yielded significantly higher temperatures than closer to the ground. The temperature was recorded every 1.2 hours using data loggers (Tinytalk-Temp, Miniature Temperature Loggers, Orion Components Ltd.) during the winter and during the following vegetation period. The sensors were insulated by an aluminium shield. Soil humidity was measured regularly using time domain reflectometry (TDR, Soilmoisture Equipment Corporation). Relative light intensity was measured on three different dates in the period from 08/20/96 to 09/02/96 at each plot. We used two Li-Cor data loggers (LI-1000) with PAR quantum-sensors. One of them, which was used as a reference for 100% light, was placed outside of the wood as close to the plot as possible. The other measured light intensity at the height of 30 cm close to the transplanted *Euphorbia amygdaloides* plants. The light conditions had to be diffuse.

Frost tolerance and frost damage

1. Frost tolerance experiments in freeze chambers were carried out at the beginning of April and in December 1996 on individuals taken from the same population that had been the source for the transplantation experiment. The plants were randomly exposed to a non-freezing control temperature (+4°C), and to -6°, -12°, -18°, -24° or -30°C (Figure 6). Additionally, some plants were treated with liquid nitrogen (-196°C).
2. All transplanted individuals were examined for frost damage in March 1996 and in December 1996.
3. Figure 7 shows the snow cover along the transect in March and November 1996. The snow cover followed more or less the west-east gradient.

The snow cover at plot 4 close to the distribution boundary was 8 cm in March 1996. This provided the opportunity to perform a further field experiment because the snow covered only parts of the native *Euphorbia amygdaloides* population. Plants were collected which were either completely covered by snow or which stood out from the snow.

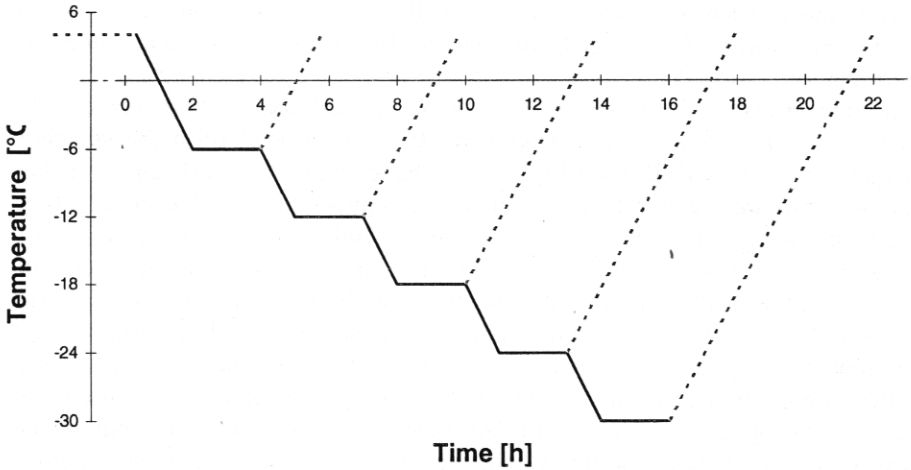


Figure 6: Freezing treatment in the frost chamber experiment: test plants were taken out of the freeze chamber after being exposed to a frost level for two hours. Afterwards the chamber with all remaining plants was cooled down to the next level.

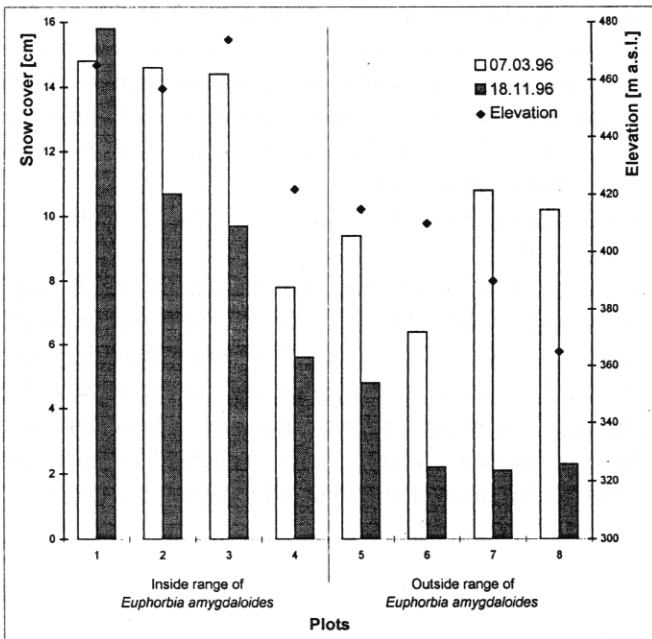


Figure 7: Snow cover distribution along the transect in March and November 1996.

In all experiments the test for frost damage was based on electrolyte leakage (Murray et al. 1989). Plant tissue was put in test tubes with demineralized water and 3 % propanol. The electrical conductivity of the solution was measured (LF 90, LF 2000, WTW) at the beginning (C_0) and then regularly for about 4 days (C_t). The more the tissue was affected by frost damage, the more rapid an increase in conductivity could be detected. The solution's conductivity after being boiled for 20 minutes (C_{boiled}) was used for reference.

Each time series of relative conductivity (RC) was fitted (SAS 6.02: proc regr) using an exponential function (1). The resulting parameter k increases with the rate of electrolyte leakage and can therefore be evaluated as a measure of frost damage:

$$RC = \frac{C_t - C_0}{C_{\text{boiled}} - C_0} = 1 - e^{-k \cdot t} \quad (1)$$

Biometrical investigations of transplanted individuals

The growth rate of the transplanted individuals was recorded during the course of the vegetation period as was the germination rate of the seeds, the seedling survival rate, fructification, seed production and seed weight.

