Integrating the Effects of Simultaneous Multiple Stresses on Plants Using the Simulation Model TREGRO

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ABSTRACT

TREGRO, a model of tree physiological response to environmental stress, was developed to examine the details of tree response to the interaction between ozone stress and nutrient deficiency. TREGRO was used to test the hypothesis that the nutrient deficiency would exacerbate growth reductions caused by ozone. When TREGRO was applied to a range of multiple-stress scenarios involving these two stresses, the simulated effects of the combined stresses in reducing red spruce (*Picea rubens* Sarg.) tree growth were less than the sum of the effects simulated when each stress was acting alone. Red spruce, a species with a growth strategy involving low maximum potential growth rates and storage in C reserves, showed simulated patterns of allocation that minimized the added effect of ozone when Mg nutrient availability was low. In contrast, ponderosa pine (*Pinus ponderosa* Dougl.), a species that allocated C to the construction of new tissue preferentially, showed little ability to ameliorate ozone stress under low nutrient conditions. In neither case were the effects of ozone increased by nutrient deficiency. It is speculated that a species which maintains maximum C demand rates considerably below rates of C supply may be better able to adjust to conditions of multiple stresses.

THERE is a wide body of work documenting the effects of individual stresses on plants. Little work has focused on identifying the response of plants to multiple stresses (Mooney et al., 1991). The presence of one stress can change the way a plant responds to a second stress. Complex interactions in the effects of multiple stresses are possible even with simple, independent mechanisms governing the action of each independent stress. The interactions can arise because a plant is an integrated organism. Without this integration the effect of the combined stresses could be the sum of the effect of each stress acting independently. With this integration the effect of one stress could increase the sensitivity to another stress, or the stresses could have antagonistic interactions, producing an effect that is less than the sum of those caused by each stress acting alone.

Two logical arguments can be constructed to predict whether stress caused by deficiency of essential soil resources increases or decreases the response of a tree to ozone. Low availability of nutrients such as N or Mg (referred to here as *nutrient stress*) could increase the effect of ozone on tree growth by forcing high expenditures of C for root growth or by decreasing photosynthesis. These effects would deplete the pool of C available within the plant to repair or ameliorate ozone damage.

Consider the response of a tree to a single stress, low availability of an essential nutrient. Nutrient stress will limit the amount of photosynthetic fixation, decreasing the pool of available C. Most plants have sufficient C to allocate to root growth, increasing the flow of nutrients and even-

tually ameliorating the stress. The resulting diversion of C to the roots instead of to increase leaf surface area will cause a loss of productivity. However, the eventual growth is larger than would be expected if root mass was not increased.

If, however, the nutrient stressed plant is also exposed to ozone, the available C for tissue building may be further reduced. Any stress that restricts the flow of C to developing C sinks within the plant will limit the pool of C that can be mobilized to grow tissue needed to ameliorate the stress. In this scenario it is possible that insufficient supplies of C would remain for root system expansion to significantly increase the nutrient supply and compensate for low nutrient availability. Simultaneous ozone and nutrient stress could deplete the C supply. The resultant limitation on the ability to grow tissue for increasing the supply of limiting resource could cause growth to be much lower than would be expected under either ozone or nutrient stress alone.

Alternatively, the effect of ozone could be reduced by nutrient stress if the nutrient deficiency reduces the growth potential of the tree, decreasing the demand for C. This condition would cause any depletion of the plant's C supply brought about by impairment of the photosynthetic apparatus to have negligible effect, since C supply would still exceed demand in this case. In this study a simulation model of tree physiological response to environmental stress, TREGRO (Weinstein et al., 1991, 1992; Weinstein and Beloin, 1990), was used to suggest which of these alternatives was the most likely.

