TIME TO CHILL: EFFECTS OF SIMULATED GLOBAL CHANGE ON LEAF ICE NUCLEATION TEMPERATURES OF SUBARCTIC VEGETATION[1]

D. J. BEERLING,[2,6] A. C. TERRY,[2] P. L. MITCHELL,[2] T. V. CALLAGHAN,[3,4] D. GWYNN-JONES,[5] AND J. A. LEE[2]

[2]Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK; [3]Centre for Arctic Ecology, University of Sheffield, Sheffield S10 2BR, UK; [4]Abisko Scientific Research Station, Abisko SE98107, Sweden; and [5]Institute of Biological Sciences, University of Wales, Aberystwyth, Ceredigion, Wales, 5Y23 5DE, UK

We investigated the effects of long-term (7-yr) in situ CO_2 enrichment (600 µmol/mol) and increased exposure to UV-B radiation, the latter an important component of global change at high latitudes, on the ice nucleation temperatures of leaves of several evergreen and deciduous woody ericaceous shrubs in the subarctic (68° N). Three (*Vaccinium uliginosum, V. vitis-idaea,* and *Empetrum hermaphroditum*) of the four species of shrubs studied showed significantly higher ice nucleation temperatures throughout the 1999 growing season in response to CO_2 enrichment and increased exposure to UV-B radiation relative to the controls. The same species also showed a strong interactive effect when both treatments were applied together. In all cases, leaves cooled to below their ice nucleation temperatures failed to survive the damage resulting from intracellular ice formation. Our results strongly suggest that future global change on a decadal time scale (atmospheric CO_2 increases and polar stratospheric O_3 destruction) will lead to increased foliage damage of subarctic vegetation by severe late spring or early autumnal frosting events. Indeed, in support of our experimental findings, there is now some evidence that increases in atmospheric CO_2 concentration over the past three to four decades may already have acted in this manner on high-elevation arboreal plants in the Swedish Scandes. The implications for vegetation modeling in a future "greenhouse" world and palaeoclimate estimates from high-latitude plant fossils dating to the high-CO_2 environment of the Mesozoic are discussed.

Key words: elevated CO_2; elevated UV-B; frost damage; global environmental change; ice nucleation temperatures; subarctic.

Current and future increases in the concentration of atmospheric CO_2 represent one of the most certain features of man's influence on the global climate system (IPCC, 1995). The rapid CO_2 increase of the past 150 yr, documented from ice cores (Friedli et al., 1986) and atmospheric measurements (Keeling et al., 1995), has, however, only been accompanied by an increase in global surface temperature of ~0.5°C (Jones et al., 1999), indicating that the relationship between the two is not straightforward and additionally may be attenuated by the oceans (Levitus et al., 2000). Therefore, in the near future natural forests and agricultural vegetation from temperate regions will be exposed to a rising atmospheric CO_2 concentration before any major changes in global temperature, with the potential for late spring and early autumnal frosting events to occur in a CO_2-rich atmosphere (Lutze et al., 1998; Wayne et al., 1998). This situation contrasts directly with that repeatedly experienced by terrestrial vegetation during glacial episodes of the Quaternary when extreme seasonally low-temperature events would have occurred under a lower-than-present atmospheric CO_2 concentration (Jouzel et al., 1993; Petit et al., 1999). The anticipated future scenario, therefore, might be regarded as one to which plants have had little opportunity to adapt in the recent past.

Low temperatures play a critical role in determining woody plant distributions through their effects on a variety of physiological and life cycle processes (Sakai and Larcher, 1987; Woodward, 1987). Significant effects of a high-CO_2 environment on plant frost sensitivity therefore could impact on the dynamics of future climate-induced shifts in species distributions. The potential for such an interaction is indicated by new work on frost damage to seedlings of the broad-leaved evergreen *Eucalyptus pauciflora* (Sieb. ex Spreng.) after growth for 3 mo in open-top chambers with (700 µmol/mol) and without (350 µmol/mol) CO_2 enrichment. *Eucalyptus pauciflora* is one of the most frost-hardy evergreen *Eucalyptus* species, yet showed greater foliage frost damage in elevated CO_2 compared to plants grown in ambient CO_2, and this was correlated with higher leaf ice nucleation temperatures (Lutze et al., 1998). Moreover, on a decadal timescale, the direct physiological action of elevated CO_2 (without warming) typically has minimal effects on the timing of bud-burst in woody taxa so it is unlikely that deciduous species will avoid the action of late-spring frosts through this mechanism (Murray and Ceulemans, 1998).