Population pattern at the distribution boundary

The pattern of plant occurrences at the distribution boundary was studied according to two different scales with respect to two different questions:

- How does population density decrease towards the distribution boundary? - Abruptly or continuously?
- How do individuals occur with respect to each other towards the distribution boundary? - In clumps, randomly or regularly?

The first question was tackled by marking areas of 1 km² around plots 1 to 8. The areas were placed in such a way that they contained only forest. At least five subareas of 1 ha each were randomly chosen. The density of *Euphorbia amygdaloides* within each hectare was estimated into six classes.

Since one hectare could be covered by more than 10'000 individuals, much smaller test areas were chosen to answer the second question. In three of the 1 ha subareas with an occurrence of *Euphorbia amygdaloides* a 10 by 10 meter section was randomly chosen. The *Euphorbia amygdaloides* plants within these 100 m² were counted and their co-ordinates registered. A test for random, regular or clump distribution was conducted according to Manly (1992) based on the nearest neighbour method.

Measurement of photosynthesis

Plants were again taken from the population near plot 1. Photosynthesis was measured with a compact minicuvette system (CMS 400, Walz) in the laboratory at four different dates.

We studied the response to light and to temperature. Different light intensities (0 to about 550 $\mu\text{mol photons/m}^2\cdot\text{s}$ PAR [Photosynthetic active radiance]) were generated using an artificial light source and different grey filters. The temperature of the cuvette was controlled. Due to problems with condensation, however, it could only be varied by 10°C above and below the ambient temperature. The temperature was changed in 4°C steps. For details of measuring photosynthesis see Schulte (1993) or Willert et al. (1995).

Important parameters of photosynthesis were estimated by using a biochemical model of photosynthetic CO₂ assimilation. The extended Arrhenius equation (2) describes the response of A_{max} to temperature (Harley & Tenhunen 1991). The curve shows the temperature optimum for photosynthesis:

$$A_{\max} = \frac{e^{-\frac{c}{R \cdot T_{\text{leaf}}}} \cdot \frac{dHa}{R \cdot T_{\text{leaf}}}}{1 + e^{-\frac{dS \cdot T_{\text{leaf}} - dHd}{R \cdot T_{\text{leaf}}}}} \quad [\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}] \quad (2)$$

A_{max}: Maximum net assimilation rate at irradiation saturation and ambient CO₂-level [μmol CO₂/m²·s]

R: Gas constant R = 0.00831 [KJ/mol·K]

dHa: Activation energy [KJ/mol]

dHd: Energy of deactivation [KJ/mol]

dS: Entropy [KJ/mol]

c: Constant

T_{leaf}: Leaf temperature [°C]

Results

Temperatures along the transect

The temperatures of all plots during January 1996, which was the coldest month of the year, are shown in Figure 8. Figure 9 shows the temperatures for February 1996, when the lowest temperatures were recorded. It was assumed that the transect would show an oceanic-continent temperature gradient and that the lowest minimum would be observed in the east. This assumption proved to be untrue. In addition to plots 5 and 6 in the eastern part, plot 2 in the west showed the lowest temperature values. The average difference between these three plots and the others was 1.5°C for January 1996 and 1.4°C for February 1996. This same phenomenon was observed throughout the winter (Figure 10).

There was little difference between the plots regarding absolute minimum temperatures. The lowest temperature of all was -18.8°C, recorded on the 7th of February at plot 4. This was followed by -18.7°C at plots 6 and -18.6°C at plot 5. The minimum temperature of the other 4 plots was only 0.9°C to 1.8°C warmer than the temperature of the coldest plot (Table 2).

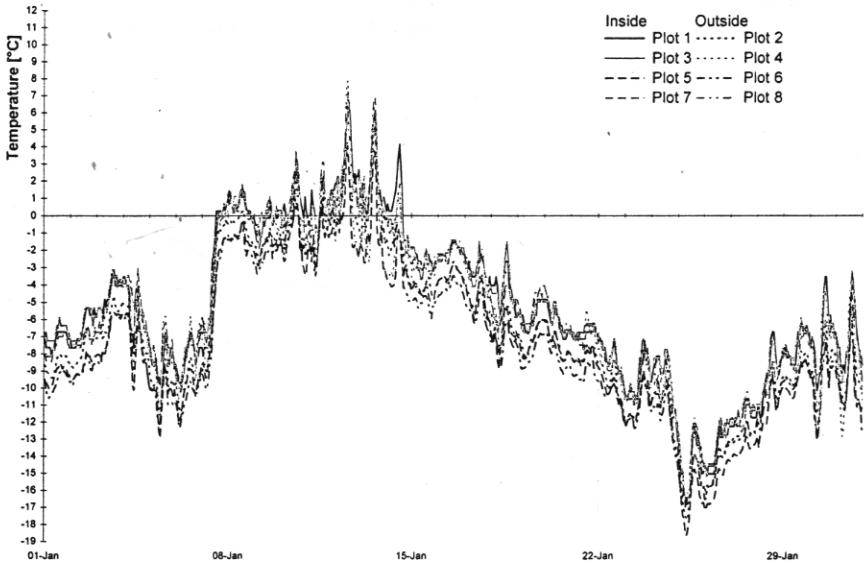


Figure 8: Air temperature along the transect, January 1996, 1.2 h interval, 30cm above ground.

