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THE RESPONSE OF TEMPERATE TREE SEEDLINGS GROWN IN ELEVATED CO₂ TO EXTREME TEMPERATURE EVENTS¹

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Abstract. Mean global temperatures have been predicted to increase in the next century; if so the frequency of extreme temperature events may also increase. Extreme temperatures may damage plant tissue and consequently limit the survival of certain plant species in a region. Elevated concentrations of CO₂ in the atmosphere alter plant allocation, physiology, and growth, and may accentuate or ameliorate the damage from extreme temperatures. In this paper we explore the interactive effects of atmospheric CO₂ concentration, nutrient levels, and exposure to extreme temperatures on seedlings of three species of temperate deciduous trees.

A 1-d exposure to extreme heat (45°C) significantly decreased conductance the following day and decreased biomass as measured at both 35 and 105 d following the extreme temperature event, regardless of atmospheric CO₂ concentration. The most shade-tolerant species, striped maple, was most severely impacted by the extreme heat event in both CO₂ environments. Furthermore, striped maple seedlings grown in elevated CO₂ concentrations had a significantly greater decrease in biomass due to the extreme heat event as compared with striped maple plants grown in ambient CO₂ concentrations at 35 d after the heat event; however, at the end of the growing season at 105 d post treatment, this difference was not significant. A one-night exposure to low temperatures (4°C) did not affect biomass for any of these species.

With an increase in global mean temperatures, the frequency of extreme temperature events, particularly hot weather events, may increase and may extend to shaded understory sites. If the frequency of extremely high temperatures increases, the role that temperature extremes may play in changing competitive interactions and thus affecting community composition may increase in importance, as these temperatures appear to severely alter plant survival and growth in some species.

Key words: diurnal heating; elevated CO₂; extreme temperatures; global change; global climate change; growth; heat stress; nitrogen deposition; nocturnal chilling recovery; temperate tree seedlings; tissue damage.

INTRODUCTION

Temperature is one of the main factors limiting the distribution of plant species worldwide. The minimum, maximum, or mean temperature may prevent the establishment of a particular plant species in a region (see Woodward 1990). Even within a season at a given location, temperature can vary rapidly as weather systems pass regionally; the frequency or timing of extreme temperatures may be lethal for particular plant species. General circulation models predict that rising concentrations of CO₂ and other greenhouse gases in the atmosphere may lead to an increase in global mean temperature of 1.5°–4.5°C in the next cen-

tury (e.g., Schneider 1989, Houghton et al. 1990, Mitchell et al. 1990, Gates et al. 1992), though this is debated (e.g., Kerr 1989). And while it is even more difficult to predict future temporal temperature patterns than the magnitude of the mean temperature rise, an increase in mean temperatures alone would indicate an increase in the probability of days with very high temperatures (Mearns et al. 1984, Wigley 1985, Rind et al. 1989). If the increase in mean temperature is accompanied by an increase in the standard deviation of temperatures, then not only would extremely hot days increase in frequency, but extremely cold temperatures might be more frequent as well (Mitchell et al. 1990, Katz and Brown 1992).

When plants are exposed to air temperatures above their preferred range, they may maintain tissue integrity either by altering their radiation exchange to keep tissue at or below air temperatures or by protecting tissues using biochemical means (Hällgren et al. 1991); otherwise scorching and tissue death may occur. Rapid synthesis of heat-shock proteins in response to a slowly

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rising temperature or a pre-exposure to a sublethal extreme temperature has been associated with an increase in thermotolerance (e.g., Schlesinger et al. 1982, Lin et al. 1984, Nover 1984, Kee and Nobel 1986, Ougham and Howarth 1988, Howarth 1991, Koppelaar et al. 1991, Vierling 1991). Transpirational cooling will dissipate heat as latent heat of evaporation (LE) (e.g., Bazzaz 1990), keeping leaf tissue at or below air temperature. However, growth in a high-CO₂ atmosphere tends to lower foliar conductance rates, which may cause higher leaf temperatures (Idso et al. 1986, 1987), and thereby lead to more damage during an extremely hot period.

Coleman et al. (1991) suggest that the lower tissue nitrogen concentrations frequently observed in plants grown in elevated-CO₂ atmospheres (reviews in Bazzaz 1990, Bazzaz and Fajer 1992) or in low-nutrient treatments may result in reduced heat-shock protein synthesis; however, no significant relationship between tissue nitrogen concentration and the plant's ability to tolerate high temperatures was observed in C₃ and C₄ annuals. Heat-shock protein synthesis may not be limited by nitrogen content as normal protein synthesis ceases during an extreme temperature event.

Both nutrient and CO₂ levels may also alter patterns of biomass allocation and plant structure, which may have indirect consequences for thermotolerance. For example, a higher (nontoxic) nutrient level may increase the ratio of transpiring leaf area to water-acquiring roots, which may reduce the plant's ability to cool, causing more damage from exposure to extreme heat. Similarly, elevated CO₂ levels may reduce the leaf area ratio (e.g., Bazzaz 1990), leading to less damage from the extreme heat event, contrary to the predicted effects of lower conductance rates in an elevated-CO₂ atmosphere.

Nighttime chilling of plants causes a slower opening of stomata the following morning for a variety of plant species (Drew and Bazzaz 1979, 1982, Bazzaz and Lerdau 1990). Further, elevation of CO₂ can ameliorate photosynthetic decline following a cold night in C₄ plants (Potvin 1985), though the implications of this on longer term growth was not discussed. The extent to which CO₂ concentrations may affect the response of temperate tree species to nighttime chilling and daytime heating, either singly or in combination, has not been adequately explored.