As yet, no information exists on whether the effect of elevated CO_2 on leaf ice nucleation temperatures observed by Lutze et al. (1998) operates in a variety of different plant taxonomic groups and functional types or whether seasonal changes in sensitivity are exhibited due to physiological cold hardening (Sakai and Larcher, 1987). Until the mechanism is understood, there is also the possibility that other features of future environmental change could exert a similar effect. Anthropogenically derived airborne acid mists in North America,

for example, have been shown to increase the susceptibility of red spruce (*Picea rubens*) to freezing injury, and this is thought to have led to its observed range contraction over the past three decades (Johnson, 1992; DeHayes et al., 1999). Another anthropogenic influence, chlorofluorocarbon emissions, leads to seasonal depletion of stratospheric ozone with a corresponding increase in the flux of UV-B radiation reaching vegetation at the earth's surface (World Meteorological Organization, 1995), particularly at the high latitudes (Müller et al., 1997; Rex et al., 1997). Given that UV-B has deleterious effects on a range of cellular and physiological processes of plants (Caldwell and Flint, 1994), it is conceivable that its action may extend to influence the freezing temperatures of plant tissues.

Here, we report results of an investigation determining the effects of long-term (7-yr) in situ exposure of woody ericaceous dwarf shrubs to atmospheric CO_2 enrichment (600 μmol/mol) and increased UV-B radiation (280–320 nm) on leaf ice nucleation temperatures. The dwarf shrub heathland community has been exposed to factorial combinations of simulated global change treatments as part of a unique and ongoing ecological experiment in Abisko, Northern Sweden (68° N) (Johanson et al., 1995; Gwynn-Jones et al., 1996, 1997). Ice nucleation temperatures were determined by measuring the heat released upon the freezing of water (exotherms) within the tissues of the leaf itself (Wisniewski, Londow, and Asworth, 1997) using a novel electronic system whereby leaves were insulated and secured onto peltier plates and then cooled at controlled rates. We selected the four community-dominant shrub species representing two deciduous (*Vaccinium myrtillus* L., *V. uliginosum* L.) and two evergreen (*V. vitis-idaea* L. and *Empetrum hermaphroditum* Hagerup) taxa. Measurements were made throughout most of the 1999 growing season in an effort to detect any seasonal component in the response.

MATERIALS AND METHODS

Experimental site description—The field experiment was conducted in the Abisko Scientific Research Station, northern Sweden (68°21′ N, 18°49′ E, 380 m above sea level). Vegetation typically comprises an open canopy birch forest community, with a field layer dominated by deciduous and evergreen ericaceous dwarf shrubs (Johanson et al., 1995). Full details of the elevated CO_2 and UV-B radiation treatment are given elsewhere (Gwynn-Jones et al., 1996, 1997). Plots of vegetation have received treatments within open-top chambers each growing season since spring 1993. Each treatment is replicated with four chambers receiving either enhanced UV-B radiation, simulating a 15% O_3 depletion, with ambient (350 ppmv) or elevated (600 μmol/mol) CO_2 concentrations or no supplementary CO_2 or UV-B radiation (controls) (Gwynn-Jones et al., 1996, 1997).

Determination of freezing exotherms—Leaves were collected from the open-top chambers and placed in individual polythene bags to minimize moisture loss from the leaf surface. Within 10 min of collection, whole detached leaves were held flat with the abaxial (lower) surface against a peltier cooling plate (ST3353-05, Marlow Industries, Texas, USA). Two thermocouples (0.75 μm diameter) were attached to the abaxial surface midway between the midrib and the margin. The leaf and thermocouples were covered with a thin plastic film, a 2-mm layer of foam and clamped beneath a 5-mm perspex cover to ensure nucleation was not triggered by the migration of ice crystals, or condensation, from other parts of the plate. The peltier plate was cooled at a set rate using a programmable power supply (SE5010, Marlow Industries) and the temperature of the leaf logged at 1-sec intervals using a data-logger (Squirrel 1000 series, Grant Instruments, Cambridge, UK). Leaf ice nucleation temperatures were determined by observing exotherms, characterized by a

near instantaneous increase in leaf temperature of between 0.5 and 1°C, due to liquid–solid phase transition during intracellular ice crystal formation, and subsequent rapid cooling back to the controlled temperature curve within ~5 sec. Pilot trials with this system indicated that ice nucleation temperatures were invariant with respect to cooling rates (3°C/h vs. 6°C/h) and showed no detectable differences between attached and unattached leaves. Natural cooling events over short periods of time on clear days at Abisko can be up to 17°C/h (e.g., from +2°C to −15°C; B. Holmgren, personal communication, University of Tromso). All ice nucleation temperatures were measured on detached leaves at a cooling rate of 6°C/h (a slower rate than that employed by Lutze et al. [1998], 8°C/h), which represents a determination of the temperature to which leaves can be supercooled (at a rate approximating natural events) until intracellular ice crystal formation occurs (sensu Sakai and Larcher, 1987). The system gave accurate, highly reproducible results (i.e., within an accuracy of ±0.1°C) and for large-leaved species it was also able to determine spatial patterns of leaf freezing with multiple thermocouples attached to different locations across the leaf surface (Terry, Quick, and Beerling, 2000).