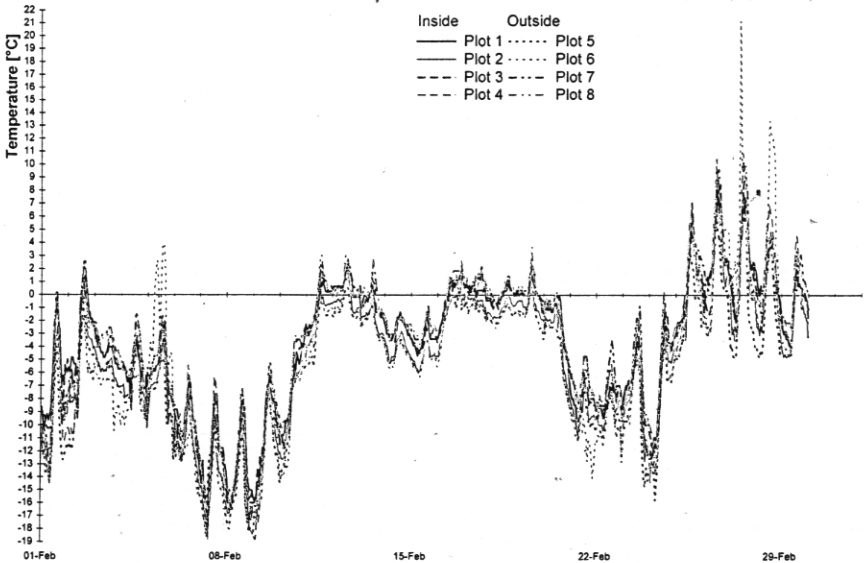


Figure 9: Air temperature along the transect, February 1996, 1.2 h interval, 30cm above ground.

Due to a failure of data loggers at plot 2, 5 and 6 in some periods in January and February and to a replacement by another logger model, the values of these plots may, to a minor degree, vary more than the others.

There were hardly any temperature differences during the summer months (Figure 10). Only plot 4 showed a higher temperature amplitude.

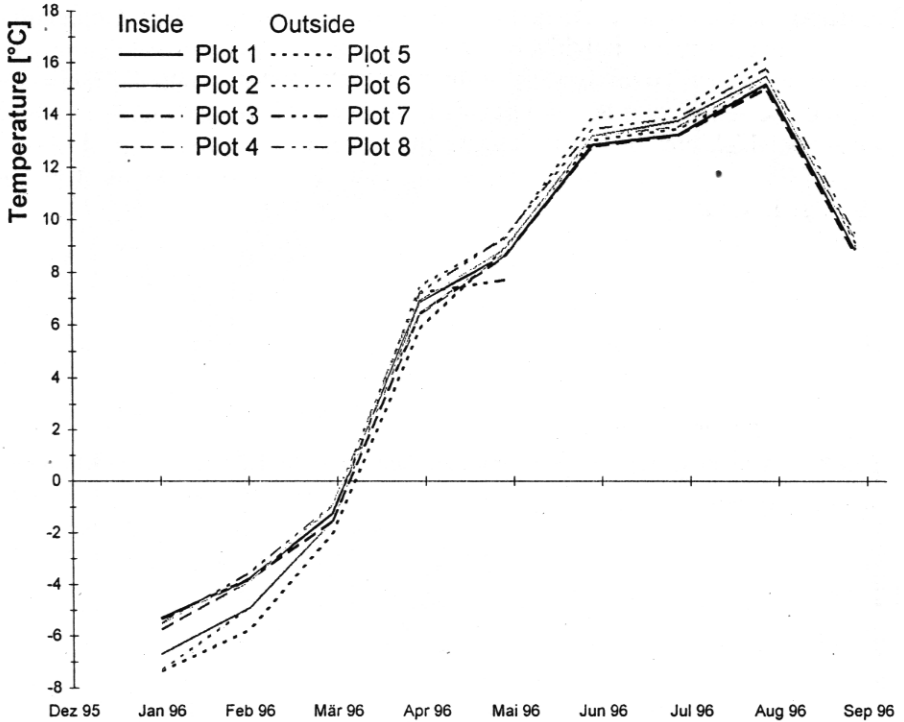


Figure 10: Mean monthly air temperature along the transect, 30cm above ground.

	Inside range of <i>Euphorbia amygdaloides</i>				Outside range of <i>Euphorbia amygdaloides</i>			
	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8
Absolute minimum temperature [°C]	-17.4	-17.9	-17.0	-18.8	-18.6	-18.7	-17.2	-17.4

Table 2: Absolute minimum temperatures along the transect, 30cm above ground.

Due to a failure of data loggers at plot 2, 5 and 6 in some periods in January and February and to a replacement by another logger model, the values of these plots may, to a minor degree, vary more than the others.

Frost tolerance and frost damage

1. Freeze chamber experiment

Frost damage to leaves after exposure to the six freezing treatments is shown in Figure 11. Only the -24°C and the -30°C treatments could be distinguished from all others within a 5% confidence interval.

Damage to different plant organs is shown in per cent of total damage (Figure 12). Damage among individual plants is highly variable. Therefore, in most cases only two groups can be statistically distinguished. For all organs the -24°C and -30°C treatments differed from all the other treatments. Only the leaves, which are the most delicate plant organs, showed a difference at -18°C as well. This lethal temperature threshold (-18°C) could also be assessed visually. The root colour changed as well, becoming dark when dead.

