*Ann Sci For* (1996) 53, 395-406

**Short note**

# The effect of excess nitrogen and of insect defoliation on the frost hardiness of bark tissue of adult oaks

FM Thomas *, R Blank

*Niedersächsische Forstliche Versuchsanstalt, Abt B, Grätzelstr 2, 37079 Göttingen, Germany*

(Received 9 December 1994; accepted 31 October 1995)

**Summary** — Deep winter frost, causing severe bark necroses, and insect defoliation are two of the causal factors for the present oak damages in northern Germany. In earlier investigations, a majority of oak stands had shown high leaf nitrogen concentrations. Therefore, the effect of nitrogen status and of insect defoliation on the frost hardiness of the bark of adult oaks was tested. At several dates during winter, samples from the living inner bark tissue were taken from adult sessile (*Quercus petraea* [Matt] Liebl) and pedunculate oaks (*Q robur* L) i) with normal or elevated leaf nitrogen concentrations, and ii) defoliated or nondefoliated in the preceding spring. Frost hardiness of bark was determined by electrolyte leakage after artificial freezing in the laboratory. During frost periods in January and February, oaks with lowered C/N ratios in bark or leaves as well as defoliated trees tended to reduced frost hardiness. Although the differences were insignificant for some temperature treatments, it is concluded that the effect of winter frost on oak damage is enhanced by a supply of excess nitrogen and by preceding insect defoliation.

**bark / frost hardiness / insect defoliation /nitrogen / oak decline / *Quercus***

**Résumé — Influence d'un excès d'azote et de la défoliation par des insectes sur la résistance au gel du liber de chênes adultes.** *Les grands froids de l'hiver qui produisent des nécroses sévères du liber, ainsi que la défoliation causée par les insectes, sont deux causes probables du dépérissement actuel des chênes en Allemagne du Nord. Dans la majorité des peuplements de chêne explorés on a détecté une forte concentration d'azote dans les feuilles. On a donc recherché l'influence de l'alimentation en azote et de la défoliation sur la résistance au gel du liber de chênes âgés. En hiver on a prélevé périodiquement des échantillons du liber de chênes sessile et pédonculé (*Quercus petraea *[Matt] Liebl et* Q robur *L), i) qui présentaient une concentration d'azote normale ou élevée dans les feuilles ou ii) qui présentaient ou non des lésions causées par la défoliation. La résistance au gel a été déter-*

---

* Present address: Universität Göttingen, Systematisch-Geobotanisches Institut, Untere Karspüle 2, 37073 Göttingen, Germany

*minée au laboratoire par la perte d'électrolytes. En janvier et en février les chênes qui avaient un faible rapport C/N dans les feuilles ou dans le liber ainsi que ceux qui présentaient des lésions importantes avaient une résistance au gel réduite. Quoique les différences trouvées n'aient pas toujours été significatives, on peut conclure que la sensibilité aux froids d'hiver est renforcée par une teneur en azote excessive et par une défoliation antérieure.*

***azote / défoliation / dépérissement du chêne / liber /*** **Quercus** ***/ résistance au gel***

## INTRODUCTION

Deep winter frost is, besides insect defoliation and drought, supposed to be one of the causal factors for the several events of decline of sessile and pedunculate oak (*Quercus petraea* [Matt] Liebl, and *Q robur* L) in northern Germany during the last 250 years. The present outbreak of damages started in 1982–1983 and culminated in 1987–1989 after three winters with severe frost. Therefore, winter frost is supposed to be the synchronizing factor of the present 'oak decline' in northern Germany (Hartmann et al, 1989; Balder, 1992; Hartmann and Blank, 1992, 1993). Up to 20% of the declining oaks showed primary bark necroses at the stem, preferably on the southern and southwestern sides (Hartmann et al, 1989; Hartmann and Blank, 1992). It is well-known that a supply of excess nitrogen can lead to a reduced frost hardiness of plant tissue (Larcher, 1985). Defoliation as well can lower the frost hardiness in the following winter (Sakai and Larcher, 1987). Since insect defoliation is one of the primary causal factors of oak decline in northern Germany, and since the majority of oak stands investigated in northwestern Germany had shown high leaf nitrogen contents as compared to literature data (Thomas and Büttner, 1992; Thomas and Kiehne, 1995), the frost hardiness of living bark tissue from adult oaks was tested in two sets of investigations: i) in trees differing in leaf nitrogen concentrations, and ii) in nondefoliated oaks versus trees defoliated in the preceding spring.

Frost hardiness was determined by electrolyte leakage after artificial freezing. In trees, this method has been widely used, for example, in stem sections of seedlings (Van den Driessche, 1969; Green and Warrington, 1978), lateral shoots (Dueck et al, 1990/1991; Sheppard et al, 1994), and pieces of twigs (Alexander et al, 1984) and needles (Aronsson, 1980; Kolb et al, 1985; Burr et al, 1990), but only rarely in bark tissue (Ashworth et al, 1983). Since in damaged oaks visible frost injury was found in the living bark, samples from this tissue were used to test frost hardiness.