Measurements of ice nucleation temperatures were made on two leaves of each species (*Vaccinium myrtillus* L., *V. uliginosum, V. vitis-idaea,* and *Empetrum hermaphroditum*) from each of two replicate open-top chambers for each treatment (four measurements per species per treatment per sampling date), and from control chambers, which received no supplementary CO_2 or UV-B. The mean ice nucleation temperature of the two leaves was the observation from each chamber used for statistical analysis. Measurements were made during three site visits (26 June–4 July, 31 July–8 August, 11–19 September) in an effort to encompass most of the 1999 growing season for the deciduous species, and an additional visit was made to capture possible late-season shifts in the two evergreen species (7–11 October).

Assessment of frost damage and leaf survival—Following cooling of leaves to below their ice nucleation temperatures, visual inspection for necrotic damage or darkening of the tissues was made resulting from the withdrawal of water from cells due to intracellular ice formation. Survival, following freezing, was tested by placing leaves with the petiole held vertically in water for 3 d and visual monitoring for regreening of the tissues.

Statistical analyses—The measurements within the same open-top chambers at several dates were analyzed with repeated-measures analysis of variance (Mead, 1988). The sources of variation arising from the factorial structure of the main treatments (CO_2, UV-B, and their interaction) were tested with a residual mean square having 4 degrees of freedom (df). The other sources of variation (date, date × CO_2 interaction, date × UV-B interaction, and date × CO_2 × UV-B interaction) were tested with a residual mean square having 8 df for deciduous species measured on three dates and 12 df for evergreen species measured on four dates. Each species was analyzed separately, and all of the sets of data were tested for homogeneity of variance and normality (Sokal and Rohlf, 1981).

Since replicate ice nucleation temperature determinations of the same species, and from the same treatment, were always very close (or identical), the residual variation was small (the residual sum of squares relative to the total sum of squares was only 0.1–1%), and so even small differences between treatments were statistically significant. Such small differences have no practical significance since the resolution of the temperature measurement system was only 0.1°C. Therefore, only differences significant at $P < 0.001$ are interpreted here since these correspond to differences between treatments of at least 0.1°C, which is the resolution of the measuring system; thus $P < 0.001$ is the level of practical significance.

RESULTS

No major differences in ice nucleation temperatures were found between evergreen and deciduous taxa in the control chambers (Figs. 1–3), and all species showed, to some extent, a decline in ice nucleation temperatures through the growing season (cf. Sakai and Larcher, 1987; Larcher, 1995). Sampling
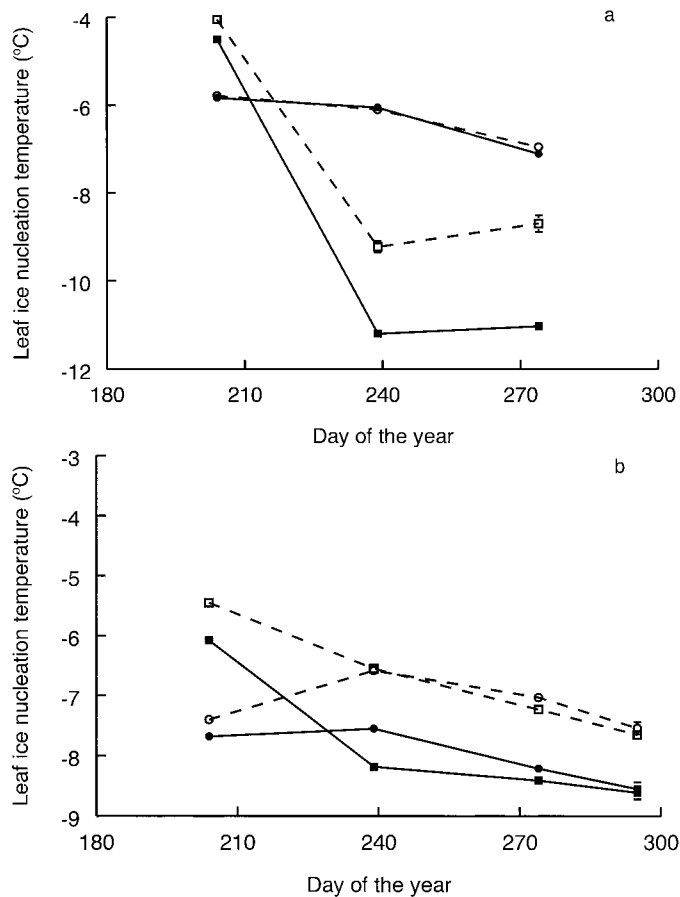
Fig. 1. Ice nucleation temperatures of leaves of (a) the deciduous shrubs *Vaccinium myrtillus* (○, ●) and *V. uliginosum* (□, ■) and (b) the evergreen shrubs *V. vitis-idaea* (○, ●) and *Empetrum hermaphroditum* (□, ■) determined through the 1999 growing season in Abisko during the 7th yr of exposure to increased CO_2 (600 μmol/L) and in the control chambers. Solid lines and solid symbols indicate data from control chambers; broken lines and open symbols indicate data from elevated CO_2 plots. All values are means ±1 SE.