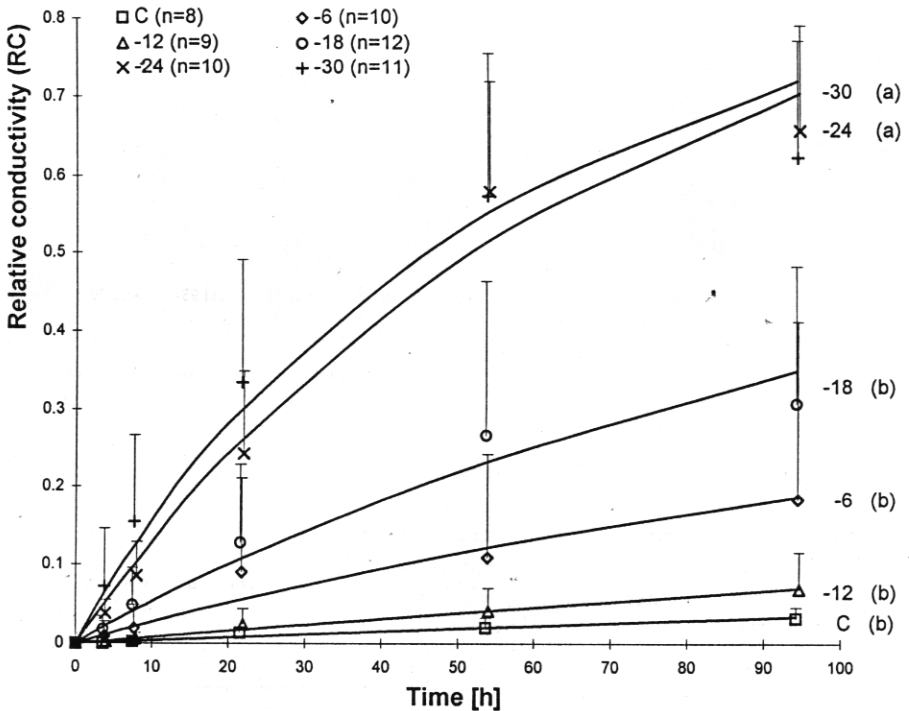


Figure 11: Exponential saturation curve according to equation (1) for the six freezing treatments of the leaves in December 1996. Bars: standard deviation.

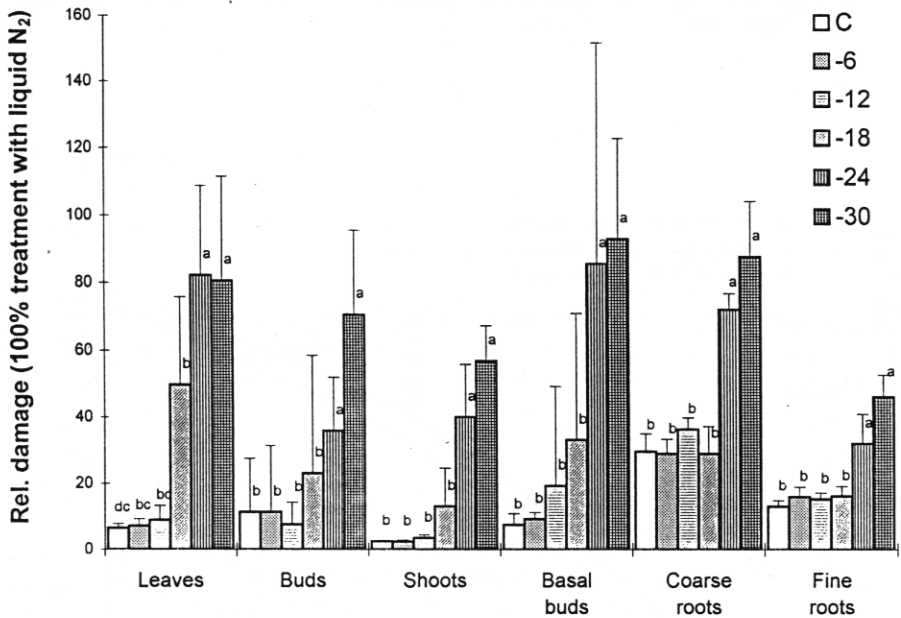


Figure 12: Relative damage to different plant organs in per cent of total damage; 100% damage was caused by treatment with liquid nitrogen, April 1996. Number of replicates (n) see below.

n	Leaves	Buds	Shoots	Basal buds	Coarse roots	Fine roots
°C	10	10	10	8	5	5
-6	10	10	10	9	6	6
-12	9	9	9	8	5	5
-18	10	10	10	8	5	5
-24	10	10	10	10	4	4
-30	10	10	10	6	5	5

2. Field experiment with transplanted individuals along the transect:

There was no significant difference in electrolyte leakage between the transplanted *Euphorbia amygdaloides* individuals, neither during the winter nor at the end of the winter (Figure 13).

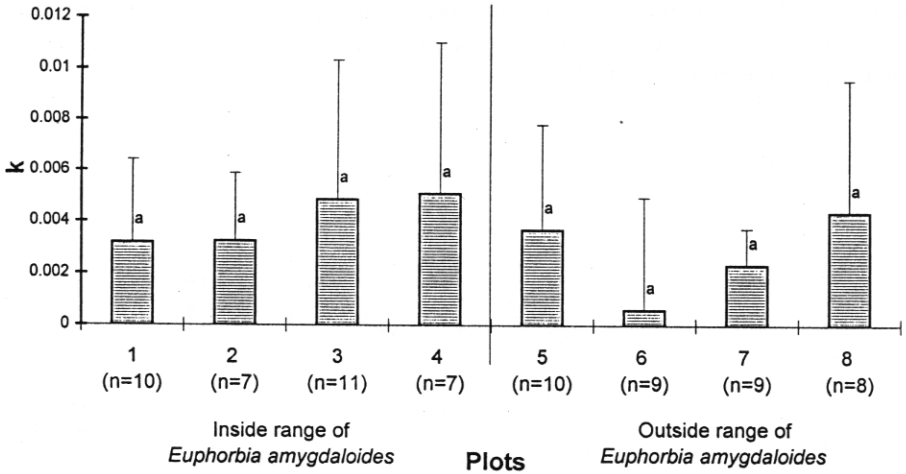


Figure 13: k as a measure of frost damage, leaves from the transplanted *Euphorbia amygdaloides* individuals, April 1996.

