Long-Term Nitrogen Fertilization Increases Winter Injury in Montane Red Spruce (*Picea rubens*) Foliage

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INTRODUCTION

Current-year red spruce (*Picea rubens* Sarg.) foliage is predisposed to winter injury by one or more types of anthropogenic pollutants, particularly acidic deposition (DeHayes, 1992). The resultant defoli-

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ation, when severe and repeated, leads to dieback and eventual mortality of affected red spruce individuals.

The role of soil-deposited pollutants in predisposing red spruce to winter injury is less well understood. Two early studies showed that large amounts of soil-applied nitrogen (N) had a positive effect on the cold tolerance of potted red spruce seedlings (Klein et al., 1989; DeHayes et al., 1989). In several field experiments on mature trees, some up to three years in duration, N fertilization had no effect, or even a slight positive influence, on the ability of red spruce foliage to survive winter conditions (Perkins and Adams, unpublished). However, given the long-term nature of forest stands, and the increasing concern over nitrogen saturation in the northeast (Abed et al., 1989), we sought to establish the relationships among soil-applied N, foliar N, winter physiology, and winter damage in high-elevation red spruce.

METHODS

Study Area and Nitrogen Treatments

This work was conducted in a series of fertilization plots established on Mt. Ascent, in southeast Vermont (42° 26′N, 72° 27′W, 762 m elevation) in 1988 (McNulty et al., 1996). Two replicate 15 m × 15 m plots of five fertilization types and levels were established in 1988, in stands dominated by red spruce. These plots were fertilized in three equal amounts applied in June, July, and August of each year to yield total annual additions of 0, 15.7, 19.8, 25.6, and 31.4 kg $N \cdot ha^{-1} \cdot yr^{-1}$ (McNulty et al., 1996). Ambient bulk precipitation added an additional 5.4 kg $N \cdot ha^{-1} \cdot yr^{-1}$ (McNulty and Aber, 1993). Monitoring within these plots has showed evidence of N-induced disruptions in foliar cations and carbon relations (Schaberg et al., 1997) and reductions in growth and increases in mortality of red spruce trees (McNulty et al., 1996).