The frost hardiness determined by the method employed depends not only on the type of organ or tissue and the time of sampling, but also on the freezing treatment itself (rate of cooling, duration of exposure, etc). Therefore, it does not reflect the actual frost hardiness of the tissue under field conditions and cannot be correlated directly with outside temperatures. As a relative parameter, however, it can be used for the comparison of two or more sets of samples taken at the same time from trees subjected to similar climatic conditions.

Since both the magnitudes and the courses of air temperatures measured at the meteorological stations used as a reference for the stands to be compared were very similar, the climatic conditions of those stands which are relevant for frost effects could be regarded as nearly equal. Therefore, the factors tested are thought to have a decisively greater influence on bark frost hardiness than stand effects which may, however, have contributed to a certain extent to the differences found between stands.

## MATERIALS AND METHODS

The investigation was carried out at the Niedersächsische Forstliche Versuchsanstalt (Lower Saxony Forest Research Station), Göttingen, Germany.

### *Investigation sites*

For the examination of the nitrogen effect, two stands of adult sessile oaks in eastern Lower Saxony (Sprakensehl and Busschewald; sampling from December 1992 to March 1993), and two stands of adult pedunculate oaks in eastern Schleswig-Holstein and eastern Lower Saxony (Eutin and Lüchow; sampling in February 1994) were chosen (fig 1). The stands differed in N concentrations and C/N ratios of leaves and bark (table I). The selected trees did not show any symptom of decline. The daily minimum air temperatures were obtained from meteorological stations of the German Meteorological Service (Deutscher Wetterdienst) which were at a distance of up to 50 km from the stands and reflected the weather situation of the region. During both winters, the courses of the minimum temperatures were similar in the regions of the stands to be compared (figs 2, 3).

The effect of earlier defoliation was investigated during January and February 1993 in one stand of ca 150-year-old sessile oaks in the Hakel Forest (forest district Pansfelde, western Saxony-Anhalt) comparing six severely defoliated and six

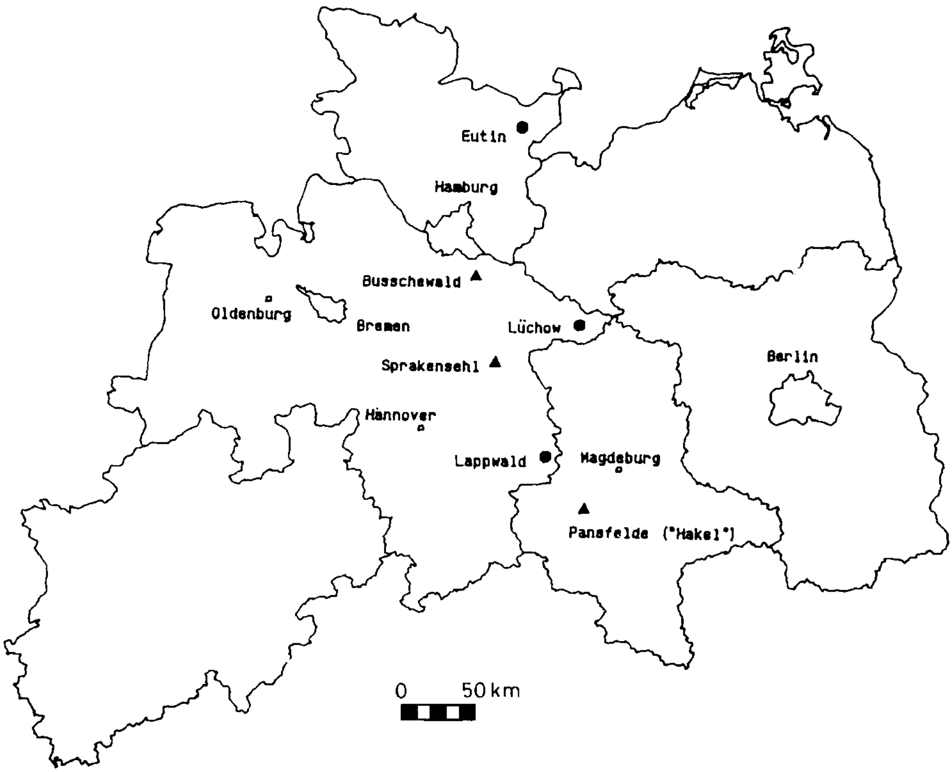

**Fig 1.** Location of the oak stands investigated in northern Germany. (●) Pedunculate oak; (▲) sessile oak.

**Table I.** Features of oaks tested for the effect of elevated nitrogen concentrations on the frost hardiness of the bark.

