

THE INFLUENCE OF CALCIUM SUPPLEMENTATION ON THE FREEZING TOLERANCE OF WOODY PLANTS

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Abstract. The effects of calcium (Ca^{2+}) supplementation on the freezing resistance of white poplar (*Populus alba*, frost hardy) and hornbeam (*Carpinus betulus*, frost sensitive) were studied by monitoring alterations in leaf fatty acids, chlorophyll fluorescence emissions, necrosis, mortality, and growth. Calcium supplementation had no significant effect on leaf fatty acids before, and from weeks 2 through 8 after, freezing. Percentages of the saturated fatty acid C16 were significantly higher in Ca^{2+} -supplemented plants immediately following freezing (day 1) only. In *P. alba*, leaf chlorophyll fluorescence and necrosis values were significantly higher and lower than in controls immediately after freezing; readings from weeks 2 through 8 did not significantly differ from controls. Leaf chlorophyll fluorescence and necrosis values in *C. betulus* after freezing were significantly higher and lower than in controls throughout the experiment. Calcium concentrations were significantly higher in supplemented plants. Lower mortality rates and root and leaf electrolyte leakage values, and higher root, shoot, and leaf dry weights and leaf area recorded in Ca^{2+} -supplemented plants indicate that freezing tolerance may be increased by application of Ca^{2+} fertilizer.

Key Words. Calcium; freezing tolerance; lipids; chlorophyll fluorescence; woody plants; fertilizer.

In temperate regions, freezing temperatures are an important factor limiting the productivity and distribution of plant species (Woodward 1988; Palta 1992). Although many plants tolerate freezing temperatures during the winter period (due to cold acclimation), significant losses still may be sustained. Freezing temperatures less than -20°C (-4°F) experienced in 1995 and 1996 in Scotland, for example, resulted in significant economic damage to the hardy ornamental woody plant industry. It has also been reported that climatic change may increase the unpredictability of weather patterns, resulting in progressively later frosts on an annual basis (Biggs 1996). Late frosts are particularly devastating to woody plants because young spring growth is unable to acclimate and therefore is extremely susceptible to frost damage (Cannel and Smith 1986).

It is widely recognized that freezing temperatures affect the composition and type of lipids present in

leaf cell membranes (Quinn 1988). An increase in the proportion of lipids to protein and in the level of unsaturated fatty acids, together with a small increase in phospholipids compared to galactolipids, is usually a consequence of low temperatures that can occur within hours following plant exposure (Lynch and Thompson 1984). Indeed, the acclimatory status of *Rhododendron* can be measured as a function of the saturation:unsaturation ratios of total foliar lipid fatty acids (Biggs 1996).

Calcium (Ca^{2+}) is involved in the control and maintenance of physiological plant responses to freezing injury such as the maintenance of membrane integrity and transport function (Legge et al. 1982). Because the plasma membrane has been proposed as the primary site of freeze-thaw injury (Steponkus 1984) and transduction of external stimuli into cellular responses (Poovaiah and Reddy 1987; Palta 1992), the progress of freezing injury can be halted by bathing or washing freeze-thaw injured tissue with a CaCl_2 solution. Research by and Meade (1989) suggests that cellular calcium may play a role in the recovery of freezing injury, which is associated with the activity of plasma membrane H^+ -ATPase. In conclusion, all studies suggest that maintenance of cellular/membrane calcium is important for frost survival (Poovaiah and Reddy 1993; Monroy and Dhindsa 1995).

The influence of Ca^{2+} supplementation on leaf fatty acid composition before, during, and after freezing injury remains unreported. Consequently, the objectives of this study were to evaluate the benefits of Ca^{2+} supplementation on freezing resistance in the woody plant species white poplar (*Populus alba*) and hornbeam (*Carpinus betulus*), which are considered to be frost hardy and frost sensitive, respectively (Sakai and Larcher 1987). Calcium nitrate was selected because it is commercially available and widely used as a fertilizer throughout the horticulture industry in the United Kingdom.

Effects on freezing resistance were determined by examining alterations to unsaturated:saturated fatty

acid components of total leaf lipids before and after freezing; quantifying damage to leaf photosynthetic capacity using *in vivo* chlorophyll fluorescence (Fv/Fm ratios); recording leaf necrosis development; determining electrolyte leakage of leaf, shoot, and root tissue immediately after freezing; and recording mortality and plant growth at the cessation of the experiment.