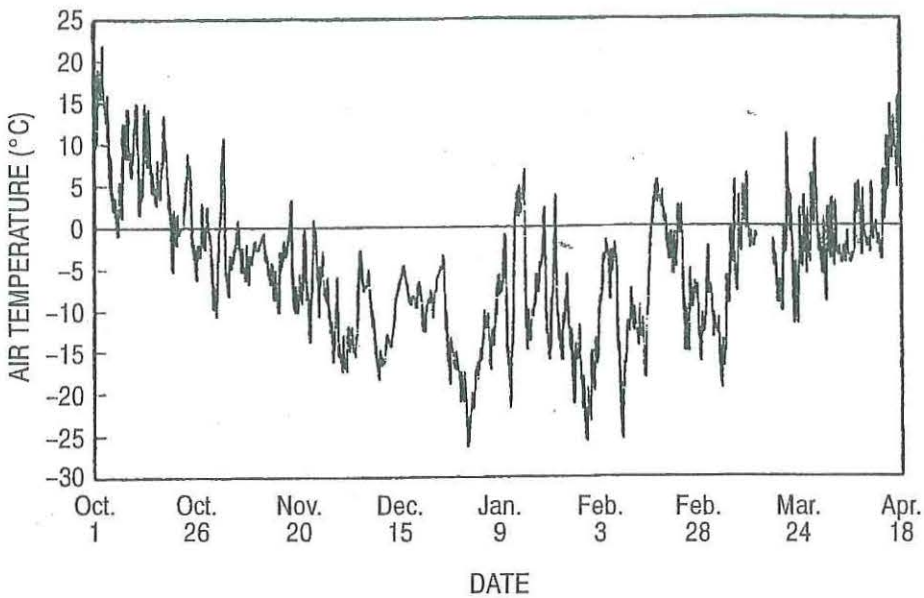
Foliar Collections

Current-year foliage was collected in August 1995. Foliar chemistry was determined according to procedures given by Schaberg et al. (1997). Monthly visits to the site were made from December 1995 through March 1996 to collect foliage for winter physiological measurements. Foliage was collected from mid-upper crown branches of three dominant trees near the center of each plot. Foliage was transferred to the laboratory in an insulated container packed with snow. The following morning, the samples were examined for presence of winter injury using a scale of 0-9 (10% injury classes). Chlorophyll fluorescence (F_v/F_m) was measured the day following collection to detect non-visual winter injury. Fluorescence of a replicate set of foliage was made after four days in the cold room (4°C) to assess potential recovery. Chlorophyll fluorescence has been demonstrated to be a rapid and quantitative measure of foliar winter damage (Adams and Perkins, 1993). Cold tolerance was established using a controlled temperature chamber using methods of Adams and Perkins (1993). Red spruce shoots were frozen at a rate of $10°C \cdot hr^{-1}$ and samples removed at various temperatures to bracket the range of expected foliar cold tolerance. Chlorophyll fluorescence was used to determine the temperature at which cold tolerance was exceeded. Dehardening capacity (the cold tolerance foliage would deharden to) and extent (the amount of dehardening) were determined by remeasuring cold tolerance of duplicate foliage samples after four days exposure to above freezing conditions in a cold room (4°C) under low light (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Rapid freezing tolerance was assessed using a Cryomed liquid-nitrogen controlled rate freezer at freeze rates of 0.0, −10.0, −12.5, and $-15.0°C \cdot min^{-1}$ after Perkins and Adams (1995). Foliar water content and desiccation rates were established gravimetrically (Perkins et al., 1993). Chlorophyll fluorescence measurements were also made as an indicator of the degree of photostress (Adams, 1996).

Slight winter injury was observed on a few trees within the plots during January 1996. Damage became visually pronounced in February, reaching a maximum in March. In both February and May (just prior to extensive abscission of affected needles), visual injury to current-year foliage on crowns of test trees within each plot was estimated on a scale of 0-9 (representing 10% damage classes of current-year foliage) from several sides, using binoculars.

Differences among physiological response and levels of winter injury were assessed using ANOVA in PC SAS. Statistical power was low, as there were only two replicate plots per N treatment. Differences were considered significant at $p \leq 0.05$. Relationships among

FIGURE 1. Air temperatures on Mt. Ascutney during the winter of 1995-96. Measurements were recorded hourly.



nutrient concentrations and measures of winter damage were established using correlation analysis in PC SAS.

RESULTS

Physiological Measurements

Since most of the physiological testing relied upon visual or chlorophyll fluorescence detection of injury induced by the test conditions, the early appearance of winter injury (January 1996) rendered most measurements after that time unreliable. Thus it is not possible to fully accredit the injury which occurred in the winter of 1995-1996 on Mt. Ascutney, Vermont to any one particular stressor. The minimum winter temperatures on Mt. Ascutney in the period of time between the two site visits did not approach the normal expected levels of cold tolerance for this species (minimum temperature $-26.5°C$, Figure 1). Cold tolerance of current-year foliage in December did not vary among N-treatments ($p = 0.78$), averaging 49.4°C for sampled trees overall (Table 1). Increasing N-treatment decreased both the temperature to which red spruce foliage dehardened ($p = 0.03$) as well as the amount of dehardening ($p = 0.03$). Trees that received the highest

TABLE 1. Winter physiological parameters of current year red spruce foliage on Mt. Ascutney during the winter of 1995-1996. Since water injury was observed beginning with the January 1996 sample, values are given for only the December 1995 sampler period. Numbers represent means ± standard error of the two replicate treatment plots (three trees per plot). Significance of the ANOVA is given.