In this paper we consider the interactive effects of elevated CO₂ and nutrients with exposure to midsummer extreme temperature events (diurnal heating and nocturnal chilling) for seedlings of three temperate tree species. The motivation for this study was to explore the ecological implications of extreme weather events for seedlings of different species grown in present-day CO₂ concentrations or elevated CO₂ concentrations expected in the future. The four temperature treatments included exposure to one hot day, one cold night, both treatments 48 h apart, or neither. The three species

examined in this study are gray birch (*Betula populifolia*), yellow birch (*Betula alleghaniensis* Britton), and striped maple (*Acer pensylvanicum*). All are common co-occurring deciduous tree species in New England forests ranging in shade tolerance: gray birch is the most shade intolerant, yellow birch is more tolerant, though it requires a gap for establishment and regeneration, and striped maple is very tolerant and can establish and remain in the understory throughout its life (Burns and Honkala 1990). These species exhibit a range of habitat preferences, so their susceptibility to extreme temperatures may differ.

We are interested in both the immediate effects of extreme temperature events and in the rates of recovery from damage due to extreme temperatures, as both processes may be affected by growth at elevated CO₂. For instance, plants grown in a high-CO₂ atmosphere may be more severely damaged by the heat event as contrasted with plants grown in low CO₂; however, high-CO₂-grown plants may have more potential to recover from the damage. We measured immediate tissue damage and conductance rates as indicators of the initial response to the extreme temperature events, and we monitored survival and growth through the first growing season as an indication of the potential recovery. Overwinter survivorship of seedlings often depends on the post-senescence stem and root biomass, as well as total carbohydrate reserves (Kramer and Kozlowski 1979, Glerum 1980).

METHODS

Growth conditions

In May 1990, seeds of gray and yellow birch, which had been collected the previous autumn at Harvard Forest, Petersham, Massachusetts, were germinated in vermiculite. On 30 May 1990, striped maple seedlings were collected at Harvard Forest at the cotyledon or two-leaf stage. On 22 June 1990, most seedlings had two leaves and were transplanted into plastic pots containing 1 L of a 1:1:1 mixture of sand, perlite, and peat. Seedlings were randomly assigned to one of two nutrient levels and received 0.12 or 1.2 g of Osmocote 14-14-14, a 3-mo slow release NPK fertilizer. The low-nutrient treatment is representative of present N mineralization rates and nutrient availability in Harvard Forest (equivalent to an N input of 40 kg·ha⁻¹·yr⁻¹), and the high-nutrient treatment represents a higher mineralization rate with extreme nitrogen deposition (equivalent to 400 kg·ha⁻¹·yr⁻¹) (Aber et al. 1989). The high-nutrient treated plants showed no signs of toxicity. On 17 August, each plant received a supplement of micronutrients (300 mL of a 0.37 g/L solution of Peter's Soluble Trace Elements). After 3 mo, when the Osmocote was calculated to be completely released, a weekly dose of 200 mL of Peter's 20-20-20 NPK fertilizer (0.42 g/L or 0.042 g/L) was applied. Weekly fertilization continued for 10 wk, at which point no

further nutrients were added, corresponding with onset of autumnal senescence.

Twelve glass-sided growth chambers oriented along an east–west axis within a glasshouse were paired into six growth blocks (GB) (see Carlson and Bazzaz 1982 for chamber design and description). Within each block, chambers were randomly assigned to one of two CO₂ levels, 350 or 700 μL/L. Seedlings of each species and nutrient level were placed in the chambers, 48 pots per chamber. The elevated-CO₂ treatment (700 μL/L) represents the predicted values for atmospheric CO₂ concentration within 100–200 yr (see Houghton et al. 1990, Mitchell and Gregory 1992), and the low-CO₂ treatment (350 μL/L) is the present-day CO₂ level. The day/night temperature regime was 28°/22° ± 2°C. Day length extended from 0600 to 1900, with natural daylight supplemented with high-intensity metal halide lamps when PAR (photosynthetically active radiation [photon flux]) levels fell below 500 μmol·m⁻²·s⁻¹.

Extreme temperature protocols

Seedlings were randomly assigned to one of four experimental temperature treatments: midsummer exposure to one hot day, maximum 45°C (HD); exposure to one cold night, minimum 4°C (CN); exposure to both extreme temperature treatments, the hot day followed by the cold night 48 h later (BE); and the control treatment of exposure to neither (NE). There were two replicates per growth block of each treatment.

On 6 September 1990, conductance rates were measured on three seedlings of each species and treatment in situ through the day, using a LI-COR 1600 Porometer (LI-COR Incorporated, Lincoln, Nebraska, USA). We measured conductance on the top, fully expanded leaf of the main stem.

A pair of growth blocks were grouped together as an Extreme Temperature Block (ETB), and for each CO₂ level, one of the chambers was randomly selected to be heated; plants were placed in the appropriate chamber to be exposed to the extremely hot day or not, at their growth CO₂ level. Plants were allowed to adjust for a minimum of 72 h after being moved.

The plants were thoroughly watered, such that the bases of all chambers were flooded the afternoon of 10 September 1990. On 11 September 1990, a brightly sunlit day, chambers due for the extreme heat event were slowly ramped up in temperature, from daybreak temperature of 28°C at 0600 to a maximal temperature of 45°C at 1100. This temperature was maintained for 4 h, at which point the temperature was slowly ramped down to the normal day temperature of 28°C by 1800, and 2 h later down to the night temperature of 22°C.