3. Snow cover had a significant effect on all investigated organs (Figure 14). k as a measure of frost damage differed in the same order of magnitude as obtained by frost treatments from -12°C to -18°C.

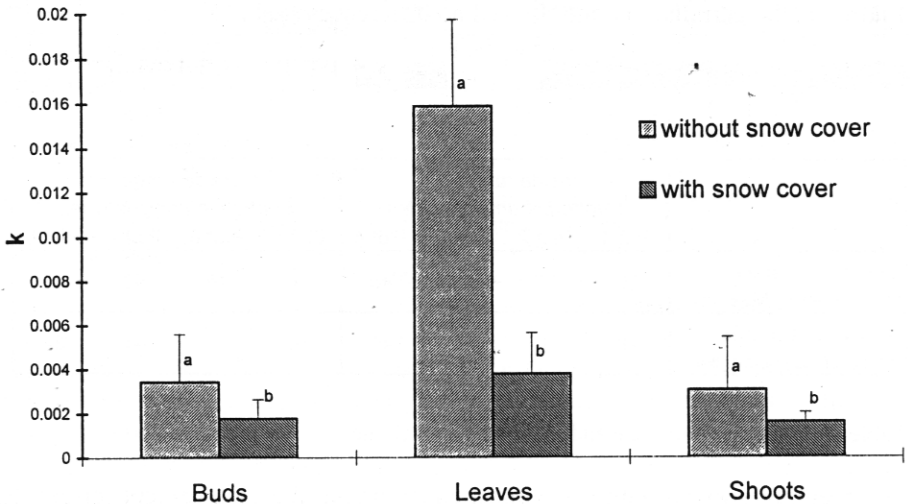


Figure 14: k as a measure of frost damage, *Euphorbia amygdaloides* individuals with and without snow cover, April 1996. (n=20)

Biometrical investigations

Two plants died in each of plots 2, 3 and 7, which was probably not due to frost damage. The plants may have been damaged when put into pots. No significant differences in vegetative growth could be detected during the entire investigation period (Figure 15).

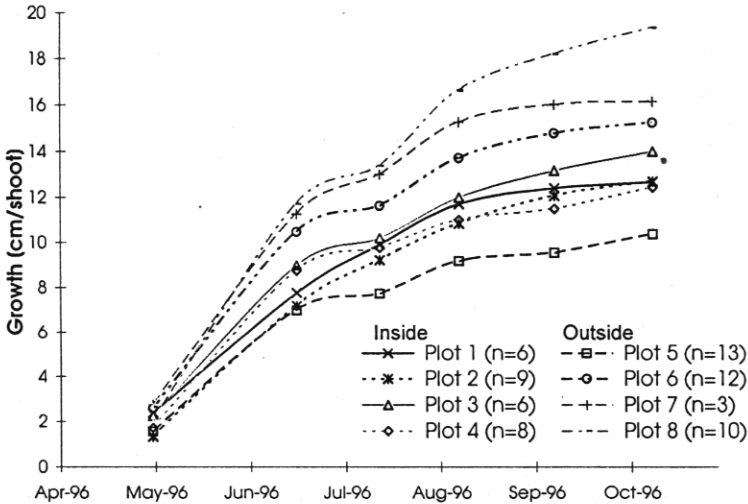


Figure 15: Growth of vegetative shoots. Reference length (0) is December 1995.

Likewise, the germination rate showed no differences (Table 3).

	Inside range of <i>Euphorbia amygdaloides</i>				Outside range of <i>Euphorbia amygdaloides</i>			
	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8
Germinated seeds (of 50 seeds) May 96	36	26	49	48	34	48	50	43
Survived seedlings (of 50 seeds) Dec. 96	26	1	34	46	16	31	41	34

Table 3: Germination rate and seedling survival rate.

The seedling survival rate was almost equal among the plots except for plot 2, where only one plant survived. No significant differences in fructification were observed (Table 4).

	Inside range of <i>Euphorbia amygdaloides</i>				Outside range of <i>Euphorbia amygdaloides</i>			
	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8
Average number of fruits per plant	8.0	0.0	13.7	10.0	10.5	7.3	18.0	6.3
Average number of fruits per generative shoot	6.8	0.0	10.2	10.0	7.8	7.3	5.3	5.6
1000-seed weight [g]	4.9		6.3	3.7	5.7	3.9	5.9	5.3

Table 4: Fructification of the transplanted *Euphorbia amygdaloides* individuals, July 1996.

Population pattern

The large-scale population pattern showed a distinct decrease in density towards the distribution boundary (Table 5).

		Inside range of <i>Euphorbia amygdaloides</i>				Outside range of <i>Euphorbia amygdaloides</i>			
Class	Plants/ha	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8
5	100000	1/5							
4	10000	3/5		1/8					
3	1000	1/5	1/5	2/8	1/5				
2	100		2/5		1/5				
1	10		1/5		1/5				
0	0		1/5	5/8	2/5	5/5	5/5	5/5	5/5

Table 5: Population densities of *Euphorbia amygdaloides* in randomly chosen 1ha squares around the experimental plots.