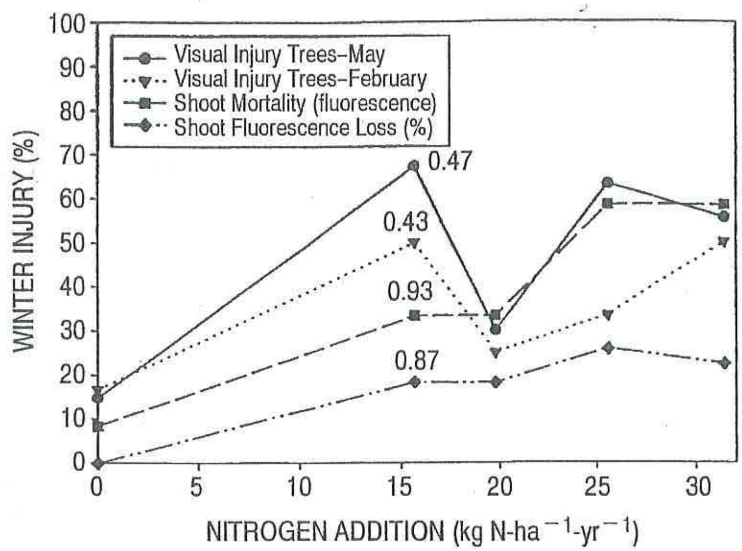
	Nitrogen Addition (kg-ha^{-1}-yr^{-1})					
Physiological Variable	0.0	15.7	19.8	25.6	31.4	p value
Cold Temperature (°C)	−43.4 ± 1.2	−43.5 ± 1.6	−42.9 ± 0.5	−45.1 ± 0.5	−44.5 ± 0.5	0.78
Dehardened Tolerance (°C)	−35.5 ± 1.1	−37.4 ± 2.1	−33.0 ± 1.5	−42.4 ± 0.3	−44.0 ± 0.1	0.03
Dehardened Extent (°C)	8.0 ± 2.3	6.1 ± 0.5	9.9 ± 1.1	2.8 ± 0.2	0.6 ± 0.4	0.05
Rapid Freeze Survival (%)	16.7 ± 11.8	22.2 ± 7.9	22.2 ± 7.9	50.0 ± 3.9	22.2 ± 7.9	0.23
Max Fluorescence (F_m)	153.6 ± 0.6	101.0 ± 7.6	186.5 ± 22.6	127.6 ± 31.9	138.4 ± 8.4	0.54
Water Content (% dw)	124.2 ± 1.6	115.0 ± 4.5	119.3 ± 0.7	112.0 ± 1.8	116.0 ± 2.6	0.31
Dessication Rate (% dw)	5.5 ± 0.6	8.2 ± 1.9	6.4 ± 0.7	8.1 ± 1.0	12.1 ± 4.8	0.01

amount of N-fertilization dehardened the least. No significant difference in susceptibility to rapid freezing was observed (p = 0.23). Similarly, water content was not significantly affected by N-addition (p = 0.31). The rate at which foliage lost water was significantly different among treatments (p = 0.01).

Winter Injury

Winter injury was observed on trees in treatment plots slightly earlier than in trees outside plots. Injury generally increased with increasing N-addition regardless of the method used to measure or express injury (Figure 2), with correlation coefficients ranging from 0.43 to 0.93. Correlations from visual estimates of winter injury were lower than those based upon fluorescence measurements. This likely arises from the fact that winter injury tended to be concentrated on southern sides of exposed tree crowns, and visual estimates were made on all sides, whereas foliage collected for physiological and damage assessments were always collected from the upper-third of the south side of the trees. It is also possible that the different forms of N-fertilization within the treatment plots contributed to the somewhat elevated levels of winter injury observed in the 15 kg $N \cdot ha^{-1} \cdot yr^{-1}$

FIGURE 2. Relationships between nitrogen treatment and winter injury on Mt. Ascutney, Vermont. Visual winter injury to current-year foliage was assessed for the crowns of three trees within each plot in February and again in May 1996. Shoot fluorescence (F_v/F_m) was measured on sampled branches in March 1996 and expressed as a percentage of fluorescence loss from the control N-treatment. Shoot mortality was calculated by placing samples of collected foliage under low light in a cold room (4°C). Foliage that did not regain typical levels of wintertime fluorescence (Fv/Fm > 0.45) was considered injured. Numbers next to lines indicate the correlation coefficient for the relationship between nutrient addition and the various measures of winter injury.



treatment. Nitrogen addition was correlated with several foliar nutrients (Table 2). The various measures of winter injury were also significantly correlated with foliar nutrients.

DISCUSSION

While several earlier studies have shown that acidic deposition can reduce the cold tolerance of current-year red spruce foliage and pointed out the importance of foliar nutrition in winter injury (see review by DeHayes, 1992), no work to date has shown that soil-applied N can increase the susceptibility of mature red spruce trees to winter injury. Many prior studies have clearly demonstrated that winter injury may be exacerbated by foliar-mediated processes. The strong relationships among N-addition, foliar nutrition and winter injury in this study show that the winter physiology of red spruce trees

TABLE 2. Correlation coefficients (r^2) among nitrogen additions or measures of injury during the winter of 1995-1996 and foliar nutrient content of current-year red spruce foliage on Mt. Ascutney (see Schaberg et al., 1997). Negative values indicate inverse relationships. Visual injury was assessed on trees in the field. Fluorescence was measured on sampled branches and is expressed as a percentage of the control N-treatment. Survival is based upon measurements of chlorophyll fluorescence after exposure of foliage to conditions that promote recovery. All relationships are highly significant ($p \leq 0.01$).