MATERIALS AND METHODS

Plant Material

Thirty white poplar (*Populus alba*) and 30 hornbeam (*Carpinus betulus*) supplied by Alba trees, Haddington, Edinburgh, UK, were potted from root trainers into 2-L (0.5-gal) pots and placed in a heated glasshouse at a temperature of $22 \pm 2^\circ\text{C}$ (72°F). Supplementary lighting by 400-W high-pressure sodium lamps (SON/T) equated to $250 \mu\text{mol}/\text{m}^2$ per second (PAR) at the tree crown and provided a 16-hour-light–8-hour-dark photoperiod. A 20 g per L solution of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ was applied to the roots of 15 trees of each species at 2-week intervals in 1997 (September 17, September 27, and October 2).

Freezing of whole plants commenced November 10, 1997, using a Merck environmental freezing chamber. The temperature was reduced by 2°C (36°F) per hour, from 10°C (50°F) to -10°C (14°F). This temperature was maintained for 4 hours, after which the temperature was raised by 2°C per hour to 10°C —a cooling regime that represents naturally occurring environmental alterations during a severe frost (Sakai and Larcher 1987). Trees were then returned to a heated glasshouse.

Analytical Techniques

Lipids were extracted from 1 g of fresh leaf material before freezing, immediately after freezing, and every 2 weeks until week 8. Leaf fatty acid samples were analyzed using gas chromatography (AMS 94, A.I. Cambridge, Cambridge, UK) and the EZ Chrom data handling system for data collation (Spec Analytical, Alloa, UK) following solvent extraction and purification as described in Boyle (1998). Three replicates per treatment were taken.

Chlorophyll Fluorescence

Before measurements were taken, 5 leaves per tree were dark adapted for 40 minutes by attaching light exclusion clips to leaf surfaces *in situ*. Chlorophyll fluorescence measurements were obtained with a portable fluorescence spectrometer (Hansatech Instru-

ments Ltd., King's Lynn, UK). In all cases, chlorophyll fluorescence measurements refer to the Fm/Fv ratios, which represent the maximum quantum yield of Photosystem II, which in turn is highly correlated to the quantum yield of net photosynthesis, providing a rapid quantitative measurement to determine plant response to freezing damage (Adams et al. 1995). Assessments were made at weekly intervals.

Leaf Necrosis

Assessments of freezing damage to leaves were estimated visually on a scale of 1 to 6 (0 = no necrosis; 1 = < 1% necrosis; 2 = 2–10% necrosis; 3 = 11–25% necrosis; 4 = 26–50% necrosis; 5 = 51–75% necrosis; 6 = > 75% necrosis). Assessments were made at weekly intervals on the same leaves used for chlorophyll fluorescence analysis.

Inductively Coupled Plasma-Emission Spectroscopy (ICP) Elemental Analysis

Root, shoot, and leaf samples of 5 trees per treatment were thoroughly washed, then dried in a convection oven at 85°C (185°F) for 48 hours before grinding through a 0.5-mm (0.02-in.) cyclone mill (Retsch, Middlesborough, UK). Samples (0.5 g) were placed into 150-mL (5-oz) volumetric flasks and digested in 20 mL (0.7 oz) of 7:1 nitric and perchloric acid. After cooling, the solutions were brought to volume with deionized water and analyzed by ICP. Calcium values are expressed as percentage of dry matter.

Cell Electrolyte Leakage

Immediately following freezing, leaves and fine root tissue were excised and placed in 30-mL (1-oz) universal bottles containing 20 mL (0.7 oz) of distilled water. Samples were stored at 22°C (72°F) for 24 hours in darkness before conductivity measurements were made using a Jenway conductivity probe and M4070 meter (BDH, Loughborough, UK). Total solute leakage was obtained by autoclaving for 1 hour at 121°C (250°F) and 0.103 MPa. Results are presented as percentage of solute leakage after 24 hours.

Plant Dry Weights and Leaf Area

At the conclusion of the experiment, surviving trees were destructively harvested, and leaf, shoot, and root dry weights were recorded after oven drying at 85°C (185°F) for 48 hours. Leaf areas were quantified using a Delta-T area meter.