Fig. 2. Ice nucleation temperatures of leaves of (a) the deciduous shrubs *Vaccinium myrtillus* (○, ●) and *V. uliginosum* (□, ■) and (b) the evergreen shrubs *V. vitis-idaea* (○, ●) and *Empetrum hermaphroditum* (□, ■) determined through the 1999 growing season in Abisko during the 7th yr of exposure to increased UV-B radiation and in the control chambers. Solid lines and solid symbols indicate data from control chambers; broken lines and open symbols indicate data from elevated UV-B plots. All values are means ±1 SE.

date had a significant effect on ice nucleation temperatures (Table 1) with three of the four species showing seasonal decline.

Effects of global change on leaf ice nucleation temperatures—Three of the four species investigated (*Vaccinium uliginosum, V. vitis-idaea*, and *Empetrum hermaphroditum*) showed significantly higher leaf ice nucleation temperatures in response to CO_2 enrichment, compared to the controls, and these were consistently higher irrespective of the time of year (Table 1, Fig. 1). The same three species also showed significant increases in leaf ice nucleation temperatures with exposure to increased UV-B radiation, and these too were irrespective of the time of year (Table 1, Fig. 2). For both the CO_2 and UV-B treatments, the effects were largest in *V. uliginosum* and least in *E. hermaphroditum*. The deciduous shrub *V. myrtillus,* which has the thinnest leaves of the four species studied, exhibited no changes in ice nucleation temperatures with CO_2 enrichment and/or increased UV-B radiation (Fig. 1).

The ANOVA identified species-specific differences in the interaction between the date of sampling and either increased
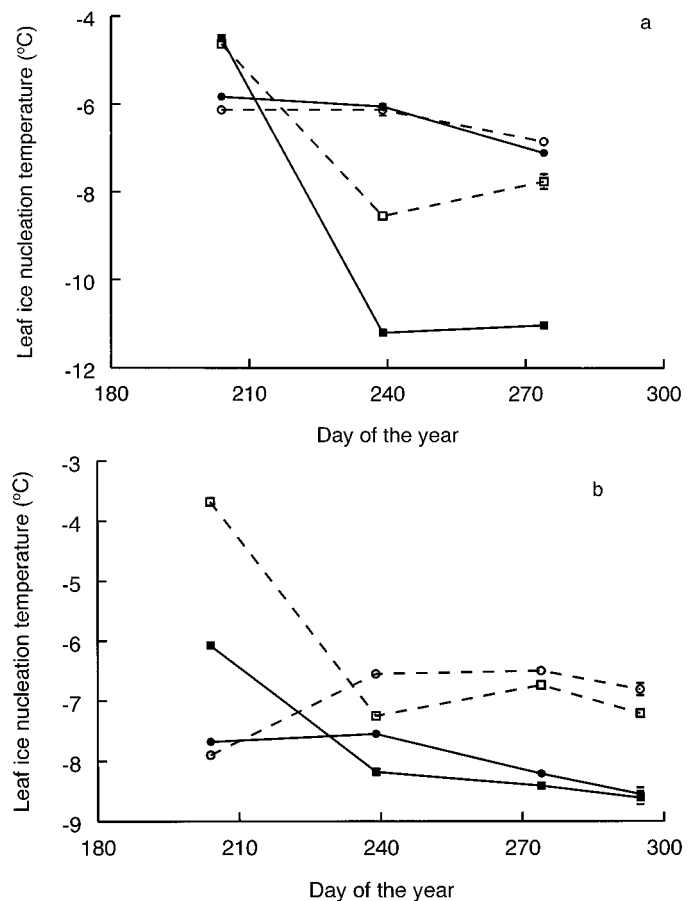
CO_2 or increased UV-B (Table 1). For increased CO_2, this date interaction was significant only for *V. uliginosum* and *V. vitis-idaea,* while for increased UV-B the interaction was significant for all three responding species.

Application of the treatments together (increased CO_2 and UV-B) increased leaf ice nucleation temperatures relative to the controls (Fig. 3). A similar effect was detected for the three-way interaction between CO_2, UV-B, and date of sampling in which only *E. hermaphroditum* showed a significant effect (Table 1).

In all cases, leaves that were cooled to below their ice nucleation thresholds showed marked necrotic damage and widespread darkening of the tissues, indicating severe cellular disruption by the formation of intracellular ice crystals. Significantly, all leaves failed to show any signs of recovery or survival 1–3 d after cooling to below their ice nucleation thresholds. Clearly, for the species examined here, freezing leaf tissues below their nucleation temperature resulted in leaf death.