Beginning at sunrise the following day, leaf-level conductance rates were measured on a subset of plants. Overall apparent damage of the heat-stress was evaluated on all heat-stressed plants. A rating of low, medium, or high was assigned to each plant. High damage was complete wilting, obvious tissue scorch, or death

of >75% of the plant's leaves; medium damage was an intermediate amount of damaged tissue; while low damage was virtually no visible damage, or <10% of tissue damaged.

Conductance rates were again measured on the second day after the heat stress. That evening all plants were placed in appropriate chambers either to be exposed to the chilling night or not, at the appropriate CO₂ level. Starting at 2300, the normal nighttime temperature of 22°C was decreased incrementally over a period of 3 h to reach a minimum temperature of 4°C for 3 h. The temperature was then increased incrementally over 3 h to the normal daytime temperature of 28°C by 0800. The coldest time was shortly before sunrise, as would generally occur in nature. Conductance rates were measured beginning at daybreak. No visible damage occurred following the cold night, so no visual damage scores were assessed.

Plants were then returned to the original day/night temperature regime of 28°/22° ± 2°C. Ten days following the cold nighttime treatment, seedlings were scored for mortality and general status. This information was used for analysis of morbidity and mortality. Plants were repositioned in the chambers in which they were originally grown.

Harvests

On 17 October 1990, 35 d after the initial extreme temperature event, one replicate of each species treatment combination from each block (six plants per treatment) was harvested in order to assess the effects of the experimental treatments following a short period of recovery and growth. The seedlings still had green foliage and had not begun apparent autumnal senescence. The plants that were harvested were measured for basal diameter, total green leaf area, and total number of leaves. Leaf area was measured with a LI-COR 3200 Leaf Area Meter. Foliage that had been severely damaged by the heat stress often remained attached to the plant although it was completely dead. This tissue was separated from the green leaf tissue if >75% of the surface area was brown. Senesced tissue that fell from the plants was not collected. The stems were cut off above the soil surface and roots were washed gently from the soil. All tissues were dried at 45°C for a minimum of 72 h and weighed.

On 17 December 1990, 105 d after the initial extreme temperature event, the remaining plants were harvested in order to measure the net seasonal carbon accumulation. At this time all leaves had senesced, so only stem height and basal diameter were measured. Plants were harvested, dried, and weighed as they were at 35 d post treatment.

Statistical analysis

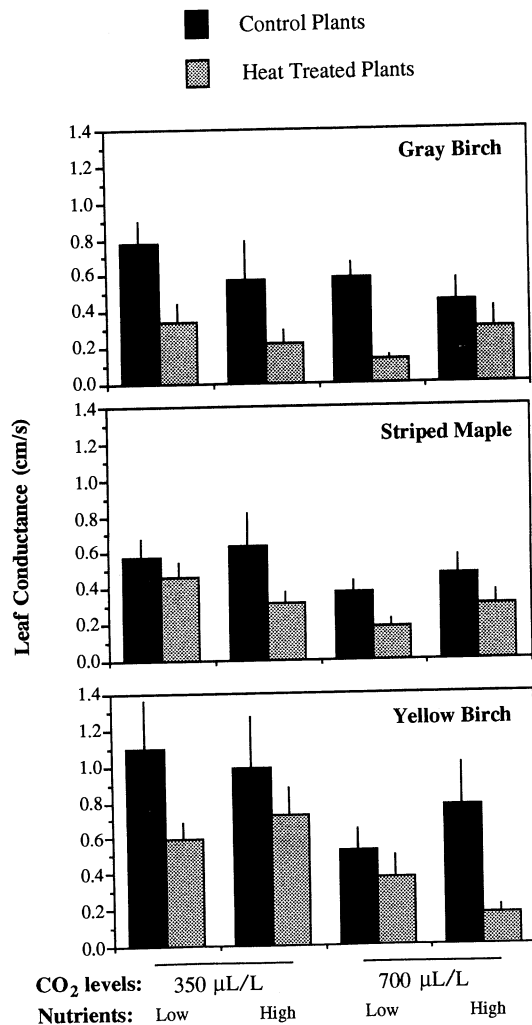
Immediate damage evaluations.—The intensity of damage due to the extremely hot day was compared

across species, CO₂ levels, and nutrient levels using log-likelihood calculations (1990 BMDP Statistical Software Package BMDP4F, Dixon 1985). Log-linear models (described in Bishop et al. 1975 and Sokal and Rohlf 1981) are additive, which is particularly useful for experimental designs in which the number of cells in each combination of treatments is preassigned. Accordingly, the initial model specified included only damage (low vs. medium vs. high) and the fixed interaction terms that could not be directly estimated (i.e., all second-, third-, and fourth-order interactions among heat treatment, species, CO₂ and nutrient level; see Sokal and Rohlf 1981, Brown 1985). Additional terms (i.e., second-order and higher interactions between damage and the treatment variables) were added sequentially to this model, and their contribution to the total G^2 determined through subtraction.