Statistical Analysis

Treatment effects were determined by analysis of variance (ANOVA) using the Genstat V program. Differences among treatment means were separated by the least significance difference (LSD) at the 0.05 level of probability. Trees not supplemented with calcium were used as controls.

RESULTS

Fatty Acid Composition of Leaf Tissue

Twenty-seven fatty acids were identified: C12 (lauric acid), C14 (myristic acid), C14:1 (N-5) (myristoleic acid), C16 (palmitic acid), C16:1 (N-7) (palmitoleic acid), C17 (margaric acid), C17:1 (N-7), C18 (stearic acid), C18:1 (N-9) (oleic acid), C18:1 (N-7) (*cis*-vaccenic acid), C18:2 (N-6) (linoleic acid), C18:3 (N-6) (γ linolenic acid), C18:3 (N-3) (α linolenic acid), C18:4, C20 (arachidic acid), C20:1 (N-9) (gondoic acid), C20:2 (N-6), C20:3 (N-6) (homo- γ linolenic acid), C20:4 (N-6) (arachidonic acid), C20:5 (N-3) (EPA), C22 (behenic acid), C22:1 (N-9) (erucic acid), C22:3 (N-3), C22:4 (N-6), C22:6 (N-3), C23, C24 (lignoceric acid). Only significant data are presented (Table 1).

Irrespective of species, Ca²⁺ supplementation had no significant effects on the percentage of unsaturated and saturated leaf fatty acids before freezing and from weeks 2 through 8 after freezing (Table 1). Percentages of the saturated fatty acid C16 were significantly higher than controls in both species ($P < 0.01$) supplemented with Ca²⁺ immediately after freezing only (day 1, Table 1). No significant effects on remaining fatty acids were recorded at day 1 (data not shown).

Chlorophyll Fluorescence

Higher chlorophyll fluorescence emission values were recorded in *C. betulus* controls compared to the reportedly more frost-tolerant controls of *P. alba* after freezing. Regardless of species, chlorophyll fluorescence emission values in Ca²⁺-supplemented plants were higher than in nonsupplemented plants throughout the experiment. In *P. alba*, chlorophyll

fluorescence emissions following freezing were reduced from approximately 0.846 to 0.25 (Ca²⁺ supplemented) and to 0.17 (controls). Although chlorophyll fluorescence values were significantly higher in the supplemented samples than in the controls ($P < 0.05$) immediately following freezing, subsequent measurements from weeks 2 through 8 were not, with values remaining relatively constant until week 8.

In the case of *C. betulus*, chlorophyll fluorescence emission values were reduced from approximately 0.795 to 0.425 (Ca²⁺ supplemented) and to 0.317 (controls), and values remained significantly higher ($P < 0.01$) in the supplemented samples than in the controls after freezing. Initially, chlorophyll fluorescence values declined until week 2, after which they steadily increased until week 8 (Figure 1).

Leaf Necrosis

Lower leaf necrosis values were recorded in *C. betulus* controls compared to controls of *P. alba* after freezing. Regardless of species, necrosis values in Ca²⁺-supplemented plants were lower than non-supplemented plants throughout the experiment. In *P. alba*, necrosis values after freezing increased from 0 to 4.40 (Ca²⁺ supplemented) and to 5.17 (controls), respectively. Although necrosis values were significantly lower in the supplemented samples than in the controls ($P < 0.05$) immediately following freezing, subsequent readings from weeks 2 through 8 were not. In the case of *C. betulus*, leaf necrosis values increased from 0 to 1.30 (Ca²⁺ supplemented) and to 2.50 (controls), respectively, after which necrosis values remained significantly lower ($P < 0.05$) than for the controls. In the case of *P. alba*, immediately after freezing, necrosis values increased until week 2 and remained relatively constant until week 8. For *C. betulus*, necrosis values increased until

Table 1. Percentage of fatty acids in leaf tissue of *P. alba* and *C. betulus* supplemented with calcium nitrate before and after freezing. All values are the mean of 3 plants.