This modeling investigation took place as part of the ROPIS (Response of Plants to Interacting Stresses) research program, which investigated whether combinations of stresses would have effects on plants that could not have been predicted based on responses to single stresses. While the results of multiple stresses on tree growth and C allocation could be identified through experimental analysis (see associated ROPIS papers, this issue), these experiments were limited in their ability to suggest the precise causes for the differences between single and multiple stress responses. Modeling can contribute to answering this question because of its capability to examine the daily progress of stress response and to identify the limits of growth of each plant tissue each day during the growing season. TREGRO was developed under the ROPIS program in collaboration with experimental approaches to assess the effects of simultaneous multiple stresses on plants. The model contains quantitative descriptions of the physiology and stress response of the plant–soil system.

Models are a means of representing what is known about a system. In complex systems such as plants, there may be no better way to represent current scientific understanding and theory than though building simulation models. In the particular case of simultaneous environmental stresses, only a mechanistic simulation model could per-

mit propagation of the effects of two different stresses according to what we know about their individual actions to test whether the observed effects could be explained without invoking additional mechanisms of stress interactions.

More importantly in this case, the TREGRO model was used to test a hypothesis concerning the way that individual stresses manifest and build in their effects within a tree. The model is a codification of a set of hypotheses defining our concept of the mechanisms of plant response to stress. The behavior produced by this model is a consequence of the form of representations of processes contained within the model. Choice of an alternative representation about the hourly and daily effect of stresses within a tree would produce different behavior. Therefore, with TREGRO the consequences of a given representation could be identified.

In addition, TREGRO was used to test the hypothesis that differences in growth strategies among species lead to different responses to multiple stresses. It is possible that different species of plants differ in their response to multiple stresses. Differences were observed in the ROPIS experiments (see other articles in this volume). The relationship of C supply to C demand may determine which type of stress interaction will prevail. A species such as red spruce, with a relatively conservative growth strategy and a surplus of C supply over demand under normal growth conditions, is likely to show less effect from ozone under nutrient stress conditions. In such a species the loss of growth potential would place such a low demand on the C supply that any reduction in photosynthesis caused by ozone would have little effect.

However, a species such as ponderosa pine uses a vigorous growth strategy in which newly fixed C is allocated to expand the tree tissues. This species would maintain C demand at nearly the level of supply, leaving little surplus of C supply over demand and little for storage reserves during the growing season. This reserve could be easily used up if the supply of new fixed C is diminished by either ozone or nutrient deficiency, leaving the tree unable to manufacture new tissue to compensate for the stress. In this species, therefore, we would expect one stress to increase the sensitivity to a second stress. To determine whether the relationship of C supply to demand could be used to predict the pattern of response to multiple stress in different species, the simulated effect of stress on red spruce and ponderosa pine were compared.

MATERIALS AND METHODS

TREGRO is a model of response of the C and nutrient acquisition and allocation dynamics of a single plant (Weinstein et al., 1991, 1992; Weinstein and Beloin, 1990). A simplification of the key aspects of these responses contained in the model are shown in Fig. 1. Details of TREGRO model equations can be found in Weinstein et al. (1992). The model simulates the rate of C fixation and the rate of C accumulation in reserve supply in the tree. The plant is divided into a number of tissue compartments: leaves (separated into classes by age), branches, stem, coarse roots (divided by soil horizon) and fine roots (divided by soil horizon). In these compartments, C may be stored as living tissue, wood, or nonstructural carbon (TNC). The demands for C by leaf, branch, stem, and root growth are calculated, with different C sinks getting different proportions of their demand
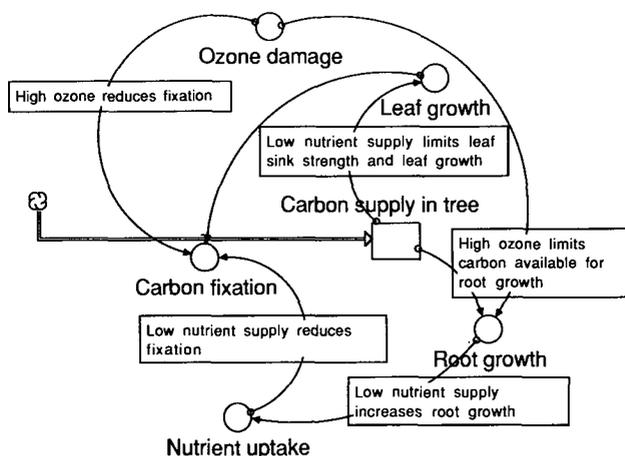
Fig. 1. Schematic of the major processes simulated in TREGRO to predict responses to high ozone levels combined with nutrient deficiencies.

met based on the phenology of growth through the year and the availability of other essential resources.