Biomass at harvests.—Biomass components at each harvest were analyzed using a randomized split-plot analysis-of-variance model with CO₂ as the (fixed) main plot factor in Data Desk 3.0 (Velleman 1989). All other treatments—species (SP), nutrient level (NL), hot day treatment (H; heat vs. no heat), and cold night treatment (C; cold vs. no cold)—were (fixed) subplot factors. Growth blocks (GB) and extreme temperature blocks (ETB) were designated as random; growth blocks were nested within extreme temperature blocks. The main effect of CO₂ was tested over the main plot error term (ETB × CO₂). All other effects and interactions were tested over the residual error. Type 3 (partial) sums of squares were calculated. Biomass components were transformed to their natural logarithms to meet assumptions of normality and homogeneity of variance. Log-transformed variates and their residuals were judged to be adequately normally distributed and homoscedastic using a combination of histograms and normality statistics, normal probability plots (Data Desk 3.0, Velleman 1989), and Scheffé–Box tests of homogeneity of variance (Sokal and Rohlf 1981). An analysis of pre-treatment plant height, leaf number, and basal diameter uncovered several plants that either did not grow or (rarely) had experienced accidental damage before we implemented the extreme temperature treatments. Those plants that had values for growth characters that were beyond 2.5 SD from the pre-treatment mean ($n = 48$) for each species × CO₂ level × nutrient treatment were eliminated from the data set.

Mortality and morbidity.—Some plants died as a result of the extreme temperature events, and several plants were very severely damaged by the extreme temperature events. These outliers were identified and were not included in the analysis of biomass responses. The effects of CO₂, nutrient level, and species on the mortality and morbidity in response to the extreme temperature events were analyzed separately in a contingency analysis for both harvests combined, using models constructed as for immediate damage evaluations.

Foliar Conductance Rates 1 Day Following the Extreme Heat Event



CO₂ and Nutrient Treatments

FIG. 1. Foliar conductance 1 d following the extreme heat event for gray birch, striped maple, and yellow birch grown in ambient (350 μL/L) or double ambient (700 μL/L) CO₂ atmospheres, with low or high nutrient amendments. Values represent the mean for one leaf each from 6–16 replicate plants for each treatment; error bars are +1 SE.

RESULTS

Immediate effects after extreme temperature events

Conductance rates were depressed substantially in the heat-stressed plants the day following the heat shock event (Fig. 1) and showed no signs of recovery 2 d after the extreme heat event. Following the cold night, no clear trends were detected as there was great variability in conductance rates (data not shown).