Fatty acid	Species	Ca ²⁺	Percentage of lipids					
			Before freezing	After freezing				
			Week 0	Day 1	Week 2	Week 4	Week 6	Week 8
C16	<i>P. alba</i>	Ca ²⁺	31.7 ^{ns}	29.44*	42.7 ^{ns}	48.2 ^{ns}	37.8 ^{ns}	28.6 ^{ns}
Palmitic acid	<i>P. alba</i>	—	32.3	22.33	42.0	43.1	37.3	26.7
	<i>C. betulus</i>	Ca ²⁺	35.6 ^{ns}	33.63*	42.7 ^{ns}	57.3 ^{ns}	47.7 ^{ns}	28.6 ^{ns}
	<i>C. betulus</i>	—	34.0	30.98	57.0	61.9	35.4	24.3

*Significant at $P < 0.01$; ns, not significant.

week 2, after which values slowly decreased until week 8 (Figure 2).

Electrolyte Leakage, Mortality, and Growth

Irrespective of species, leaf and root electrolyte leakage values were significantly lower ($P < 0.05$) in plants supplemented with Ca^{2+} than in the controls, with the exception of leaf tissue of *C. betulus*, for which electrolyte leakage values were not significantly different (Table 2). Similarly, significantly higher root, shoot, and leaf areas were recorded in Ca^{2+} -supplemented *C. betulus* plants but not for *P. alba*, where values were higher—but not significantly higher than the controls. Supplementation with Ca^{2+} reduced mortality rates from 70% to 40% (*C. betulus*) and from 90% to 80% (*P. alba*). Lower plant mortality and root and shoot electrolyte leakage values between controls demonstrate *C. betulus* to be more freezing resistant than *P. alba*.

Nutrient Analysis

Calcium supplementation increased ($P < 0.05$) Ca^{2+} concentrations in leaf, root, and shoot tissue of both *P. alba* and *C. betulus*. In the case of *P. alba*, leaf, root, and shoot calcium concentrations were raised from 0.66, 0.40, and 1.32 to 0.82, 0.58, and 1.51 percentage dry matter, respectively. In the case of *C. betulus*, leaf, root, and shoot concentrations were raised from 0.75, 0.33, and 0.70 to 1.10, 0.79, and 1.64 percentage dry matter, respectively. Higher concentrations of Ca^{2+} in root, shoot, and leaf tissue of *C. betulus* than of *P. alba* indicate that *C. betulus* accumulated Ca^{2+} ions at a more rapid rate than did *P. alba* in response to supplementation. Indeed, initial Ca^{2+} concentrations in shoot and leaf tissue of *C. betulus* were lower than those found in *P. alba*.

DISCUSSION

Lower necrosis, mortality rates, and root and leaf electrolyte values, combined with higher chlorophyll fluorescence values and root, shoot, and leaf dry weights and larger leaf areas in Ca^{2+} -supplemented plants at the conclusion of the experiment indicate that the freezing tolerance of *C. betulus* and *P. alba* may be increased by application of Ca^{2+} fertilizer.

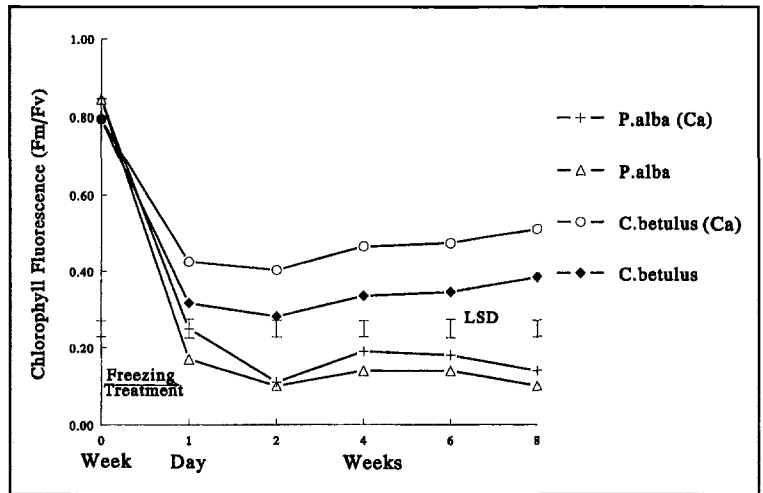


Figure 1. Leaf chlorophyll fluorescence (Fm/Fv) following freezing. All values mean of 5 leaves, 12 trees per treatment. Calcium-treated plants. LSD = Least significant difference at $P \leq 0.05$.