	Foliar Element			
	N	Ca	Mg	Al
N Addition	0.71	−0.93	−0.89	−0.49
Visual Injury (May 1996)	0.86	−0.85	−0.81	−0.66
Visual Injury (Feb 1996)	0.52	−0.84	−0.73	−0.41
Fluorescence (F_v/F_m)	0.90	−0.94	−0.97	−0.74
Foliar Survival	0.80	−0.88	−0.84	−0.46

may also be impacted via soil-mediated processes, although it is unknown whether nitrogen itself or another nutrient affected by the N-fertilization, that is the important nutrient regulating winter injury.

Because of the unexpected early appearance of winter injury, the physiological measurements done in the course of this work provide few clues as to the mechanism(s) that caused the damage. Seedling studies have shown that cold tolerance is enhanced by N-fertilization (Klein et al., 1989; DeHayes et al., 1989). This study showed no clear effect of N on cold tolerance. However, dehardening was clearly positively affected (trees receiving higher N-fertilization dehardened less). Interestingly, although winter injury does not seem to be caused by desiccation, increasing N tended increase the rate at which foliage loses water.

CONCLUSIONS

Although foliar-mediated processes that cause winter injury are clearly recognized and reasonably well understood, soil-mediated processes have been largely overlooked. These results indicate that, in addition to the foliar effects of acidic deposition, nitrogen deposition to soils can also influence winter physiological processes and winter injury susceptibility in current-year red spruce foliage. With continued deposition of N-containing compounds to northern forests, it is likely that we will observe further changes in summer and winter physiology and growth of trees in high-elevation sites.

REFERENCES

Aber, J.D., Nadelhoffer, K.J., Steudler, P., and Melillo, J.M. 1989. Nitrogen saturation in northern forest ecosystems. Bioscience 39: 378-386.

Adams, G.T. 1996. Wintertime Photostress in Red Spruce Foliage. M.S. Thesis, University of Vermont, Burlington, VT.

Adams, G.T. and T.D. Perkins. 1993. Assessing cold tolerance in *Picea* using chlorophyll fluorescence. Environ. Exp. Bot. 33: 377-382.

DeHayes, D.H. 1992. Winter injury and developmental cold tolerance of red spruce. In: C. Eagar and M.B. Adams (Eds.). The Ecology and Decline of Red Spruce in the Eastern United States. Springer-Verlag, New York.

DeHayes, D.H., M.A. Ingle, and C.E. Waite. 1989. Nitrogen fertilization enhances cold tolerance of red spruce seedlings. Can. J. For. Res. 19: 1037-1043.

Klein, R.M., T.D. Perkins, and H.L. Myers. 1989. Nutrient status and winter hardiness in red spruce foliage. Can. J. For. Res. 19: 754-758.

McNulty, S.G. and J.D. Aber. 1993. Effects of chronic nitrogen additions on nitrogen cycling in a high elevation spruce-fir stand. Can. J. For. Res. 23:1252-1263.

McNulty, S.G., J.D. Aber, and S.D. Newman. 1996. Nitrogen saturation in a high elevation New England spruce-fir stand. For. Ecol. Mgmt. 84: 109-121.

Perkins, T.D. and G.T. Adams. 1995. Rapid freezing induces winter injury symptomatology in red spruce foliage. Tree Physiol. 15: 259-266.

Perkins, T.D., G.T. Adams, S. Lawson, and M.T. Hemmerlein. 1993. Cold tolerance and water content of current-year red spruce (*Picea rubens* Sarg.) foliage over two winter seasons. Tree Physiol. 13: 119-144.

Schaberg, P.G., T.D. Perkins, and S.G. McNulty. 1997. Effects of chronic low-level N additions on gas exchange, shoot growth and foliar elemental concentrations of mature montane red spruce. Can. J. For. Res. 27: 1622-1629.