Scores of the apparent damage assessed the day fol-

**Frequency of Damage Ratings
Evaluated Immediately After Extreme Heat Event**

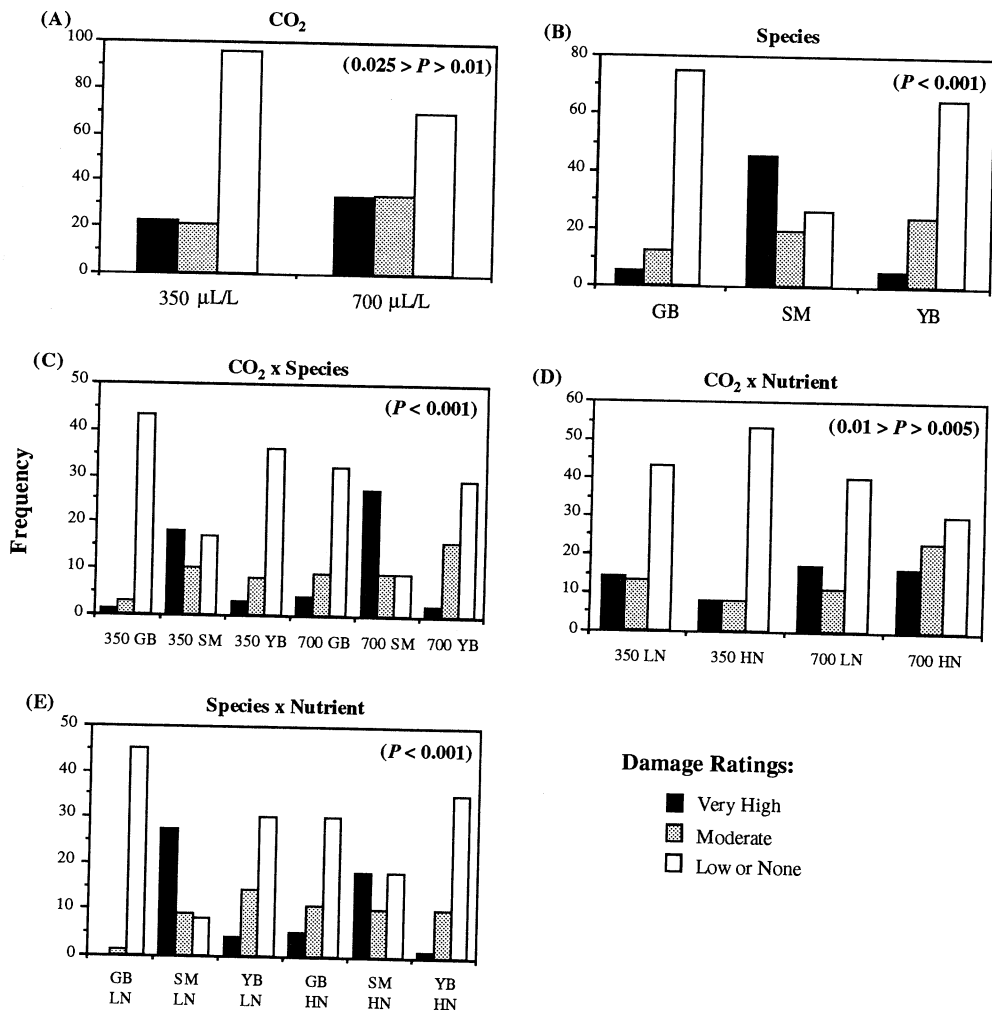


FIG. 2. Frequencies of the scores of immediate damage after the extreme heat event: very high, moderate, or low. Only the significant interactions are shown; the probability associated with the log-likelihood (G^2) test of association is indicated. Tallies of damage ratings are shown (A) by CO₂ level, summed across all species and nutrient levels; (B) by species, combining CO₂ and nutrient levels; (C) for each CO₂-species combination, combining the nutrient levels; (D) for each CO₂-nutrient level combination, summing across the three species; and (E) for each species-nutrient combination, summing across CO₂ levels. In each panel the total number of heat-treated plants evaluated for the damage rating frequencies was 288. Abbreviations are as follows: 350 = ambient CO₂ concentration; 700 = elevated CO₂ concentration; GB = gray birch; SM = striped maple; YB = yellow birch; LN = low-nutrient treatment; HN = high-nutrient treatment.

lowing the extremely hot day indicated that the plants grown in the high-CO₂ atmosphere suffered more damage than did the plants grown in the low-CO₂ atmosphere (0.025 > P > 0.01) (Fig. 2A). Additionally, apparent damage due to the heat stress was significantly different among the species (P < 0.001) (Fig. 2B). In particular, striped maple appeared most significantly damaged by the heat stress, with the highest number of individuals in the categories of high and intermediate damage. There were significant interactions between species and CO₂ (P < 0.001), CO₂ and nutrient level (0.01 > P > 0.005), and species and nutrient

level (P < 0.001) (Fig. 2C, D, and E respectively). There was no visible damage due to the extremely cold night.

Mortality and morbidity

Significantly more striped maple seedlings died due to the extreme heat event than did seedlings of either of the birch species (0.025 > P > 0.01). The high-nutrient striped maple had the most extreme damage due to the heat treatment (as identified by 2.5 standard deviations from the mean), causing a significant species by nutrient interaction (0.01 > P > 0.05).

TABLE 1. Biomass of tree seedlings at 35 and 105 d after the extreme temperature events, corrected for block and chamber effects. Sample size is six in most cases, except when mortality reduced this to no less than three. Abbreviations are as follows: BE, exposure to both extreme temperature events; CN, exposure to cold night only; HD, exposure to hot day only; HN, high-nutrient treatment; LN, low-nutrient treatment; NE, exposure to neither extreme temperature.

Species	Nutr. trts.	Temp. trts.	350 $\mu\text{L/L}$				700 $\mu\text{L/L}$								
			Stem mass (g)	SE	Root mass (g)	SE	Leaf mass (g)	SE	Stem mass (g)	SE	Root mass (g)	SE	Leaf mass (g)	SE	
35 d after the extreme temperature events															
Gray birch	LN	BE	0.304	0.008	0.733	0.020	0.648	0.012	0.416	0.010	0.815	0.022	0.777	0.019	
		HD	0.325	0.008	0.660	0.018	0.755	0.014	0.440	0.011	1.027	0.028	0.762	0.018	
		CN	0.298	0.008	0.650	0.017	0.672	0.012	0.332	0.008	0.891	0.024	0.672	0.016	
		NE	0.346	0.009	0.736	0.020	0.727	0.013	0.411	0.010	0.947	0.026	0.592	0.014	
	HN	BE	2.692	0.068	1.989	0.053	3.002	0.654	4.034	0.101	3.509	0.096	3.663	0.088	
		HD	3.592	0.499	2.333	0.283	4.203	0.509	4.446	0.834	3.730	0.542	4.540	0.603	
		CN	3.773	0.096	2.782	0.075	4.484	0.083	4.593	0.115	4.669	0.128	4.831	0.116	
		NE	3.561	0.090	2.387	0.064	4.429	0.082	4.901	0.123	4.891	0.134	5.182	0.125	
	Striped maple	LN	BE	0.409	0.150	0.426	0.143	0.891	0.256	0.559	0.206	0.594	0.168	0.977	0.305
			HD	0.585	0.173	0.525	0.142	0.891	0.214	0.380	0.164	0.457	0.124	0.506	0.233
			CN	0.171	0.004	0.232	0.006	0.495	0.203	0.571	0.104	0.619	0.052	0.875	0.128
			NE	0.260	0.014	0.548	0.099	0.634	0.282	0.568	0.112	0.751	0.028	0.628	0.213
HN		BE	3.129	0.637	1.076	0.049	3.415	0.564	2.299	0.521	1.357	0.148	2.935	0.832	
		HD	3.016	0.426	0.967	0.034	3.369	0.378	3.413	0.467	1.289	0.098	3.743	0.667	
		CN	3.497	0.510	1.128	0.052	3.744	0.450	7.087	0.177	2.825	0.078	7.783	0.187	
		NE	4.500	0.689	1.642	0.125	4.721	0.654	4.727	1.142	2.163	0.470	5.187	1.094	
Yellow birch		LN	BE	0.295	0.007	0.510	0.014	0.666	0.012	0.437	0.011	0.661	0.018	0.689	0.017
			HD	0.382	0.010	0.501	0.013	0.599	0.011	0.335	0.008	0.769	0.077	0.785	0.196
			CN	0.346	0.009	0.527	0.014	0.711	0.013	0.446	0.011	0.843	0.023	0.699	0.017
			NE	0.573	0.143	0.698	0.069	0.679	0.017	0.407	0.010	0.734	0.020	0.700	0.017
	HN	BE	3.565	0.091	2.346	0.063	4.885	0.090	3.917	0.098	3.576	0.098	4.717	0.637	
		HD	4.018	0.102	1.934	0.052	5.125	0.095	3.585	0.090	3.359	0.092	4.724	0.612	
		CN	4.021	0.102	3.085	0.083	4.890	0.090	4.166	0.104	4.560	0.125	5.374	0.129	
		NE	3.018	0.353	2.754	0.361	4.143	0.470	4.054	0.101	3.819	0.105	5.217	0.125	
	105 d after the extreme temperature events, following completion of autumnal senescence														
	Gray birch	LN	BE	0.499	0.012	1.071	0.045			0.535	0.020	1.273	0.057		
			HD	0.451	0.010	0.926	0.039			0.997	0.238	1.544	0.190		
			CN	0.487	0.011	1.044	0.044			0.534	0.020	1.206	0.054		
NE			0.911	0.290	1.413	0.161			0.569	0.021	1.302	0.058			
HN		BE	4.646	1.150	4.205	0.899			5.292	1.135	5.133	1.004			
		HD	4.450	0.868	3.625	0.557			7.761	0.289	6.861	0.307			
		CN	6.634	0.153	5.458	0.231			7.841	0.292	7.254	1.110			
		NE	6.507	0.150	4.882	0.207			7.799	0.290	6.131	0.848			
Striped maple		LN	BE	0.543	0.219	0.743	0.214			0.357	0.013	0.707	0.032		
			HD	0.449	0.234	0.639	0.229			0.279	0.010	0.470	0.021		
			CN	0.793	0.277	1.053	0.245			0.946	0.312	1.275	0.297		
			NE	0.945	0.253	1.275	0.235			0.661	0.196	1.063	0.154		
	HN	BE	3.896	0.703	2.513	0.259			4.770	1.369	3.177	0.570			
		HD	5.614	0.129	2.318	0.098			4.546	1.283	3.001	0.495			
		CN	6.955	0.160	4.221	0.179			7.655	1.236	5.022	0.601			
		NE	5.855	0.843	3.215	0.301			8.599	0.320	6.204	0.278			
	Yellow birch	LN	BE	0.516	0.012	0.768	0.033			0.545	0.020	0.863	0.039		
			HD	0.449	0.010	0.777	0.033			0.823	0.165	1.036	0.193		
			CN	0.484	0.011	0.737	0.031			0.616	0.023	1.142	0.051		
			NE	0.794	0.184	0.981	0.191			0.617	0.023	1.101	0.049		
HN		BE	4.911	0.639	3.654	0.357			6.396	1.462	5.838	1.168			
		HD	6.305	0.145	4.193	0.178			6.078	0.226	5.242	0.235			
		CN	6.053	0.848	4.695	0.528			5.880	0.219	6.963	0.312			
		NE	4.118	1.058	3.800	0.796			6.335	0.236	5.502	0.247			