Only C16 (*C. betulus*) fatty acids were increased in Ca^{2+} -supplemented plants immediately following freezing, indicating that the increased tolerance of the woody plants tested was not due to any effects on leaf fatty acids.

In response to extracellular freezing, plant cells dehydrate and eventually collapse, causing the solidified cell membrane to fracture and solutes within the cell to be lost. Additionally, enzymes in the cytosol may affect unsaturated bonds between membrane lipids, resulting in peroxidation of lipids and impairment of membrane semipermeability (Levitt 1980). Ultimately, such damage becomes visibly manifest as leaf necrosis, plant death, or both (Sakai et al. 1968). Electrolyte leakage is widely used to measure freezing damage as well as to quantify species resistance to cold and freezing injury (McKay 1992). Similarly, measurement of chlorophyll fluorescence is now regarded as a rapid method for mass screening of plants in breeding programs to evaluate the response of crop species to chilling and freezing (Percival et al. 1998). Higher and lower leaf chlorophyll fluorescence and necrosis values, respectively, after freezing, and reduced root and leaf electrolyte values of Ca^{2+} -supplemented plants may have resulted from a number of alterations in a variety of plant physiological and biochemical processes influenced by calcium that were not explored in this investigation (e.g., cell-wall strengthening [Legge et al. 1982] and increased enzymatic activity [Monroy et al. 1993; Berbezzy et al. 1996]).

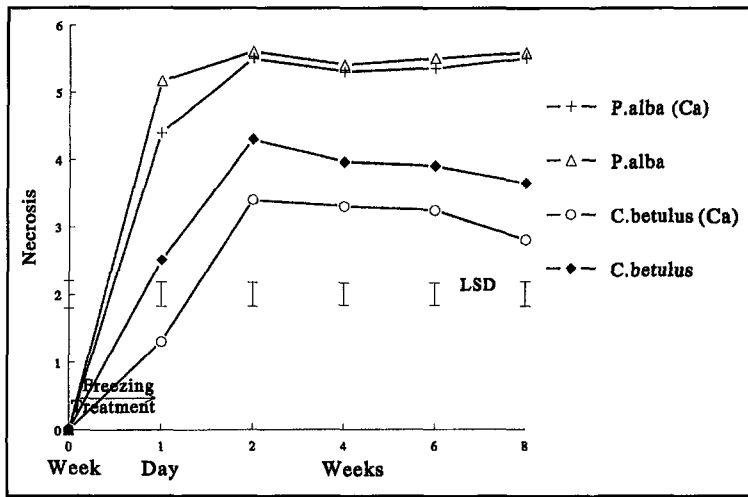


Figure 2. Leaf necrosis following freezing. All values mean of 5 leaves, 12 trees per treatment. Calcium-treated plants. LSD = Least significant difference at $P \leq 0.05$.

In *C. betulus*, chlorophyll fluorescence values increased and necrosis values decreased from week 2 onwards, with values higher and lower, respectively, in Ca^{2+} -supplemented plants. This effect can be accounted for by the fact that between weeks 2 and 4, trees produced a flush of growth that increased leaf area and effectively reduced the proportion of yellow and necrotic tissue. Additionally, abscission of badly necrotic leaves left healthy, largely undamaged leaves for chlorophyll fluorescence studies. The benefits of calcium supplementation in maintaining a higher rate of photosynthetic integrity and in turn photosynthesis are likely to have a major effect on the rate of recovery from freezing injury and be of particular importance for subsequent plant growth rates.

Results demonstrate that *C. betulus* is more resistant to freezing than is *P. alba*. This is contrary to

what has previously been observed (Sakai and Larcher 1987). Freezing resistance is known to be affected by a number of factors, such as developmental stage, bark thickness, leaf morphology, and species provenance, which may have accounted for this result (Levitt 1980; Palta 1992). Alternatively, increased resistance to freezing injury in *C. betulus* may occur as a result of higher accumulation of calcium ions in roots, shoots, and leaves. Before supplementation, lower Ca^{2+} concentrations in shoot and leaf tissue were recorded for *C. betulus* than for *P. alba*; however, following treatments, higher Ca^{2+} concentrations in supplemented *C. betulus* plants were found. Consequently, between species, accu-

mulation rates of Ca^{2+} following supplementation may be important in conferring freezing resistance.