| *Oak species* | *Forest district* | *No of trees* | *Tree age (years)* | *Date of harvest (leaf analyses)* | *Leaf N ($mg\ g^{-1}DM$)* | *Leaf C/N ($g\ g^{-1}$)* | *Date of harvest (bark analyses)* | *Bark N ($mg\ g^{-1}\ DM$)* | *Bark C/N ($g\ g^{-1}$)* |
|---|---|---|---|---|---|---|---|---|---|
| Sessile oak | Sprakensehl | 4 | 97 | August 1992 | 24.1 ± 0.5 | 19.3 ± 0.5 * | 5 Jan 1993 | 4.84 ± 0.03 | 92.6 ± 2.9 * |
| Sessile oak | Busschewald | 4 | 101 | July 1992 | 30.1 ± 1.8 * | 15.8 ± 0.9 | 5 Jan 1993 | 5.40 ± 0.21 * | 77.6 ± 2.6 |
| Pedunculate oak | Lüchow | 12 | 140 | July 1994 | 25.2 ± 0.6 | 20.0 ± 0.5 | 14 Feb 1994 | 5.43 ± 0.24 | 84.9 ± 3.8 * |
| Pedunculate oak | Eutin | 12 | > 100 | July 1994 | 26.4 ± 0.7 | 19.3 ± 0.5 | 14 Feb 1994 | 6.03 ± 0.23 | 74.6 ± 3.0 |

* Significantly higher in comparison of the stands in Sprakensehl and Busschewald, or Lüchow and Eutin, respectively.

nondefoliated trees, and, in January and February 1994, in one stand of 130-year-old pedunculate oaks in the forest district Lappwald (eastern Lower Saxony) comparing 12 severely defoliated and three nondefoliated trees (fig 1). Defoliation had been caused by larvae of *Tortrix viridana* L and/or *Operophthera brumata* L in the preceding spring (after bud-burst in May). In July, however, after flushing of dormant buds, the foliage was almost completely reestablished. Apart from defoliation, no visible symptoms of injury occurred.

### *Sampling, determination of frost hardiness, chemical analyses*

After removal of the outer bark, samples with a diameter of 10 mm and ca 5 mm thick were taken from the inner living bark with a cork borer at breast height from the southwestern sides of the trunks where, in February, frost hardiness had been shown to be lower than at the opposite side (Thomas and Hartmann, 1992). Sampling was carried out on the same day on the trees to be compared. The bark samples were transferred in a cold bag to the laboratory and cooled in a cryostat with a cooling rate of 5 °C $h^{-1}$ according to Kolb et al (1985). The samples were cooled down to two freezing levels: −10 or −15 °C, respectively, and −25 °C. At freezing temperatures higher than −10 °C, sometimes only a very small response is obtained from samples taken during winter (Thomas and Hartmann, 1992). Air temperatures around −25 °C had caused primary bark necroses in the severe winters of 1985–1987 (Hartmann and Blank, 1992). Each desired freezing level was maintained for 30 min before removal of the samples. The control samples were kept in a refrigerator at ca +5 °C. Three replicates were employed for control and each freezing treatment.

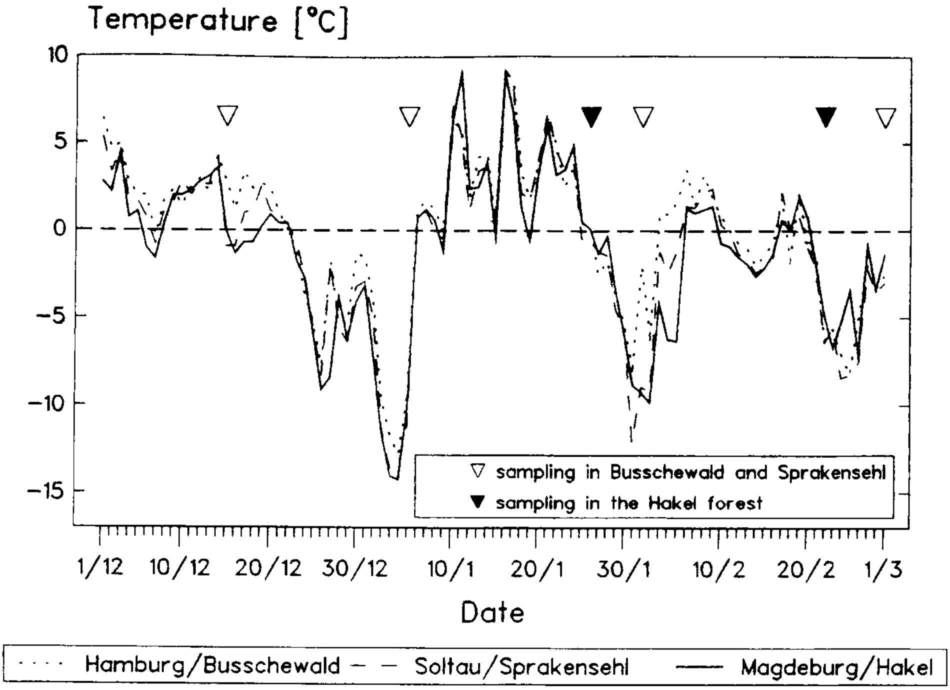

**Fig 2.** Daily minimum air temperatures measured at the meteorological stations of Hamburg-Fuhlsbüttel (reference station for Busschewald), Soltau (reference station for Sprakensehl), and Magdeburg (reference station for the Hakel Forest) from December 1992 to February 1993, and dates of sampling of bark tissue.