Biomass after 35 d of recovery

Biomass measured 35 d after the extreme temperature events indicated that elevated CO_2 levels significantly increased root and stem biomass ($P = 0.0048$ and 0.0365), and the high nutrient level significantly increased all measures of biomass ($P < 0.0001$) (Tables

1 and 2). The root and live leaf biomass of the species differed significantly ($P < 0.0001$), while the stem mass was not significantly different among the species.

The extreme heat treatment significantly decreased both root and stem biomass as well as the number of live leaves (data not shown), whereas the extreme cold treatment did not significantly affect the biomass of the

TABLE 2. Results of analysis of variance for various growth parameters of temperate tree seedlings exposed to extreme temperatures and elevated CO₂ concentrations. Factors and models are described in *Methods*. Only *P* values <0.10 are shown.

Source*	After 35 d			After 105 d	
	Root mass	Stem mass	Live leaf mass	Root mass	Stem mass
GB		
ETB0072	.0977
CO ₂	.0048	.0365	.0737
GB × CO ₂0042	.0170
ETB × CO ₂
NL	.0000	.0000	.0000
CO ₂ × NL	.05900000	.0000
SP	.00000147	...
CO ₂ × SP0000	.0000	.0000
NL × SP0880
CO ₂ × NL × SP0001	.0000	.0982	.0000
ET
H	.0000	.00600000	.0002
C
H × C
CO ₂ × ET0386	.0571
H	.0583	.0129	.00180766
C
H × C
NL × ET
H	.0705
C
H × C
CO ₂ × NL × ET0762	.0662
H0809
C
H × C
SP × ET0056
H	.0109	.02660004	.0002
C
H × C	.05610968
CO ₂ × SP × ET
H	.0221	.0005	.0001
C
H × C
N × SP × ET
H08610942
C
H × C06930444
CO ₂ × NL × SP × ET
H0005	.0637	...
C
H × C
r ²	88.2	93.8	93.8	94.2	97.7

* C = cold treatment, ET = extreme temperature treatment, ETB = extreme temperature block, GB = growth block, H = heat treatment, NL = nutrient level, SP = species.

tree seedlings. Due largely to the response of striped maple, high-CO₂-grown plants tended to be more severely affected by the heat event, having a significantly lower stem, root, and live leaf biomass and live leaf area due to the heat event as contrasted to plants grown in ambient CO₂ conditions (Fig. 3) ($P = 0.0129, 0.0583, 0.0018$, and 0.0308). Striped maple had the greatest

reduction in biomass due to the heat event ($P = 0.0109$ and 0.0266 for root and stem respectively). In particular, high-CO₂-grown striped maple was most severely impacted by the extreme heat event, giving rise to a significant interaction of growth CO₂ atmosphere by species by heat event for root, stem, and live leaf mass ($P = 0.0221, 0.0005, 0.0001$).