Attempts to improve cold hardiness through plant breeding may be limited by the nature of freezing tolerance, which is thought to be a quantitatively inherited trait controlled by many genes (Palta 1996). The use of calcium supplementation to reduce freezing damage to urban trees could easily be adopted by arboriculturists. Such a step would require no capital investment and only small adjustments to standard planting procedures—the cost of which would be negligible compared to the risk of reduced growth and tree losses. Also, calcium is a nontoxic mineral nutrient, and plant cells can tolerate high concentrations when it is applied extracellularly (Palta and Lee-Stadleman 1983). Previous research has shown because of calcium's effects on membrane stability, ap-

Table 2. Root and leaf electrolyte leakage immediately following freezing at -10°C (14°F) and growth of *P. alba* and *C. betula* at week 8 supplemented with calcium nitrate. All values are the mean of 5 plants.

Species	Ca^{2+}	Electrolyte leakage		Mortality (%)	Week 8			
		Day 1			Root dry weight (g)	Shoot dry weight (g)	Leaf dry weight (g)	Leaf area (cm)
		Leaf	Root					
<i>P. alba</i>	Ca^{2+}	45.09*	38.66*	80	4.58 ^{ns}	5.70 ^{ns}	0.96 ^{ns}	119 ^{ns}
<i>P. alba</i>	—	55.64	48.04	90	4.07	5.23	0.94	116
LSD		5.63	6.01		0.97	1.02	0.95	10.38
<i>C. betulus</i>	Ca^{2+}	32.45 ^{ns}	32.35*	40	5.15*	3.82*	1.33 ^{ns}	151*
<i>C. betulus</i>	—	30.69	44.46	70	4.06	2.73	1.01	123
LSD ²		4.17	5.22		0.65	0.71	0.60	13.52

¹Least significant difference.

*Significant at $P < 0.05$; ns, not significant.

plications of the mineral can increase cold-storage time for such fruits as avocados, mangoes, cherries, and apples (Anderson and Campbell 1995; Palta 1996) by reducing chilling injury.

Further work is now planned to determine the effectiveness and feasibility of commercially produced calcium compounds (calcium nitrate, sulfate, and chloride) singly and in combination to improve cold tolerance of woody perennials and to investigate the mode of application (root drench and foliar spray) and time of applications (dormancy or bud flush) conferring maximal resistance to freezing damage.

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Acknowledgments. SAC are partially funded by the Scottish Office Agriculture Environment and Fisheries Department.

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Résumé. Les effets d'un apport supplémentaire de calcium (Ca^{2+}) sur la résistance au gel du peuplier blanc (*Populus alba*) et du charme européen (*Carpinus betulus*) ont été étudiés en suivant les altérations des acides gras foliaires, les émissions fluorescentes de la chlorophylle, les nécroses, la mortalité et la croissance. L'apport de calcium n'a pas d'effet significatif ni sur les acides gras insaturés ni saturés avant ainsi que de deux à huit semaines après le gel comparativement aux arbres témoins. Sans tenir compte des pourcentages d'acides gras saturés selon les espèces, les C16 étaient significativement plus élevés chez les plantes suppléées en Ca^{2+} immédiatement (1 jour) après le gel seulement. Aucun effet significatif sur les acides gras restants n'a été enregistré le jour 1. Chez *P. alba*, les valeurs de fluorescence de la chlorophylle foliaire et de nécrose étaient significativement plus élevées et basses respectivement que celles des témoins, et ce immédiatement après le gel ; les lectures subséquentes entre les semaines 2 et 8 ne différaient pas significativement de celles des témoins. D'un autre côté, pour *C. betulus* après le gel, les valeurs de fluorescence de la chlorophylle foliaire et de nécrose étaient significativement plus élevées et basses respectivement que celles des témoins, et ce sur l'ensemble de l'expérience. Les concentrations en calcium étaient significativement plus élevées chez les plantes ayant eu un apport supplémentaire. À la fin de l'expérience, les plantes suppléées en Ca^{2+} ont enregistré des valeurs plus basses de taux de mortalité et de perte en électrolyte racinaire et foliaire, ainsi que des valeurs plus élevées de surface foliaire et de masse sèche de racines, de pousses et de feuilles. Les résultats indiquent que la tolérance au gel peut être accrue par l'application d'engrais contenant du Ca^{2+} ; les implications de cela en regard des bénéfices pour l'industrie arboricole sont discutées.