High levels of ozone or low levels of nutrient supply will reduce the fixation of C. The model then estimates the change in the pattern of C allocation caused by this reduction in C source and the effect the stress has on each of the sinks. Low nutrient supply will limit leaf sink strength and leaf growth in the model, but will increase the root sink strength. High ozone will limit the supply of C available for root growth. Since photosynthesis, ozone damage, C partitioning, and nutrient uptake are the major plant processes affected by ozone exposure and nutrient deficiency, the details of the simulation of these processes are presented below.

Photosynthesis is modeled as two conductances of CO_2 flow into the leaf at the stomate (stomatal conductance) and at the cell membrane (mesophyll conductance) in series and is proportional to the gradient of CO_2 from the atmosphere to the mesophyll cells (Lohammer et al., 1980; Running, 1984).

$$\text{Photosynthesis} = \Delta\ CO_2\ [(k_s k_m)/(k_s + k_m)] \qquad [1]$$

where $\Delta\ CO_2$ is the gradient from atmosphere to carboxylation site (kg CO_2 m^{-3}), k_s is the stomatal conductance (cm s^{-1}), and k_m is the mesophyll conductance to CO_2 (cm s^{-1}). The time step for photosynthesis is hourly.

The calculated stomatal conductance of a leaf class during each hour depends on the maximum stomatal conductance possible under optimal growing conditions, the soil water availability, and the water vapor pressure deficit (VPD). Mesophyll conductance is a function of light, temperature, N, and Mg concentrations, the age of the foliage, and cumulative ozone dose. Light energy depends on the position of the foliage in the canopy (two positions, open sun and shade) for each age class of foliage. All leaves within a given age class and position are assumed to receive the same light level. The total photosynthesis in the canopy is the sum the photosynthesis of each of these groups of foliage.

Cumulative ozone uptake (g) was calculated on an hourly basis as a function of stomatal conductance.

$$\Sigma\ O_3 = \frac{(\text{ozone} - \text{threshold})\ (g_s)}{\text{leaf\_area}}\ 4.2 \times 10^{-5} \qquad [2]$$

where $\Sigma\ O_3$ is the cumulative ozone uptake (g), ozone is the ambient hourly ozone level (μg L^{-1}), threshold is the accumulation ozone necessary to begin plant injury (μg L^{-1}), g_s is the average stomatal conductance to water vapor for a given hour (cm s^{-1}), leaf_area is the leaf area for this particular leaf class

(m^2), and 4.2×10^{-5} is a constant used to convert from $\mu g \, L^{-1}$ to $g \, O_3 \, h^{-1} \, m^{-2}$.

Ozone was assumed to have a cumulative effect on photosynthesis and an instantaneous effect on respiration (Pell, 1987). The first effect is the increase of maintenance respiration (Amthor and Cumming, 1988), presumably to detoxify ozone (Coyne and Bingham, 1981). In the second effect photosynthesis was reduced in each leaf class by a reduction of mesophyll conductance (Reich et al., 1985; Sasek and Richardson, 1989) after the cumulative ozone exposure of the leaf class exceeded a threshold of 63 000 mg L^{-1} h. Based on observations made during the experimental exposure of red spruce trees to ozone, mesophyll conductance was reduced continuously at a rate of 1% for each additional 3600 mg L^{-1} h of exposure.