Biomass 105 d after extreme events

The reduction in stem and root biomass due to the extreme heat alone was still highly significant at the end of the growing season ($P = 0.0001$ and 0.0002). The exposure to the cold night alone did not significantly affect root or stem biomass; however, the subsequent exposure to the cold night following the hot day further decreased root mass significantly ($P = 0.0386$) and stem mass marginally ($P = 0.0571$), as contrasted to the heat exposure alone. Striped maple had the greatest reduction in root and stem biomass due to the extreme heat event ($P = 0.0004$ and 0.0002). The interaction of exposure to elevated CO_2 and the extreme heat event, which had yielded significant reductions in biomass at 35 d post heat treatment, was no longer significant at the end of the season (Fig. 3). High- CO_2 -grown seedlings had only a marginally greater reduction in stem biomass due to exposure to the extreme heat event as compared to ambient- CO_2 -grown plants ($P = 0.0766$). Likewise, the previously significant interaction of CO_2 , species, and heat treatment was no longer significant by the end of the season.

DISCUSSION

The extreme temperature events did significantly affect seedling growth and physiology. In particular, the high-temperature treatment decreased conductance and caused the apparent death of considerable plant tissue in some individuals. After 35 d of recovery from the extreme temperature event, biomass of the heat-treated plants was significantly less than that of controls, due to a combination of tissue death and reduced growth. At the end of the growing season, after 105 d of recovery, plants that had been exposed to the heat treatment were still significantly smaller than non-heat-treated plants.

The extremely cold night had no visually apparent effect on the plants, nor on the biomass of plants measured at either 35 d or 105 d after the cold night. These species seem quite able to survive a near-freezing night in the middle of the growing season. Perhaps the short-term reduction in stomatal conductance the day following a cold night, which Drew and Bazzaz (1979, 1982) and Potvin (1985) had observed, did not affect the longer term measurements of growth after 35 and 105 d. However, by the second harvest, plants that received a cold night following the extremely hot day were marginally smaller than expected based on the response to either extreme cold or heat alone.

Damage from the heat treatment appeared more severe for plants grown in an elevated- CO_2 atmosphere, in particular striped maple, both immediately after the extreme heat event and after 35 d of recovery and regrowth (Figs. 2 and 3). The mechanisms causing the observed patterns of immediate heat-induced damage are unknown; perhaps the actual tissue temperatures during the heat event were higher in plants growing in

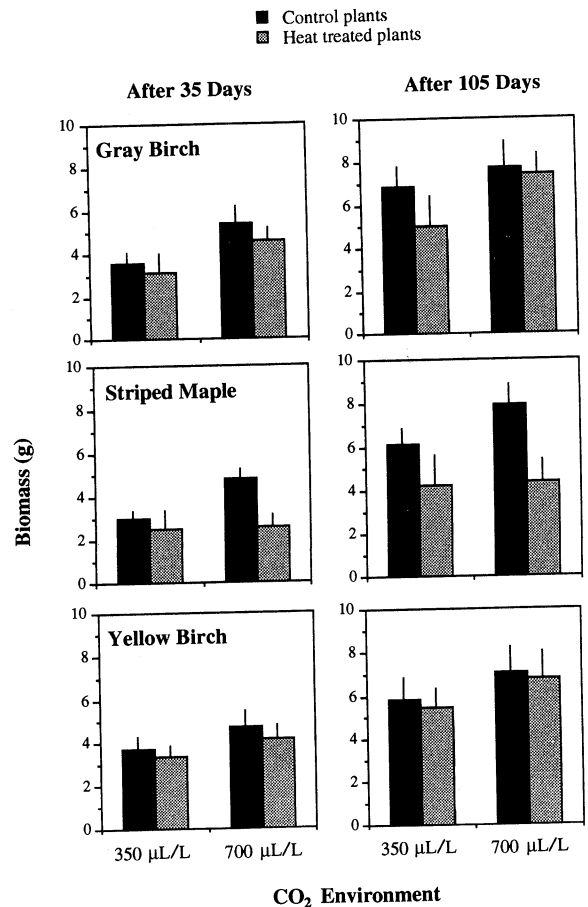


FIG. 3. Dry nonfoliar biomass (stem and root mass combined) of seedlings grown in two different CO_2 concentrations at 35 and 105 d after the extreme temperature events, focusing on the effects of the extreme heat event (comparing plants exposed to either the hot day or both extreme events to those plants exposed to only the cold night or neither extreme event). At 105 d post treatment, autumnal senescence was complete. Values are the means for both nutrient treatments combined, so the sample size for each bar is 24, unless reduced slightly by mortality. Means have been corrected for block and chamber effects. Error bars represent $+1$ SE.

elevated- CO_2 atmospheres due to a difference in transpirational cooling capacities. Control plants in elevated CO_2 had lower conductance rates than those in ambient CO_2 levels ($P = 0.0268$; $F = 5.089$) and striped maple had lower conductance rates than the two birches ($P = 0.0201$; $F = 5.629$) (Fig. 1). Though high- CO_2 -grown striped maple had the lowest conductance rates and suffered the most damage from the extremely hot day, a causal relationship cannot be concluded. Further, plants with a high ratio of conducting leaf area to active root tissue may be even more susceptible to extreme heat, because they may have a limited capacity to maintain high conductance rates for the duration of a heat event.