As a measure of the freezing damage, the electrolyte leakage of the tissue was determined according to Ritchie (1991). After thawing in a refrigerator, each sample was infiltrated with 5 mL of 3% propanole in highly purified water and incubated in this solution for 24 h at 25 °C (yielding about 90% of the leachable solute as had been tested in preliminary studies). After incubation, the conductivity of the solution was measured, and the tissue was killed by autoclaving at 120 °C for 20 min. After that, incubation and determination of the conductivity were repeated. From the ratios of the conductivity values before and after autoclaving obtained from treatment and control samples, an index of injury, $I_t$, was calculated for each freezing treatment according to Flint et al (1967), the range of this index being 0% (no freezing damage) to 100% (tissue completely killed). In the bark samples, the nitrogen concentrations and C/N ratios were determined with a C/N analyzer (two replicates per tree) for each sampling date.

### *Statistics*

The results are given as means with standard errors. For statistical analyses, the Mann-Whitney ranked sum test (U-test) was employed. Correlation coefficients were tested against the distribution of *t*-values. The significance level was 5% in each case.

## RESULTS

### *Frost hardiness of bark tissue from oaks differing in the C/N ratios of bark or leaves*

In January 1993, after ca 2 weeks of permanent frost with temperatures down to

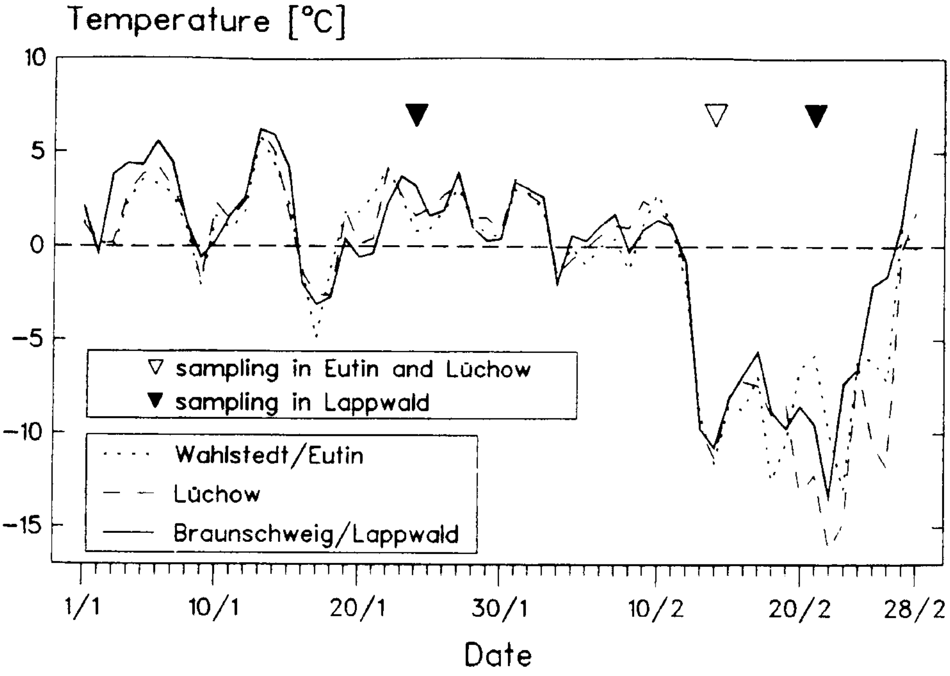

**Fig 3.** Daily minimum air temperatures measured at the meteorological stations of Wahlstedt (reference station for Eutin), Lüchow, and Braunschweig (reference station for Lappwald) in January and February 1994, and dates of sampling of bark tissue.

about −13 °C, the indices $I_t$ were higher in the bark tissue from the sessile oaks in Busschewald as compared to samples from Sprakensehl, indicating lower frost hardiness. For the −10 °C treatment, the difference was significant (fig 4a). At the beginning of February, the tendency was the same, but the differences failed to be significant. At the other sampling dates, no distinct differences could be detected. In the oaks of Busschewald, as compared to the trees in Sprakensehl, the nitrogen concentrations were significantly higher and the C/N ratios significantly lower not only in the leaves harvested in the preceding summer, but also in the bark tissue sampled in January (table I). From bark tissue taken in February, similar values were obtained, but the differences were statistically insignificant. For the tissue sampled in January and February, however, a significantly negative correlation was found between C/N ratios and freezing damage at −10 °C (fig 5). In contrast, only a weak correlation was found if the whole set of samples taken between December and March was considered ($r = -0.35$).

In late winter 1993–1994, deep frost did not occur until mid-February (fig 3). The bark tissue from the pedunculate oak stand in Eutin which had lower C/N ratios in the bark (table I) and tended to higher nitrogen concentrations and lower C/N ratios of the leaves showed, at the −10 °C treatment, a significantly higher index of injury than samples from the stand in Lüchow (fig 4b).