DISCUSSION

Our observations demonstrate that the long-term (7-yr) in situ growth of subarctic vegetation in a CO_2-enriched atmo-
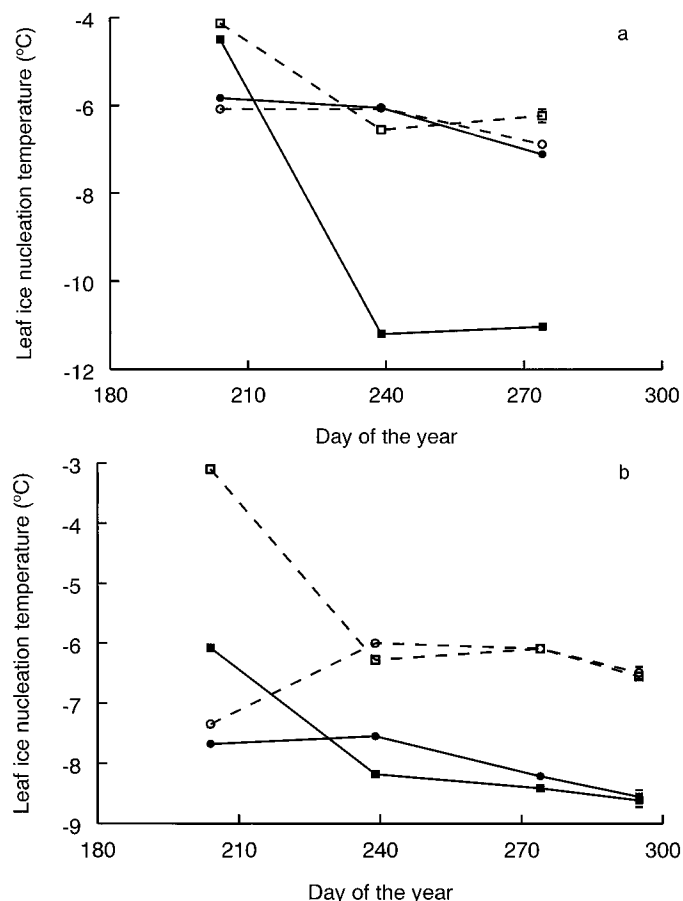
Fig. 3. Ice nucleation temperatures of leaves of (a) the deciduous shrubs *Vaccinium myrtillus* (○, ●) and *V. uliginosum* (□, ■) and (b) the evergreen shrubs *V. vitis-idaea* (○, ●) and *Empetrum hermaphroditum* (□, ■) determined through the 1999 growing season in Abisko during the 7th yr of exposure to CO_2 enrichment and increased UV-B radiation together and in the control chambers. Solid lines and solid symbols indicate data from control chambers; broken lines and open symbols indicate data from elevated CO_2 and UV-B plots. All values are means ±1 SE.

B and CO_2. The CO_2 effect is consistent with the earlier report of Lutze et al. (1998) for the evergreen *Eucalyptus pauciflora,* which suffered severe necrotic tissue damage as a result of frosting during growth with CO_2 enrichment. The phenomenon is evident in both evergreen and deciduous species and persists throughout the growing season, and so cannot be regarded as a transient feature of plant–climate interactions or confined to a particular growth habit. As the frequency of extreme events increases due to the greenhouse effect altering natural climate variability (Rind, Goldberg, and Ruedy, 1989; Katz and Brown, 1992), we suggest that the foliage of vegetation, especially in the high latitudes, where stratospheric ozone shows strong seasonal depletion (Müller et al., 1997; Rex et al., 1997), might experience significant increases in frost sensitivity over the coming decades as a result of future global environmental change. High-elevation vegetation of the Swedish Scandes, including dwarf shrubs, has responded retrogressively to the past four to five decades of instrumentally recorded cooling through defoliation, retarded growth, and some locally restricted retraction of the tree lines (Kullman, 1998).

In terms of plant performance, these effects imply that future severe late and early season freezing events in a CO_2-rich atmosphere will likely curtail the length of the growing season, especially for high latitude vegetation exposed to a progressively higher flux of UV-B radiation. A reduction in the length of the growing season will be particularly important for sub-arctic plants that already experience a short-growing season and could lead to reduced biomass production and affect plant cover (Larcher, 1995). Late spring freezing injury abruptly terminates photosynthetic carbon gain (Roden, Egerton, and Ball, 1999; Terry, Quick, and Beerling, 2000) and early autumnal freezing events cause the loss of stored nonstructural assimilates and ~50% of leaf mineral nutrients that are usually recovered during normal senescence (Körner and Larcher, 1988). Indeed, experimental defoliation of those dwarf shrub species showing higher ice nucleation temperatures in response to the CO_2 and UV-B treatments (Figs. 1–3) severely reduced resource resorption, leading to a significant reduction in subsequent leaf and shoot growth (Ekstein, Karlson, and Weih, 1998). These effects will operate in addition to CO_2-related reductions in photosynthetic efficiency experienced by vegetation due to increased photoinhibition following natural freezing events (Roden, Egerton, and Ball, 1999).