Zusammenfassung. Der Einfluß von Kalzium (Ca^{2+}) Düngung auf die Frostresistenz der Weißpappel (*Populus alba*-frosthart) und Hainbuche (*Carpinus betulus*-frostempfindlich) wurde durch eine Überwachung der Veränderungen in den Fettsäuren der Blätter, der Chlorophyll-Fluoreszenz-Emissionen, der Nekrosis, Sterberate und des Wachstums studiert. Die Kalziumdüngung zeigte im Vergleich mit Kontrollen keine signifikanten Auswirkungen auf gesättigte oder ungesättigte Fettsäuren vor und nach 2 bis 8 Wochen nach einem Frost. Unabhängig von

dem Anteil der Arten bei den gesättigten Fettsäuren waren C16 in Ca^{2+} gedüngten Pflanzen direkt nach dem ersten Frosttag wesentlich höher. In diesem ersten Tag gab es keinen signifikanten Einfluß auf die anderen, vorhandenen Fettsäuren. Bei Palba waren die Werte für Blattchlorophyllkonzentration und Nekrose im Vergleich zu den Kontrollen und direkt nach dem Frost deutlich höher und niedriger. Spätere Messungen zwischen der 2. und 8. Woche zeigten keinen Unterschied zu den Kontrollen. Alternativ dazu waren die Werte für Blattchlorophyllkonzentration und Nekrose bei *C. betulus* während des ganzen Experiments höher und niedriger als die Kontrollen. Die Calciumkonzentration war in gedüngten Pflanzen wesentlich höher. Als Ergebnis dieses Experiments wurde bei calciumgedüngten Pflanzen folgendes festgestellt: geringere Sterberate, die Werte für Wurzel- und Blattelektrolytverlust, höhere Trockengewichte für Wurzeln, Trieb, Blatt und Blattfläche. Die Ergebnisse zeigen, daß die Frosttoleranz durch die Düngung von Calcium vergrößert werden kann. Die Auswirkungen auf die Baumpflegeindustrie wurden hier dargestellt.

Resumen. Fueron estudiados los efectos de suplementos de Calcio (Ca^{2+}) sobre la resistencia a las heladas del álamo plateado (*Populus alba*-resistente a las heladas) y hornbeam (*Carpinus betulus*-sensible a las heladas) para monitorear las alteraciones en los ácidos grasos del follaje, emisiones de clorofila, necrosis, mortalidad y crecimiento. Los suplementos de calcio no tuvieron efecto significativo sobre cualquiera de los ácidos grasos saturados y no saturados antes y después de las 2-8 semanas siguientes al enfriamiento, comparados con los controles. Independientemente de los porcentajes de ácidos saturados de las especies, C16 fue significativamente mayor en el Ca^{2+} suplementado solamente a las plantas inmediatamente después de la helada (1 día). No fueron registrados efectos significativos en los ácidos grasos remanentes en un día. En *P. alba*, la clorofila de las hojas y los valores de necrosis fueron significativamente mayores y menores que los controles, respectivamente, inmediatamente después de la helada; las lecturas subsiguientes de las semanas 2-8 no difirieron significativamente de los controles. Alternativamente, los valores de clorofila y necrosis, en *C. betulus* después de la helada, fueron significativamente más altos y más bajos que los controles, respectivamente, a través del experimento. Las concentraciones de calcio fueron significativamente mayores en las plantas suplementadas. En la conclusión del experimento fueron registradas tasas de mortalidad más bajas, merma en los valores de electrolitos de raíz y hojas y mayores pesos secos de raíz, brotes y hojas y área foliar en las plantas suplementadas con Ca^{2+} . Los resultados indican que la tolerancia a las heladas puede ser incrementada con la aplicación de fertilizante con Ca^{2+} ; cuyas implicaciones para el beneficio de la industria de la arboricultura son discutidas.