The numbers indicate the plots. The median density is indicated in black, the range is indicated in grey. The fractions correspond to observations. For example, 100 to 1000 plants per ha were observed two times within 1km² around plot 3. No plants were observed six times.

Black: median, grey: range of density, fractions: fractions of observations.

The highest densities were observed around plot 1. All investigated populations were significantly clumped on the scale of 100 m² plots. Figure 16 gives an example.

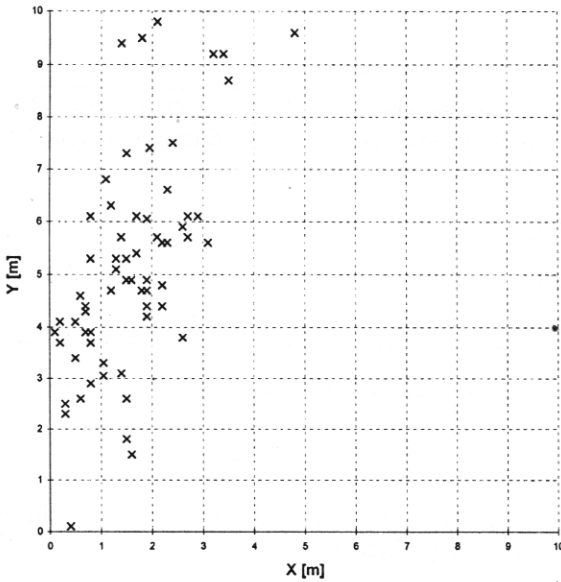


Figure 16: Distribution pattern of all (63) *Euphorbia amygdaloides* plants in a 10m by 10m area, which was randomly located near experimental plot 2.

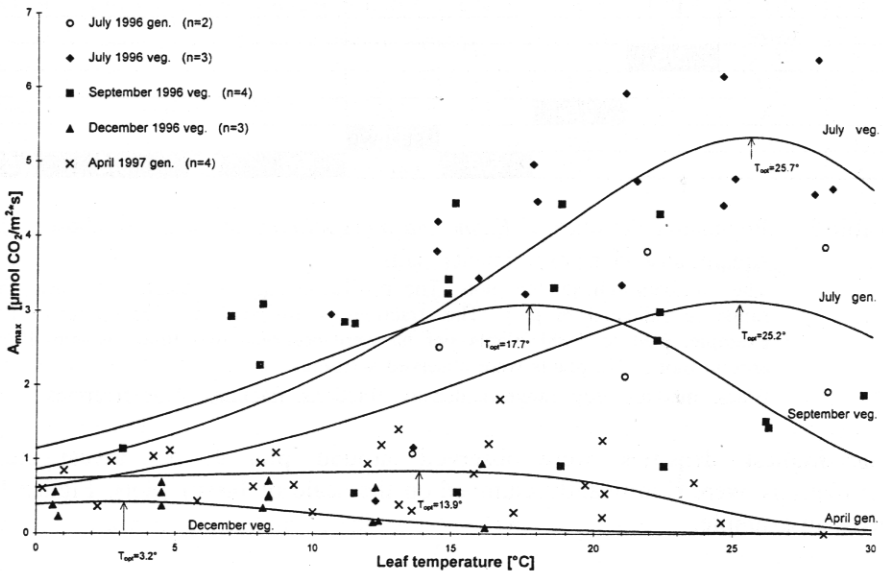


Figure 17: Response of A_{max} (maximum net assimilation rate at irradiation saturation (~550 $\mu\text{mol photons PAR/m}^2\cdot\text{s}$) and ambient CO_2 -level) to changes in temperature. Modelled optimum curves according to equation 2.

Measurement of photosynthesis

Figure 17 shows the maximum net assimilation response (A_{\max}) to changes in temperature. Responses of individual plants showed great variability. The acclimation of the plants in colder seasons is obvious, as the temperature optimum of A_{\max} drops from 26°C in summer to 18°C in autumn and down to only 3°C in winter. It increases again in April to 14°C.

Discussion

We conclude that winter minimum temperature quite probably determines the north-eastern range limit of *Euphorbia amygdaloides*. Temperatures measured in the field reached values that caused damage to plant tissues in freeze chamber experiments. The threshold temperature was about -18°C for leaves and some degrees lower for buds and shoots (Figure 12). At -24°C and below all plant organs were damaged significantly. These values indicate that the frost tolerance of *Euphorbia amygdaloides* approaches the lower limits for temperate herbs (Till 1956, Bannister 1976, Burke et al. 1976, Larcher & Bauer 1981).

Temperatures below -18°C were also recorded in half of the field plots (Table 2). However, these extremes neither resulted in killing the test plants nor were there significant differences in frost damage among the transplanted individuals (Figure 13). An explanation for the discrepancy between laboratory and field data could be a microclimatic variability within the plots. The individual plant organs may have experienced higher temperatures than measured in a height of 30 cm. To collect more precise information, sensors would have to be placed in direct contact to the plant's organs. Apart from requiring a much higher expenditure of plot equipment this design would have created much more artificial conditions. For example, fixed sensors could have influenced the shoots' movements and prevented their bending under snow weight.

Assuming that microclimatic variability is only a minor influence, the question arises as to whether the field conditions met the requirements of a field study. The winter of 1995/1996 might simply not have been severe enough to cause significant damage. The results of our freeze chamber experiments indicate that a winter with extremes of around -24°C would have been necessary to kill the test plants. Nevertheless, the weather conditions met another requirement for frost damage, namely low snow cover. The significant influence of snow cover (Figure 14) indicates that the plants can endure much lower temperatures with sufficient snowfall. A snow cover of more than 20 cm rarely allows the temperature to drop below -5°C (Sakai & Larcher 1987: 16). Since precipitation was exceptionally low in January (Figure 4) the winter can be considered to have offered almost optimum conditions for this field experiment.