### Frost hardiness of bark tissue from defoliated and nondefoliated oaks

Compared to nondefoliated trees, defoliated sessile and pedunculate oaks tended to

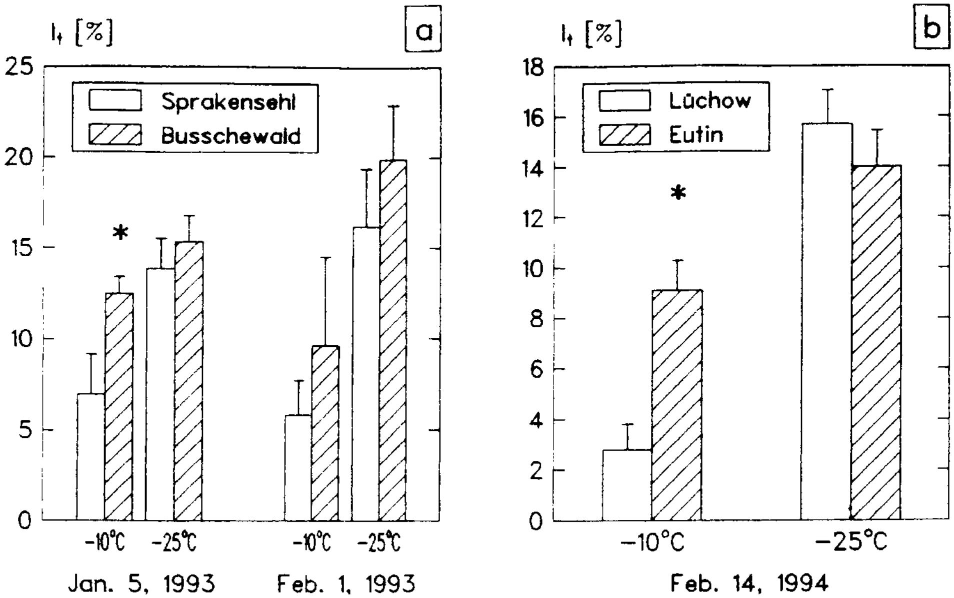

**Fig 4.** Index of injury, $I_t$, obtained from bark tissue of sessile (**a**) and pedunculate (**b**) oaks by artificial freezing at different temperatures, in comparison of oaks with 'normal' N status (Sprakensehl, Lüchow) and high N status (Busschewald, Eutin) (see table I). * Significantly higher within the same freezing treatment.

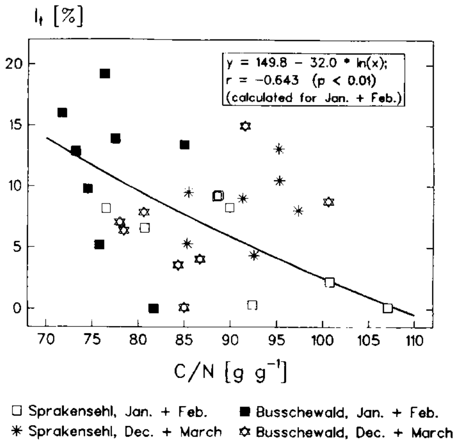

**Fig 5.** Indices of injury, $I_t$, after the −10 °C treatment, and C/N ratios of bark tissue sampled in winter 1992–1993 from sessile oaks with 'normal' N status (Sprakensehl) and high N status (Busschewald). The regression curve was calculated for the samples taken in January and February.

increase freezing damage of the bark tissue. The differences were significant for two treatments (fig 6). In one case, nondefoliated oaks showed a significantly lower frost hardiness (fig 6b). This was, however, during a period with relatively mild temperatures (cf fig 3) when, possibly, optimum frost hardiness had not yet developed.

## DISCUSSION

During winter, the $I_t$ values obtained in this investigation were rather low. In samples from sessile oaks, the maximum mean value found was 19.9 ± 3.0% (Busschewald, 1 February 1993), and in samples from pedunculate oak, it was 16.5 ± 2.6% (Lappwald, 26 January 1994), determined after freezing at −25 °C. Generally, the $I_t$ values of bark from sessile oaks were, at compa-