As the temperature rose during the extreme heat event, vapor pressure deficits must have increased,

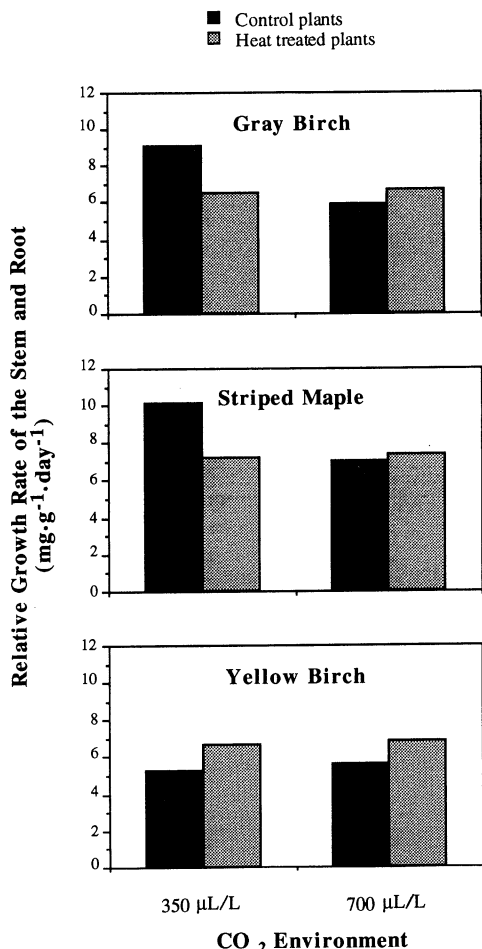


FIG. 4. Relative growth rate of the root and stem only for seedlings grown under different CO₂ concentrations calculated for the interval between 35 and 105 d after the extreme temperature events.

which alone may have affected gas exchange. However the bases of the chambers were flooded prior to the heating event, which should have minimized this difference. Even in natural ecosystems, rapidly rising temperatures would necessarily be associated with higher vapor pressure deficits.

The effects of the extreme heat event were still highly significant at the end of the growing season ($P < 0.001$ and 0.002 for root and stem mass respectively). It is impossible, however, to distinguish among the effects of species, nutrients, and CO₂ levels on the rates of recovery per se after the extreme temperature events as the immediate responses to the extreme temperatures were different among the groups of plants. With different levels of damage among the treatments, recovery began from different points. Biomass measured at the end of the growing season indicates the net response to the extreme temperatures including a 105-d recovery period.

The relative growth rate (RGR) of the stem and root biomass between the first and second harvest ($RGR =$

$\ln(\text{Biomass}_{\text{stem and root at 35 d}}) - \ln(\text{Biomass}_{\text{stem and root at 105 d}})/70 \text{ d}$; Hunt 1990) is an indication of the late-season growth potential in the various treatments. RGR was lower for the non-heat-treated plants grown in elevated CO₂ than for those grown in ambient CO₂ (Fig. 4). This is likely due to the larger sizes of the elevated-CO₂ plants observed at the 35-d harvest. The heat treatment must have damaged plant tissue, reducing the late-season RGR for gray birch and striped maple in ambient CO₂. While in spite of the comparable level of damage in elevated CO₂ (or more severe in the case of striped maple), the late-season RGR was higher for the heat-treated plants than for the non-heated plants. The elevated-CO₂ atmosphere may have partially ameliorated the damage from the extreme heat event. It is unclear why yellow birch shows a slightly different pattern.

Though the nutrient treatment had a highly significant effect on biomass at 35 and 105 d after the extreme temperature treatments, there were no highly significant interactions between nutrient treatment and other factors. There was no indication that response and recovery from the extreme temperatures was altered by the nutrient level.

The striped maple, particularly among those grown in high CO₂ levels, had the most individuals with a very high rating of damage immediately following the heat event. And further, striped maple had the highest morbidity and mortality as evidenced by the extreme-heat-induced outliers. This species is the most shade-tolerant species in this study, and so it may be least adapted to high temperatures associated with open exposed sites. By contrast, the annual species used by Coleman et al. (1991), *Abutilon theophrasti* and *Amaranthus retroflexus* in particular, may be adapted to more open, old-field environments that frequently experience intense heat, and thus the marginal effects due to the high-CO₂ environment may have been minimal. A shade-intolerant, gap-occupying species may naturally experience fluctuating extreme temperatures and consequently be less susceptible to extreme heat or cold, as contrasted with an understory, shade-tolerant species that usually experiences moderated temperature conditions. With global warming, even understory sites may experience extremely high temperatures.

The implications of the heat-induced damage in the high-CO₂-grown striped maple may be quite significant in the future, as it has been predicted that the growth enhancement due to elevated CO₂ persists for striped maple, while in most other species the enhancement declines (Bazzaz et al. 1993). So, while controlled-environment experiments may predict that the CO₂ fertilization effect would lead to a great increase in striped maple biomass, single days of extremely high temperatures could largely offset it. Further, in a future, warmer world, the frequency of such extremely high temperature events may be even higher, exacerbating their effect.

At the end of the season there was a highly significant decrease in biomass due to the extreme heat event for plants, particularly striped maple, grown in both ambient and double-ambient CO₂ environments. Further, striped maple suffered the highest morbidity rates observed in this study. Clearly, heat stress could be an important factor in controlling seedling regeneration in temperate forests. In a future world with a higher frequency of extreme temperature events, the impact of weather patterns on community composition and survival in natural ecosystems may be increasingly significant.

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