The effect of snow on plant distribution has been pointed out by various authors. With respect to the Hainleite area, Köhler (1967) and Meusel et al. (1978: 11) suggested to take snow cover into consideration to understand plant

distribution. Uemura (1989) showed that snow exerted a strong influence on the distribution pattern of evergreen plants in Japan.

It should be possible to assess the risk of frost damage at low snow cover by combining both parameters into an index. It is astonishing that such an index has not yet been proposed in plant geography (cf. Tuukkanen 1980). In analogy to the simplest possible humidity index, in which the moisture factor H is defined as a ratio of monthly precipitation in mm to monthly mean temperature in $^{\circ}\text{C}$, an index for frost risk could be derived with F as a reciprocal of H . F should be formulated for the coldest month and would decrease (negative sign) with increasing frost risk. F should be valid only for mean temperatures below a threshold (e.g. 0°C) and the influence of precipitation should be weakened by adding a constant to monthly precipitation (e.g. +10). It would be worthwhile to test such an index for coincidences with ranges of European plant species.

We would like to include a few comments on using standard climatic data recorded at a height of two meters by weather stations for correlations with plant ranges. In the case of forest plants such as *Euphorbia amygdaloides*, the question arises whether temperatures at the undergrowth level are comparable to temperatures in the open field. It is often stated that the tree canopy of a forest reduces the heat loss of the herbal layer, resulting in more evenly levelled temperatures (Sakai & Larcher 1987: 9). Comparing the minimum temperature in the field of -18.8°C recorded at plot 4 (Table 2) with the minimum temperature of -15.5°C measured by the official weather station of Leinefelde at the standard height of two meters above the ground reveals that the canopy effect does not fully compensate the low temperature closer to the ground. This observation is consistent with values given by Sakai & Larcher (1987: 11) that temperature near to the ground may be 5°C lower than at a height of 2 m and consistent with the measurement in Leinefelde at ground level of -19.1°C . The comparison reveals that forest herbs may experience minimum temperatures of 3.3°C lower than temperatures measured in standard weather stations in the surrounding area. This effect should be taken into consideration when trying to correlate isotherms with plant ranges of forest plants.

Apart from having lethal effects on plants, frosts affect production processes even when they are only moderate. Restriction of photosynthetic production at low temperatures is shown clearly in Figure 17. During seasons of freezing events photosynthesis capacity reaches only 8.0% of the maximum which was found in July. This decrease of photosynthesis can be clearly interpreted as an effect of frost hardening (Larcher & Bauer 1981). In the midst of winter photosynthetic activity is extremely low, indicating that its contribution to the annual dry matter production is negligible. In this respect evergreen leaves do not seem to be advantageous for *Euphorbia amygdaloides*. Of course, this is true for only part of the total range of the species. In regions with mild winters, photosynthetic production in this season will be greater.

The test plants experienced frosts even in April, which might explain their low photosynthetic activity in this month due to an inhibitory effect after freezing, even if the event occurred several nights before (Larcher & Bauer 1981). Nevertheless, a warm spring is likely to support the *Euphorbia amygdaloides* occurrence. Jäger (1970) gives an interpretation of the north-eastern boundary as being coincidental with the 8°C-April isotherm, which indicates a requirement for warm springs. It is quite probable that warm springs are more favourable to respiration processes resulting in a break down of stored carbohydrates for early bud burst and vegetative growth rather than photosynthetic dry matter production.

In summer, the lack of differences in vegetative growth (Figure 15) corresponds to the marginal differences in summer temperature (Figure 10). Other studies showing differences in growth rates along transects have used much steeper climatic gradients (Woodward & Pigott 1975, Friend & Woodward 1992: 89ff.).

Whether a species is able to maintain a viable population at a particular site is not only dependent on its survival and production capacity but also on its ability to propagate and multiply. The observed temperatures had no lethal effects on the plants' buds from the previous year (Figure 12). Freezing events in early spring did not harm the flowers, which can be concluded by the normal seed production of most test plants (Table 4). Germination and the seedling survival were not reduced either (Table 3), with the exception of plot 2. It is likely that the death of all but one seedling in this plot was due to the comparatively low temperatures there, which were probably caused by a more open canopy (Table 1). This observation, together with the direct frost damage to adults, can explain why the species is mostly confined to forests in the study area and only occasionally grows in forest gaps or at forest edges.

From the study's results we conclude that *Euphorbia amygdaloides* could also survive outside of its range at plots 5 to 8, provided that winters are no more severe than during the study period. The question arises whether the actual distribution limit along the transect is identical to the potential limit, which may be located further to the east. Without being able to answer this question we have evidence that it is impossible to answer it on the scale of our study. Variability in microclimate among plots 1 to 4 within the *Euphorbia amygdaloides* range is at least as high or even higher than between plots 5 to 8 outside the range. It can be assumed that at least some sites outside the actual range would meet the requirements for supporting a viable *Euphorbia amygdaloides* population. We suppose that the presence of such sites follows a probability function. For *Euphorbia amygdaloides* the probability of the existence of suitable habitats within the range gradually decreases towards the actual distribution limit. Outside the actual distribution boundary, the probability of encountering suitable habitats greatly decreases. If we assume a lower probability of suitable sites and therefore greater distances between them, it becomes even more improbable that these distances can be spanned by dispersal agents. From this point of view the actual distribution limit is only a

sharp bend in the probability function. Consequently, the actual distribution limit would not be a line but a zone with a geographical extension. The potential distribution limit would be the point where probability is zero. This definition differs considerably from the traditional formulation for the potential distribution as the range that could be developed when competition from other plants is excluded (Jäger 1972, Walter & Straka 1970).