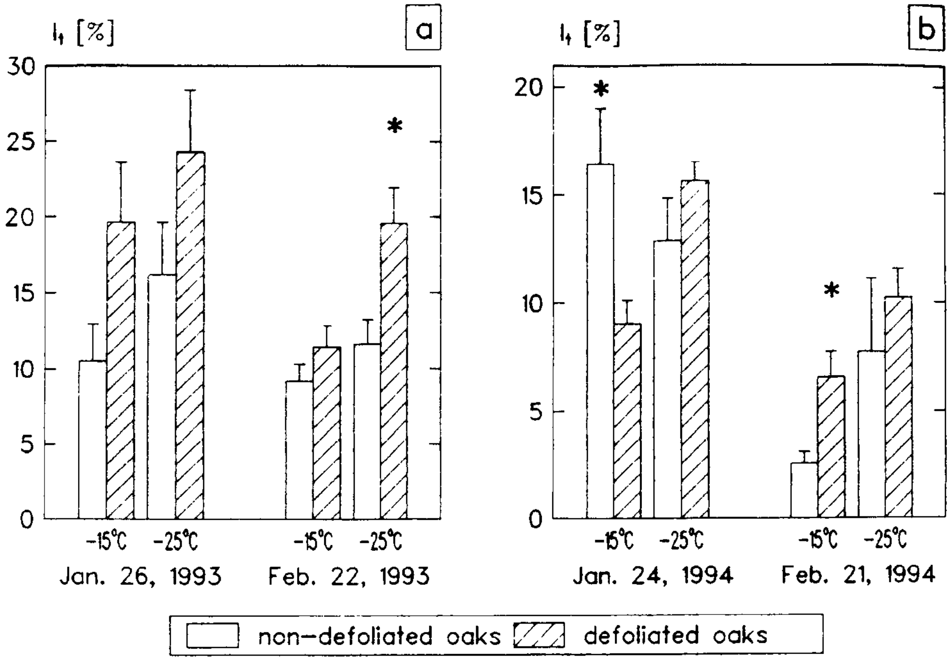

**Fig 6.** Index of injury, $I_t$, obtained by artificial freezing of bark tissue from nondefoliated and defoliated trees from stands of sessile oak (Hakel Forest [**a**]) and pedunculate oak (Lappwald [**b**]). * Significantly higher within the same freezing treatment.

rable sampling dates, higher than $I_t$ values of bark from pedunculate oak. This finding is in accordance with the commonly held opinion that sessile oak is more susceptible to winter frost than pedunculate oak (cf Ellenberg, 1986).

The low $I_t$ values generally found in January and February point to a relatively high extent of hardening. Distinct higher $I_t$ was not detected before early spring, after the presumed onset of dehardening. Figure 7 gives a compilation of maximum $I_t$ values determined with the above-stated method (but by freezing between –20 and –30 °C) at different dates during winter for the sun-exposed (southwestern) and shaded (northeastern) side of the trunk of sessile oaks in Sprakensehl, showing the course of hardening and dehardening of the tissue. The highest $I_t$ value found in this series of tests was 55%, obtained at the end of April.

In both oak species, bark tissue with a lower C/N ratio sampled during cold periods showed, mainly after the –10 °C treatment, tendencies of a reduced frost hardiness (figs 2–4). The finding of increasing freezing damage at –10 °C with decreasing C/N ratios of the bark tissue sampled from sessile oak in January and February 1993 (fig 5) points to a connection between excess nitrogen supply and frost hardiness of the bark. For the sampling dates in December 1992 and March 1993, it can be assumed that frost hardiness of the tissue was not at its maximum (fig 7) and that, therefore, significant differences were prevented. At temperatures lower than –10 °C, the frost effect presumably outweighed the

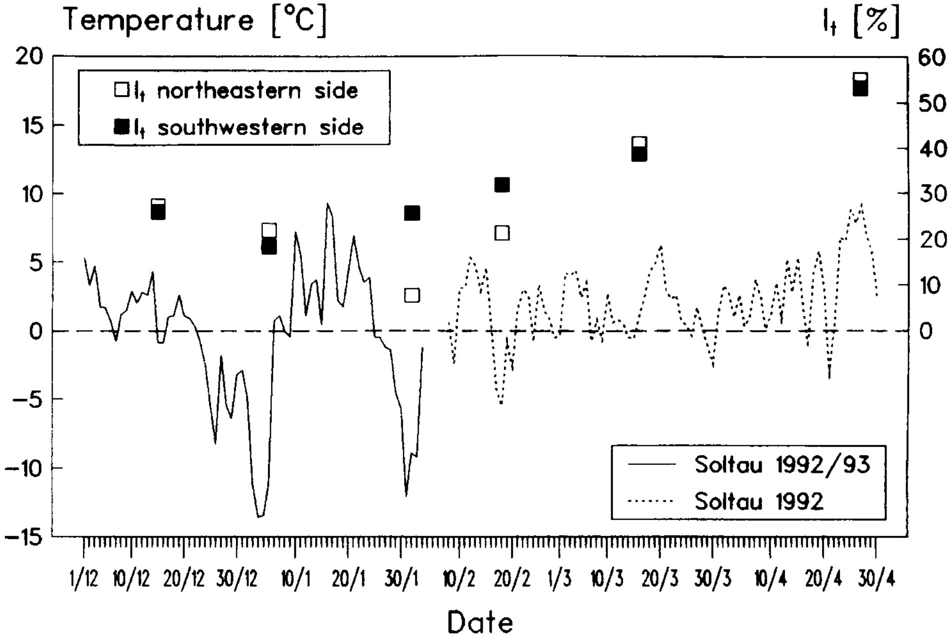

**Fig 7.** Daily minimum air temperatures measured at the meteorological station of Soltau, and maximum indices of injury, $I_t$, obtained at various dates after artificial freezing (between –20 and –30 °C) of bark samples taken from different sides of trunks of 96-year-old sessile oaks growing in the forest district of Sprakensehl. Combined data from mid-February to April 1992, and from December 1992 to early February 1993.

nitrogen effect. This could be the reason for the finding of only insignificant differences at these freezing levels.