The phenomenon of a CO_2-enriched atmosphere raising ice nucleation temperatures of leaves has implications for modeling the redistribution of vegetation in a future high-CO_2

sphere has the capacity to increase the temperatures at which leaves freeze. Moreover, we have shown for the first time that this phenomenon can be brought about by the exposure of vegetation to an increased flux of UV-B radiation, as well as CO_2 enrichment, and that there is an interaction between UV-

TABLE 1. Summary of ANOVA results showing the significance of the treatment effects on the ice nucleation temperatures of each species. Only significances of $P < 0.001$ are of practical significance (i.e., represent a treatment effect of $>0.1°C$, the resolution of the measuring system).

| | | Deciduous species | | | Evergreen species | |
Source	df	*Vaccinium mytillus*	*Vaccinium uliginosum*	df	*Vaccinium vitis-idaea*	*Empetrum hermaphroditum*
CO_2	1	ns	***	1	***	***
UV-B	1	ns	***	1	***	***
$CO_2 \times$ UV-B interaction	1	ns	*	1	*	**
Residual mean square	4	0.0045	0.0061	4	0.0212	0.0080
Date	2	***	***	3	***	***
$CO_2 \times$ date interaction	2	ns	***	3	***	**
UV-B $\times$ date interaction	2	**	***	3	***	***
$CO_2 \times$ UV-B $\times$ date interaction	2	ns	*	3	*	***
Residual mean square	8	0.0060	0.0162	12	0.0079	0.0069
Total sum of squares	23	5.300	147.83	31	70.00	16.72

* $P = 0.05$; ** $P = 0.01$; *** $P = 0.001$; ns, not significant.

world and for interpreting the palaeoclimates from high-latitude plant fossils dating to the high-CO_2 environment (Berner, 1997; Ekart et al. 1999) of the Mesozoic and early Tertiary (250–50 million years ago, Mya). Global-scale plant biogeography models use minimum temperatures as a key climatic determinant of the distribution of major types of woody vegetation (e.g., Prentice et al., 1992; Foley, 1994). For broadleaved evergreen trees and shrubs of temperate regions, particular emphasis is placed on the critical freezing temperatures at which leaf damage occurs (Prentice et al., 1992). Therefore, any increased sensitivity of this group of plants to freezing injury under elevated CO_2, as shown here, strongly suggests that the capacity of these types of models to make predictions of future changes in their distributions in a high-CO_2 "greenhouse" world will be limited. Such predictions may be further compromised by neglecting to consider the separate and interactive effects of increased exposure to UV-B radiation on plant frost sensitivity.

From a palaeoclimatic perspective, the potential for a high-CO_2 environment to increase the sensitivity of extant plant groups to frost injury implies that the palaeobiology and climatic limits of vegetation growing in a Mesozoic palaeoatmosphere 900–1500 ppmv CO_2 were rather different from those seen today (Beerling, 1998a, b). Continental palaeotemperatures, typically estimated from the occurrence of ancient high latitude plant fossils by direct extrapolation of the current climatic limits of nearest living relatives (e.g., Greenwood and Wing, 1995), could, for example, be too low by up to several degrees. This is without the possibility of CO_2 selection operating over millions of years. Moreover, current general circulation climate models similar to those used for predicting future climates are unable to reproduce high-latitude temperatures calculated in this way for ancient greenhouse climates (Sloan and Pollard, 1998). This difficulty would clearly be further compounded if palaeotemperature estimates reconstructed from plant fossils were revised upwards, as might be required after accounting for the CO_2 effects on tissue supercooling identified here.

A rise in leaf freezing temperatures implies that CO_2 and UV-B impair the ability of a plant to supercool its leaf tissues and preliminary measurements indicate a similar effect is exerted on other tissues with bud-freezing temperatures of *Pinguicula* being higher than those of plants grown in elevated CO_2 (A. C. Terry et al., unpublished data). A mechanistic explanation for this newly identified action of CO_2 and UV-B on plants remains to be identified. Lutze et al. (1998) postulated that the phyllosphere supported greater populations of ice-nucleating bacteria, and this effect might be operating in the leaves of vegetation at Abisko. However, we note that enhanced UV-B has been shown to decrease populations of phyllosphere organisms. Another possibility is that freezing injury is linked to the dynamics of physiologically active and labile calcium pools through changes in number of calcium-binding sites available as the composition of lipids of the cell walls and plasma membranes shifts (Senser and Beck, 1984; DeHayes et al., 1997). Plant growth in elevated CO_2 has been shown, for example, to increase the lipid contents and degree of unsaturation of thykaloid membranes (Sgherri et al., 1998), and there is evidence for UV damage to plant cellular membranes (Murphy, 1983). Clearly, the mechanism underpinning the phenomenon should now be sought.