$$g_{mO_3} = g_m - O_3\text{slope} \, (\Sigma \, O_3 - O_3\text{threshold}) \quad [3]$$

where g_{mO_3} is the altered mesophyll conductance caused by ozone exposure (cm s^{-1}), g_m is the average mesophyll conductance for a given hour (cm s^{-1}), O_3slope is the slope of the response to cumulative ozone uptake (cm s^{-1} g O_3^{-1}), $\Sigma \, O_3$ is the cumulative ozone uptake (g O_3), and O_3threshold is the threshold of cumulative ozone uptake below which ozone does not affect mesophyll conductance (g O_3). Similarly, for respiration:

$$R_{\text{leaf}O_3} = R_{\text{leaf}} + O_3\text{slope}_R \, (O_3 - O_3\text{threshold}_R) \quad [4]$$

where $R_{\text{leaf}O_3}$ is the altered respiration rate of leaf tissue caused by ozone exposure (g h^{-1}), O_3slope$_R$ is the slope of the response to hourly ozone [g h^{-1} ($\mu g \, O_3 \, L^{-1}$) $^{-1}$], O_3 is the hourly ozone atmospheric concentration ($\mu g \, O_3 \, L^{-1}$), and O_3threshold$_R$ is the threshold of hourly ozone atmospheric concentration below which ozone does not affect respiration ($\mu g \, O_3 \, L^{-1}$). The effect of ozone on respiration each hour is assumed to be independent from any other hour (all damage is repaired by the end of the hour), and so $R_{\text{leaf}O_3}$ is reset to R_{leaf} at the beginning of each hour.

Based on observations made during the experimental exposure of red spruce trees to ozone, leaf respiration was increased each hour by 0.5% per $\mu g \, L^{-1}$ ozone concentration above a threshold of 40 $\mu g \, L^{-1}$. Although detoxification of ozone is not explicitly modeled, it was assumed to occur through the elevation of respiration rates, which can return to normal levels once ozone concentrations fall below 40 $\mu g \, L^{-1}$. There is an indirect effect in the model of ozone exposure on growth partitioning, due only to the rules of C allocation and growth, explained below.

In order to use C to build structural tissue within the plant, the model assumes a given amount of each nutrient for each gram of tissue built must be available. Further, photosynthesis is affected by deficiency of N or Mg. Nutrient is supplied by daily uptake and from internal storage pools. The time step for nutrient uptake, C allocation, and plant growth is daily.

Nutrient uptake was calculated with a modified version (R.D. Yanai, unpublished) of the Nye and Tinker model (Baldwin et al., 1973; Nye and Tinker, 1977). This model is suitable to calculating nutrient uptake by long-lived plants because it considers the effect of daily root growth and mortality on uptake. In the model, established roots are assumed to have depleted nutrient supplies in the rhizosphere. The modification to the Nye and Tinker model corrects for the uptake by newly grown roots not yet in steady state with the soil. Plant uptake (mol $cm^{-2} \, h^{-1}$) is the sum of contributions by old (U_{old}) and new (U_{new}) roots:

$$\text{Uptake} = U_{\text{old}} + U_{\text{new}} \quad [5]$$

Solute uptake by established roots, U_{old} (mol $cm^{-2} \, h^{-1}$), is the product of root surface area, solute concentration at the root surface, the root absorbing power and the time interval:

$$U_{\text{old}} = L \, 2\pi r_o \, \alpha \, C_o \, \Delta t \quad [6]$$

where L is the root length (cm), r_o is the radius of the root (cm), α is the root absorbing power (cm s^{-1}), C_o is the concentration of substance at the root surface (mol cm^{-3}), and Δt is the model time step (h). The contribution to solute uptake by new root growth, U_{new} (mol $cm^{-2} \, h^{-1}$), is calculated as the amount of solute mined from depletion zone as this zone attains the steady state:

$$U_{\text{new}} = A \, \Delta L \quad [7]$$

where ΔL is the root growth since the last iteration (cm h^{-1}) and A is the amount of solute removed from the depletion zone per unit length of root (mol cm^{-1}). Parameters required in the calculation of nutrient uptake are root radius and uptake kinetics, the diffusivity of the solute, the concentration of solute in solution and in exchangeable form, and the rate of water movement to the root surface. In this implementation of the model we set water uptake to a constant rate. The rate of water uptake affects nutrient uptake since bulk flow of solution delivers solute to the surface of the root. The interroot distance, active root length, and new root growth are calculated daily by the model and used in the simulation of solute uptake.