The finding of higher N concentrations in the bark tissue of pedunculate oaks, compared to sessile oaks, is in accordance with the fact that the leaves of pedunculate oak also often show higher N concentrations (cf Van den Burg 1985, 1990). The differences in leaf nitrogen concentrations and C/N ratios between the stands to be compared were not extreme. However, more distinct differences between meteorologically comparable stands were not detected. In 37 oak and mixed oak stands investigated in Lower Saxony and Schleswig-Holstein from 1990 to 1992, mean leaf nitrogen concentrations were not below 22 mg $g^{-1}$ DM and mean leaf C/N ratios not above 21.5 g $g^{-1}$ in healthy trees. In 23 stands, however, leaf nitrogen concentrations exceeded the upper threshold of the 'normal range' which can be assumed, according to Van Den Burg (1985, 1990), to be 25 mg N $g^{-1}$ DM in sessile oak and 27 mg N $g^{-1}$ DM in pedunculate oak. The elevated nitrogen concentrations were accompanied by increased ratios of N/P and N/Mg, pointing to nutritional disharmonies (Thomas and Büttner, 1992; Thomas and Kiehne, 1995). The differences in leaf nitrogen concentrations and C/N ratios between the stands in Eutin and Lüchow found in July 1994 were small. Previous investigations in the same forest districts, however, had revealed larger discrepancies (24.6 mg N $g^{-1}$ DM and C/N 20.2 in Lüchow, and 30.6 mg N $g^{-1}$ DM and C/N 17.2 in Eutin).

The discussion on the effect of excess nitrogen on forest dieback in Europe has been stimulated by Nihlgård (1985) who stated that, besides other adverse effects, "increased amounts of leaf-nitrogen cause a decrease in frost hardiness". Indeed, it had been shown that Scots pine and Norway spruce trees fertilized with nitrogen showed reduced frost hardiness of needles and higher amounts of injured needle cells, probably due to winter frost, compared to control trees (Aronsson, 1980; Soikkeli and Kärenlampi, 1984). Fumigation of Scots pine saplings with ammonia did reduce the frost hardiness of the needles after freezing at −10 °C and below (Dueck et al, 1990/1991). Our data give evidence to a decrease in frost hardiness of the bark of broad-leaved deciduous trees with elevated leaf nitrogen concentrations. However, the extent of the contribution of excess nitrogen to forest damage, and to oak decline in particular, remains to be clarified as does the physiological mechanism of impairment. Possibly, the increased demand of carbon skeletons due to the need of enhanced nitrogen assimilation after excess nitrogen uptake leads to a reduction in the contents of soluble sugars and/or carbohydrate derivatives, serving as cryoprotectants (see later).

In both oak species, insect defoliation in spring tended to increase the damage caused by artificial freezing of bark tissue sampled during frost periods of the following winter. However, the connection between spring defoliation and decrease in frost hardiness has to be confirmed by further tests. A decrease in frost hardiness can be due to lowered carbohydrate contents. The cold resistance of roots, rhizomes, xylem and phloem tissue and buds from several trees and shrubs was found to correlate with the concentration of carbohydrates, especially of sugars (Parker, 1962; Kaurin et al, 1981; Korotaev, 1994). Leaf loss caused by spring defoliation of the oaks investigated could, together with the following reestablishment of leaf biomass by flushing of dormant buds, have led to an impaired replenishment of the carbohydrate pool, thereby affecting cold hardiness in two possible ways: i) by a decrease in the concentrations of soluble sugars, leading to increased susceptibility to dehydration caused by extracellular freezing; and/or ii) by a decrease in the concentrations of cryoprotectants (eg, sugar alcohols), resulting in increased susceptibility to intracellular freezing. Since it has been shown that freezing damage to bark tissue mainly is due to extracellular ice formation (Ashworth et al, 1983; Malone and Ashworth, 1991), it seems that the role of soluble sugars is more important. Further investigations are necessary for a better understanding of the underlying physiological mechanisms.

The findings of adverse effects of ample N supply and of spring defoliation on frost hardiness of the bark still need further experimental confirmation; however, they are in good accordance with results of tree ring analyses of 600 adult oaks in northern Germany having shown that severe growth reductions are accompanied by the combined occurrence of at least two of the factors insect defoliation, drought, and deep winter frost, the latter being regarded as the synchronizing factor for the outbreak of the present oak damages in northern Germany (Hartmann and Blank, 1992).

## ACKNOWLEDGMENTS

This research was supported by the German Bundesministerium für Forschung und Technologie (BMFT), Project no 0339382A. The authors are responsible for the content of the publication. We would like to thank Pr Dr M Popp and Dipl-biol T Große Schulte, University of Münster, Institute of Applied Botany, for the analyses of C and N of bark tissue sampled from the stands in Busschewald and Sprakensehl.

## REFERENCES

Alexander NL, Flint HL, Hammer PA (1984) Variation in cold-hardiness of *Fraxinus americana* stem tissue according to geographic origin. *Ecology* (Durham) 65, 1087-1092

Aronsson A (1980) Frost hardiness in Scots pine (*Pinus silvestris* L). II. Hardiness during winter and spring in young trees of different mineral nutrient status. *Stud Forest Suecica* 155, 1-27

Ashworth EN, Rowse DJ, Billmyer LA (1983) The freezing of water in woody tissues of apricot and peach and the relationship to freezing injury. *J Am Soc Hort Sci* 108, 299-303

Balder H (1992) Europaweite Eichenschäden durch Frost. *Allg Forst Z* 47, 747-752

Burr KE, Tinus RW, Wallner SJ, King RM (1990) Comparison of three cold hardiness tests for conifer seedlings. *Tree Physiol* 6, 351-369