Conclusion—Our work, and that of others, indicates that increased leaf ice nucleation temperatures is a probable effect of future global environmental change on terrestrial vegetation. Indeed, we cannot exclude the possibility that changes in leaf ice nucleation temperatures of woody plant foliage may have already occurred over the past 200 yr of CO_2 increase and more recent ozone depletion. The data also suggest that if minimum land surface temperatures increase as a result of global warming then the potential for frost injury to temperate and polar/arctic vegetation will remain. At present, we consider that the effects on subarctic plants will be mainly confined to curtailing plant resource acquisition towards the end of the growing season. However, the cumulative nature of this effect may ultimately lead in the long term to a reduction in plant performance and cover with a loss of competitive ability. Quantitative inclusion of the interaction between atmospheric CO_2 and foliage frost sensitivity into vegetation modeling studies, and into palaeoclimatic temperature estimates from plant fossils, requires elucidation of the underlying mechanism.

LITERATURE CITED

BEERLING, D. J. 1998a. The future as the key to the past for palaeobotany? *Trends in Ecology and Evolution* 13: 311–316.

———. 1998b. Atmospheric carbon dioxide, past climates and the plant fossil record. *Botanical Journal of Scotland* 51: 49–68.

BERNER, R. A. 1997. The rise of land plants and their effect on weathering and atmospheric CO_2. *Science* 276: 544–546.

CALDWELL, M. M., AND S. D. FLINT. 1994. Stratospheric ozone reduction, solar UV-B radiation and terrestrial ecosystems. *Climatic Change* 28: 375–395.

DeHAYES, D. H., P. G. SCHABERG, G. J. HAWLEY, C. H. BORER, J. R. CUMMING, AND G. R. STRIMBECK. 1997. Physiological implications of seasonal variations in membrane-associated calcium in red spruce mesophyll cells. *Tree Physiology* 17: 687–695.

———, P. G. SCHABERG, G. J. HAWLEY, AND G. R. STRIMBECK. 1999. Acid rain impacts on calcium nutrition and forest health. Alteration of membrane associated calcium leads to membrane destabilization and foliar injury in red spruce. *BioScience* 49: 789–800.

EKART, D. D., C. E. CERLING, I. P. MONTAÑEZ, AND N. J. TABOR. 1999. A 400 million year carbon isotope record of pedogenic carbonate: implications for paleoatmospheric carbon dioxide. *American Journal of Science* 299: 805–827.

EKSTEIN, R. L., P. S. KARLSON, AND M. WEIH. 1998. The significance of resorption of leaf resources for shoot growth in evergreen and deciduous woody plants from a subarctic environment. *Oikos* 81: 567–575.

FOLEY, J. A. 1994. Net primary productivity of the terrestrial biosphere: the application of a global model. *Journal of Geophysical Research* 99: 20773–20783.

FRIEDLI, H., H. LÖTSCHER, H. OESCHGER, U. SIEGENTHALER, AND B. STAUFFER. 1986. Ice core record of the $^{13}C/^{12}C$ ratio of atmospheric CO_2 in the past two centuries. *Nature* 324: 237–238.

GREENWOOD, D. R., AND S. L. WING. 1995. Eocene continental climates and latitudinal temperature gradients. *Geology* 23: 1044–1048.

GWYNN-JONES, D., O. L. BJORN, T. V. CALLAGHAN, C. GEHRKE, U. JOHANSON, J. A. LEE, AND M. SONESSON. 1996. Effects of enhanced UV-B radiation and elevated concentrations of CO_2 on a subarctic heathland. *In* C. Körner and F. A. Bazzaz [eds.], Carbon dioxide, populations and communities, 197–207. Academic Press, San Diego, California, USA.

———, J. A. LEE, AND T. V. CALLAGHAN. 1997. Effects of enhanced UV-B radiation and elevated carbon dioxide concentrations on a sub-Arctic forest heath ecosystem. *Plant Ecology* 128: 242–249.

IPCC (Intergovernmental Panel on Climate Change). 1995. Climate change 1995. J. T. Houghton, L. G. Meira Filho, B. A. Callander, N. Harris, A. Kattenberg, and R. H. Moss [eds.]. Cambridge University Press, Cambridge, UK.

JOHANSON, U., C. GEHRKE, L. O. BJÖRN, T. V. CALLAGHAN, AND M. SONESSON. 1995. The effects of enhanced UV-B radiation on a subarctic heath community. *Ambio* 24: 106–111.

JOHNSON, A. H. 1992. The role of abiotic stresses in the decline of red spruce

in high elevation forests of the eastern United States. *Annual Reviews of Phytopathology* 30: 349–367.

JONES, P. D., M. NEW, D. E. PARKER, S. MARTIN, AND I. G. RIGOR. 1999. Surface air temperature and its changes over the past 150 years. *Reviews of Geophysics* 37: 173–199.

JOUZEL, J. ET AL. 1993. Extending the Vostok ice-core record of palaeoclimate to the penultimate glacial period. *Nature* 364: 407–412.

KATZ, R. W., AND B. G. BROWN. 1992. Extreme events in a changing climate: variability is more important than averages. *Climate Change* 21: 289–302.