TREGRO contains no explicit control of C partitioning as a function of environmental stress. Instead, C and nutrients are allocated to the various growing tissues according to priorities of tissue type that represent the relative strength of the sink at different times of the year (Table 1). Aboveground portions of the plant have first access to newly fixed C, while belowground portions have first access to nutrients for growth. Tissues can grow at phenologically regulated maximum rates only if carbon and nutrients are abundant. Insufficient C or nutrients limit growth.

It is important to note that under this allocation calculation a given tissue with a low priority and large sink size can receive most of the C being allocated if all the tissues with higher priorities have a lower sink size and receive all the C they demand. In addition, a set of priorities does not mean that only one tissue can be allocated C on a given day. If the higher priority tissues receive all the C they demand, the lower priority tissues will

Table 1. Sink priorities for allocation of newly fixed photosynthate.†

Growth period	Allocation to existing tissues			Allocation to new tissues‡					
	Respiration	Leaf TNC	Other TNC	Buds	Leaf	Branch	Stem	Coarse root	Fine root
Pregrowth	1	2	3	−§	—	—	—	—	—
1	1	2	5	3	—	—	—	—	4
2	1	2	6	—	3	4	—	—	5
3	1	2	7	—	—	3	4	5	6
4	1	2	4	—	—	—	—	—	3

† Numbers represent the order in which each sink will receive newly fixed photosynthate. A sink with a lower number will receive photosynthate before one with a higher number. After the needs of a sink are fully satisfied and if newly fixed photosynthate remains, C will be given to fill the needs of the next highest priority sink. If the rate at which high priority sinks use photosynthate is low, more C may actually be given to low priority tissues during a time step.
‡ Allocation to new tissues for growth includes C losses due to associated growth respiration.
§ Indicates growth periods in which the respective tissue is not permitted to grow.

receive portions of the remaining C being allocated that day (allocation in the model occurs only once a day).

This simple scheme is capable of reproducing changes in shoot/root ratio observed under ozone stress and nutrient stress (Weinstein et al., 1991). Ozone is often observed to cause a decrease in root growth, while nutrient deficiency causes root growth to increase relative to growth of shoots (Anderson et al., 1991; Oshima et al., 1979). In the model, under ozone stress growth of below ground tissues is limited by carbon availability so that more growth occurs aboveground than belowground, while under nutrient stress growth of aboveground tissues is limited by nutrient availability so that belowground tissues grow at a faster rate.

We selected Mg as the limiting nutrient for simulating nutrient deficiency because this nutrient was observed to decline with acidity of precipitation (Sherman and Fahey, 1990) and because it has feedbacks on photosynthesis as well as growth. For each simulation, we used a constant soil solution concentration of Mg to define nutrient availability in the soil. This method ignores feedback between plant uptake of nutrients and soil nutrient availability, which would have complicated interpretation. The model can also be run with this feedback enabled, with the soil model calculating the nutrient concentration of the soil solution at each time step.

Parameter values for red spruce in TREGRO (Weinstein et al., 1992) were set to represent one of the control trees in the ROPIS experiment, selected because its height and leaf mass were average among the population. The biomass of the various tissue compartments was known for the time of harvest, at the end of 1988 (Laurence et al., 1989; Kohut et al., 1988). Since the mass of tissues at the beginning of the experiment, two growing seasons earlier, was unknown (a destructive harvest would have left the experiment without a tree), they were estimated. The estimates were made using known masses of the leaf classes at the end of 1988, assuming no leaf shedding had occurred since the season the leaves were produced, and using growth rates calculated from radial growth of the stem. Photosynthetic parameters were known from measurements (Amundson et al., 1990, 1991). Respiration rates were assumed to be the same function of temperature and tissue mass for all tissues: 0.25% of mass per day at 20 °C, with a Q_{10} of 2 (Kozlowski et al., 1991; Waring and Schlesinger, 1985). Data from an experimental exposure of ponderosa pine was used to set the photosynthetic and respiration rates and to calibrate the maximum C allocation rates, as explained below, for construction of a ponderosa pine parameter file (J. Weber, 1993, personal communication). The initial tree size for the ponderosa pine simulation was adjusted so that the simulated tree would initially be comparable in size to the red spruce tree. The same weather data was used for simulations of both trees to minimize the influence of differences in the pattern or magnitude of light, temperature, humidity, and ozone.