Deutscher Wetterdienst (1992–1994) *Monatlicher Witterungsbericht.* Deutscher Wetterdienst, Offenbach

Dueck TA, Dorel FG, Ter Horst R, Van der Eerden LJ (1990/1991) Effects of ammonia, ammonium sulphate and sulphur dioxide on the frost sensitivity of Scots pine (*Pinus sylvestris* L). *Water Air Soil Pollut* 54, 35-49

Ellenberg H (1986) *Vegetation Mitteleuropas mit den Alpen.* 4th edn. Ulmer, Stuttgart, Germany

Flint HL, Boyce BR, Beattie DJ (1967) Index of injury – a useful expression of freezing injury to plant tissues as determined by the electrolytic method. *Can J Plant Sci* 47, 229-230

Green LM, Warrington IJ (1978) Assessment of frost damage in radiata pine seedlings using the diffusate electroconductivity technique. *NZ J For Sci* 8, 344-350

Hartmann G, Blank R (1992) Winter frost, insect defoliation and *Agrilus biguttatus* Fabr as causal factors of oak decline in northern Germany. *Forst Holz* 47, 443-452 [In German with English summary]

Hartmann G, Blank R (1993) Etiology of oak decline in northern Germany. History, symptoms, biotic and climatic predisposition, pathology. In: *Recent Advances in Studies on Oak Decline* (N Luisi, P Lerario, A Vannini, eds), Proceedings International Congress, Selva di Fasano (Brindisi), Italy, 13–18 September 1992, Dipartimento di Patologia vegetale, Bari, Italy, 277-284

Hartmann G, Blank R, Lewark S (1989) Oak decline in northern Germany – distribution, symptoms, probable causes. *Forst Holz* 44, 475-487 [In German with English summary]

Kaurin Å, Junttila O, Hansen J (1981) Seasonal changes in frost hardiness in cloudberry *(Rubus chamaemorus)* in relation to carbohydrate content with special reference to sucrose. *Physiol Plant* 52, 310-314

Kolb TE, Steiner KC, Barbour HF (1985) Seasonal and genetic variations in loblolly pine cold tolerance. *For Sci* 31, 926-932

Korotaev AA (1994) Investigations of frost resistance of tree roots. *Forstarchiv* 65, 93-95 [In German with English summary]

Larcher W (1985) Frostresistenz. In: *Handbuch der Pflanzenkrankheiten* (B Rademacher, ed), vol 1, pt 5. Parey, Berlin and Hamburg, 177-259

Malone SR, Ashworth EN (1991) Freezing stress response in woody tissues observed using low-temperature scanning electron microscopy and freeze substitution techniques. *Plant Physiol* 95, 871-881

Nihlgård B (1985) The ammonium hypothesis – an additional explanation to the forest dieback in Europe. *Ambio* 14, 2-8

Parker J (1962) Seasonal changes in cold resistance and free sugars of some hardwood tree barks. *For Sci* 8, 255-262

Ritchie GA (1991) Measuring cold hardiness. In: *Techniques and Approaches in Forest Tree Ecophysiology* (JP Lassoie, TM Hinckley, eds), CRC Press, Boca Raton/Ann Arbor/Boston, 557-582

Sakai A, Larcher W (1987) *Frost Survival of Plants.* Ecological Studies 62. Springer-Verlag, Berlin and New York

Sheppard LJ, Leith ID, Cape JN (1994) Effects of acid mist on mature grafts of Sitka spruce. I. Frost hardiness and foliar nutrient concentrations. *Environ Pollut* 85, 229-238

Soikkeli S, Kärenlampi L (1984) The effects of nitrogen fertilization on the ultrastructure of mesophyll cells of conifer needles in northern Finland. *Eur J For Pathol* 14, 129-136

Thomas FM, Büttner G (1992) The nutritional status of oaks in Lower Saxony (northern Germany). *Forst Holz* 47, 464-470 [In German with English summary]

Thomas FM, Hartmann G (1992) Frost hardiness of bark of adult sessile oaks on sun exposed and shaded sides of trunks. *Forst Holz* 47, 462-464 [In German with English summary]

Thomas FM, Kiehne U (1995) The nitrogen status of oak stands in northern Germany and its role in oak decline. In: *Nutrient Uptake and Cycling in Forest Ecosystems* (LO Nilsson, RF Hüttl, UT Johansson, eds), Kluwer, Dordrecht/Boston/London, 671-676

Van Den Burg J (1985) Foliar analysis for determination of tree nutrient status – a compilation of literature data. *Rijksinstituut voor Onderzoek in de Bos- en Landschapsbouw "De Dorschkamp",* Report no 414, Wageningen, the Netherlands

Van Den Burg J (1990) Foliar analysis for determination of tree nutrient status – a compilation of literature data. 2. Literature 1985–1989. *"De Dorschkamp",* Institute for Forestry and Urban Ecology, Report no 591, Wageningen, the Netherlands

Van Den Driessche R (1969) Measurement of frost hardiness in two-year-old Douglas fir seedlings. *Can J Plant Sci* 49, 159-172