Our concept is supported by the results of the distribution pattern along the transect (Table 5). The probability of finding *Euphorbia amygdaloides* within a randomly located square of 1 ha decreases from plot 1 to 4, as do the number of individuals within 1 ha. It is likely that the trend could be demonstrated even more by including further transects in other regions. A consequence of this concept for plant range studies would be to shift the focus from autecology to population biology.

However, on a scale of 100 m² the *Euphorbia amygdaloides* population is unlikely to follow a probability function because all investigated squares revealed a clumped pattern. Most probably, clumping is an effect of the species dispersal biology. *Euphorbia amygdaloides* seeds are spread by ants (van der Pijl 1982). It can be assumed that myrmecochory is responsible for long distance dispersal, ensuring settlement in suitable site conditions. By contrast, autochory may be responsible for a clumped growth pattern at a given site. In order to study the future development of this growth pattern we have marked each of the 100 m² squares with magnets.

Although there is evidence that winter temperature is the key factor limiting the *Euphorbia amygdaloides* range in this area there are of course other possible explanations. For example, high summer temperatures may favour an increased floral initiation, resulting in more abundant flowers in the subsequent year (Pigott & Huntley 1981, Owens 1991). Temperature can affect all stages of the life cycle, beginning with the seed bank and ending with seed production. Woodward & Williams (1987) described the transition from one stage to the next as a probability function influenced by temperature. More long-term studies would be useful to assess these effects. Furthermore, temperature can also have an additive quality (Went 1957, Cleary & Waring 1969). The calculation of heat sums could be useful for an interpretation of the north-western boundary of the *Euphorbia amygdaloides* range in Europe.

Finally, the question remains whether this study allows us to make predictions in the larger context of global climatic change. Such predictions are, of course, highly uncertain due to the uncertain trend of all climatic parameters and due to the high acclimation potential of plants (Skre 1993). Further uncertainty factors must be taken into consideration with respect to our probability concept of range limits. However, if absolute temperature minima increase and/or snow in January becomes more abundant, the *Euphorbia amygdaloides* range will probably expand towards the east.

Despite all the difficulties of our approach, we consider further field studies on a local scale in combination with laboratory tests to be useful.

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The importance of plant ranges in understanding the life history of plant species was early recognised by Prof. H. Meusel, who died on the 3rd of January 1997. It is to his memory that we dedicate this paper.

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Plenary discussion

Statement: N. KRÄUCHI

I have a minor remark with respect to the setup of your meteorological instruments. I strongly suggest to measure temperature according to standardized manuals. Temperature is commonly measured at 2 meters above ground. If you measure at different heights within the forest stand and in the open field you need to transform the data for comparison. I would further suggest to add meteorological stations in the open field as close as possible to the sites in the forest.

Statement: H. BRUELHEIDE

Exactly this has been tried by forest-ecologists in Göttingen. They had to establish a net of weather stations inside the forests in the Harz Mountains, because they could not compare any station outside the forest with the climatic conditions inside. Additionally, they established stations nearby in the open field.

Statement: N. KRÄUCHI

These microclimatic differences of the forested and non-forested nearby site was the main reason to use two meteorological stations for each plot of the Long-term Forest Ecosystem Research (LTFER) Programme in Switzerland.

Statement: F. KLÖTZLI

I also wanted to point out the fact. I'm not against the fact that you measure it near the plant or on the plant but you should have a reference point on the international level of measurements. What I want to say is also endorsing your conclusion: one year is not enough, that's clear, we don't have to discuss that. But you will see *Euphorbia amygdaloides* is behaving in a rather crazy way. It is very resistant, it is not browsed by roe-deer or so, it's not attacked by insects, it's influenced by pathogens e.g. from fungi such as ascomycetes. So you get jumps in its appearance, that's our experience. You have chosen a "funny plant". It's not because you have done a funny work, no, you have just chosen a plant which in an absolutely unpredictable way is very resistant, very stable and all of a sudden it is gone.

Statement: H. BRUELHEIDE

We have not known before our studies that *Euphorbia amygdaloides* tolerates such low temperatures, so this is one of the main results of our work.

Statement: F. KLÖTZLI

That's what I meant to say, it's a very stable plant but all of a sudden it's not here anymore. And then it jumps and that's why you get such "funny" boundaries. The plant is well chosen in a way as you have said, the criteria are clear, but if you continue you will have a lot of surprises with it.

Statement: A. BORHIDI

I would like to suggest, that perhaps it would be useful to make additional temperature measuring points on the surface of the leaves.

Answer: H. BRUELHEIDE

Yes, of course, but you have to remember that we already used 32 data loggers for this experiment (8 for air temperature, 24 for soil temperature). Since there is the risk of theft if you just put them in the field, increasing the number of plots could be rather expensive.

Question: N. KRÄUCHI

Have you tried to figure out for what reason so many plants disappeared in some plots? Were there fungi?

Answer: C. SCHULZ

Unfortunately, we don't have any evidence for parasites influencing the survival of our plants. It is also possible that plants at plot 2 suffered low temperatures.

Answer: H. BRUELHEIDE

This was probably due to the open canopy. The plot with the largest damage to seedlings was plot 2, where we recorded just 8 % relative light intensity. Maybe the seedlings experienced frost due to heat emission in the night (radiation cooling) which we were unable to detect by measuring air temperature. We did not recognise this inappropriate feature of plot 2 because we chose all the plots during the winter.