KEELING, C. D., T. P. WHORF, M. WAHLEN, AND J. VAN DER PLICHT. 1995. Interannual extremes in the rate of rise of atmospheric carbon dioxide since 1980. *Nature* 375: 666–670.

KÖRNER, C., AND W. LARCHER. 1988. Plant life in cold climates. *In* S. P. Long and F. I. Woodward [eds.], Plants and temperature, 25–57. Symposia of the Society for Experimental Biology XXXXII, Co. Biologists Ltd., Cambridge, UK.

KULLMAN, L. 1998. Tree-limits and montane forests in the Swedish Scandes: sensitive biomonitors of climate change and variability. *Ambio* 27: 312–321.

LARCHER, W. 1995. Physiological plant ecology. Springer-Verlag, Berlin, Germany.

LEVITUS, S., J. I. ANTONOV, T. P. BOYER, AND C. STEPHENS. 2000. Warming of the world ocean. *Science* 287: 2225–2229.

LUTZE, L. J., J. S. RODEN, C. J. HOLLY, J. WOLFE, J. J. G. EGERTON, AND M. C. BALL. 1998. Elevated atmospheric [CO_2] promotes frost damage in evergreen tree seedlings. *Plant, Cell and Environment* 21: 631–635.

MEAD, R. 1988. The design of experiments. Cambridge University Press, Cambridge, UK.

MÜLLER, R., P. J. CRUTZEN, J. U. GROOß, J. M. RUSSEL, H. GERNANDT, D. S. MCKENNA, AND A. F. TRUCK. 1997. Severe chemical ozone loss in the Arctic during the winter 1995–1996. *Nature* 389: 709–712.

MURPHY, T. M. 1983. Membranes as targets of ultraviolet radiation. *Physiologia Plantarum* 58: 381–388.

MURRAY, M. B., AND R. CEULEMANS. 1998. Will tree foliage be large and live longer? *In* P. G. Jarvis [ed.], European forests and global change. The likely impact of rising CO_2 and temperature, 94–125. Cambridge University Press, Cambridge, UK.

PETIT, J. E. ET AL. 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* 399: 429–436.

PRENTICE, I. C., W. CRAMER, S. P. HARRISON, R. LEEMANS, R. A. MOSERUD, AND A. M. SOLOMAN. 1992. A global biome model based on plant physiology and dominance, soil properties and climate. *Journal of Biogeography* 19: 117–134.

REX, M. ET AL. 1997. Prolonged stratospheric ozone loss in the 1995–1996 Arctic winter. *Nature* 389: 835–838.

RIND, D., R. GOLDBERG, AND R. RUEDY. 1989. Change in climate variability in the 21st century. *Climate Change* 14: 5–37.

RODEN, J. S., J. J. G. EGERTON, AND M. C. BALL. 1999. Effect of elevated [CO_2] on photosynthesis and growth of snow gum (*Eucalyptus pauciflora*) seedlings during winter and spring. *Australian Journal of Plant Physiology* 26: 37–46.

SAKAI, A., AND W. LARCHER. 1987. Frost survival of plants. Responses and adaptation to freezing stress. Ecological Studies 62. Springer Verlag, Berlin, Germany.

SENSER, M., AND E. BECK. 1984. Correlation of chloroplast ultrastructure and membrane lipid composition to the different degrees of frost resistance achieved in leaves of spinach, ivy and spruce. *Journal of Plant Physiology* 117: 41–55.

SGHERRI, C. L. M., M. F. QUARTACCI, M. MENCONI, A. RASCHI, AND F. NAVARILZZO. 1998. Interactions between drought and elevated CO_2 on alfalfa plants. *Journal of Plant Physiology* 152: 118–124.

SLOAN, L. C., AND D. POLLARD. 1998. Polar stratospheric clouds: a high latitude warming mechanism in an ancient greenhouse world. *Geophysical Research Letters* 25: 3517–3520.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry: the principles and practice of statistics in biological research, 2nd ed. Freeman, New York, New York, USA.

TERRY, A. C., W. P. QUICK, AND D. J. BEERLING. 2000. Long-term growth of Gingko with CO_2 enrichment increase leaf ice nucleation temperatures and limits recovery of the photosynthetic system from freezing. *Plant Physiology* 124: 183–190.

WAYNE, P. M., E. G. REEKIE, AND F. A. BAZZAZ. 1998. Elevated CO_2 ameliorates birch response to high temperature and frost stress: implications for modelling climate-induced geographic range shifts. *Oecologia* 114: 335–342.

WISNIEWSKI, M., S. E. LONDOW, AND E. N. ASHWORTH. 1997. Observations of ice nucleation and propagation in plants using infrared video thermography. *Plant Physiology* 113: 327–334.

WOODWARD, F. I. 1987. Climate and plant distribution. Cambridge University Press, Cambridge, UK.

WORLD METEOROLOGICAL ORGANIZATION. 1995. Scientific assessment of ozone depletion. Report number 37. WMO, Geneva, Switzerland.