Maximum potential C allocation rates were adjusted until the simulation approximated the observed masses of tissues after 3 yr of tree growth were simulated. Only these maximum potential allocation rates were manipulated for the calibration. Since it is nearly impossible to measure maximum potential allocation rates (these rates may be achieved on only 1 d of the growing season), it was not inappropriate to calibrate the model behavior by adjusting these rates. This calibration did not impair the capability of the model to be used to examine the most likely pattern of multiple stress response or the relationship between this response and the C supply and demand.

Predictions of the red spruce tissue C contents during the baseline simulation without ozone are shown in Fig. 2, including the detail of seasonal patterns in growth and C mobilization. Each year a large quantity of C accumulated in the branches prior to leaf bud break. This C was rapidly mobilized to create a new

leaf class in the late spring. At this point C reserves were at their lowest point, as seen in a drop in biomass in branches and coarse roots, the principle storage organs. Following leaf expansion, C was used to build branch and stem structure. Only when this growth slowed in the simulation was there sufficient C to support fine root growth. As a consequence, fine root growth was predicted to occur immediately after leaf break, and the duration of growth was short. It reoccurred only after branch and stem growth had ceased for the year. Deprived of C for growth during leaf growth, normal senescence caused fine root mass to decline. Coarse root growth was predicted to occur gradually through the later portion of the year, but it, too, was suppressed during the growth of branches and stems. As more and more leaves were built each year the amount of C that was fixed and built into structure increased exponentially through the 3 yr.

Data from the field tree harvested and measured in 1988 and the estimated biomass for 1987 were available for comparison with simulated biomass (indicated as the actual growth rates in Fig. 2). The estimated biomass for 1986 was used as the starting point for the simulation. TREGRO was able to simulate the measured total tree biomass at the end of 1988 within 3.5% for each tissue. Since we used the field data on the ozone effect on photosynthesis to calibrate the ozone response relationship in the model the comparison between the simulated and actual effect of ozone on growth does not provide any insight into the validity of the model. It does, however, demonstrate that the model produces behavior consistent with the data on which it is based. Because the ROPIS experiment made no attempt to control nutrient supply to the plant as it is controlled in the simulation, we cannot compare these simulations under nutrient stress with the results of the experiment.

RESULTS

Simulated Effects of Multiple Stress on Red Spruce Growth and Carbon Allocation.

To test the interaction of stresses in a red spruce tree as represented in TREGRO, five concentrations of Mg in soil solution and five scenarios of ozone concentrations were used. To simulate nutrient stress, the minimum Mg concentration that produced no nutrient limitation to growth was found (5×10^{-8} mol cm^{-3}), and that concentration was reduced by 40, 80, 84, and 88% to produce increasing Mg deficiency. To simulate ozone exposures, measured hourly ambient ozone concentrations from the field site in Ithaca, NY, were used, with these hourly concentrations multiplied by factors of 0, 1, 1.5, 2, or 2.5. The "base case" tree is that with the highest Mg concentration and zero ozone exposure. All combinations of the two stresses were simulated for 3 yr of growth starting from the initial conditions of 1986.

Both ozone and nutrient stress reduced tree growth. Figure 3 arrays the total biomass of the 25 simulated trees along two stress axes. The stress scenarios used in this study differed in the magnitude of growth effects. The most severe nutrient stress resulted in a tree that was 56% of the size of the unstressed tree; the most severe ozone stress resulted in a tree that was 80% as large as the unstressed tree. The combined stresses resulted in growth reductions that were less than additive. For example, under the most severe stresses (6×10^{-9} mol cm^{-3} Mg and 2.5 times ambient ozone), the combined effect was a tree with 49% of the biomass of the unstressed tree. Additive reductions would have predicted a tree with 36% of the unstressed tree (56% remaining after the nutrient effect, minus 20%
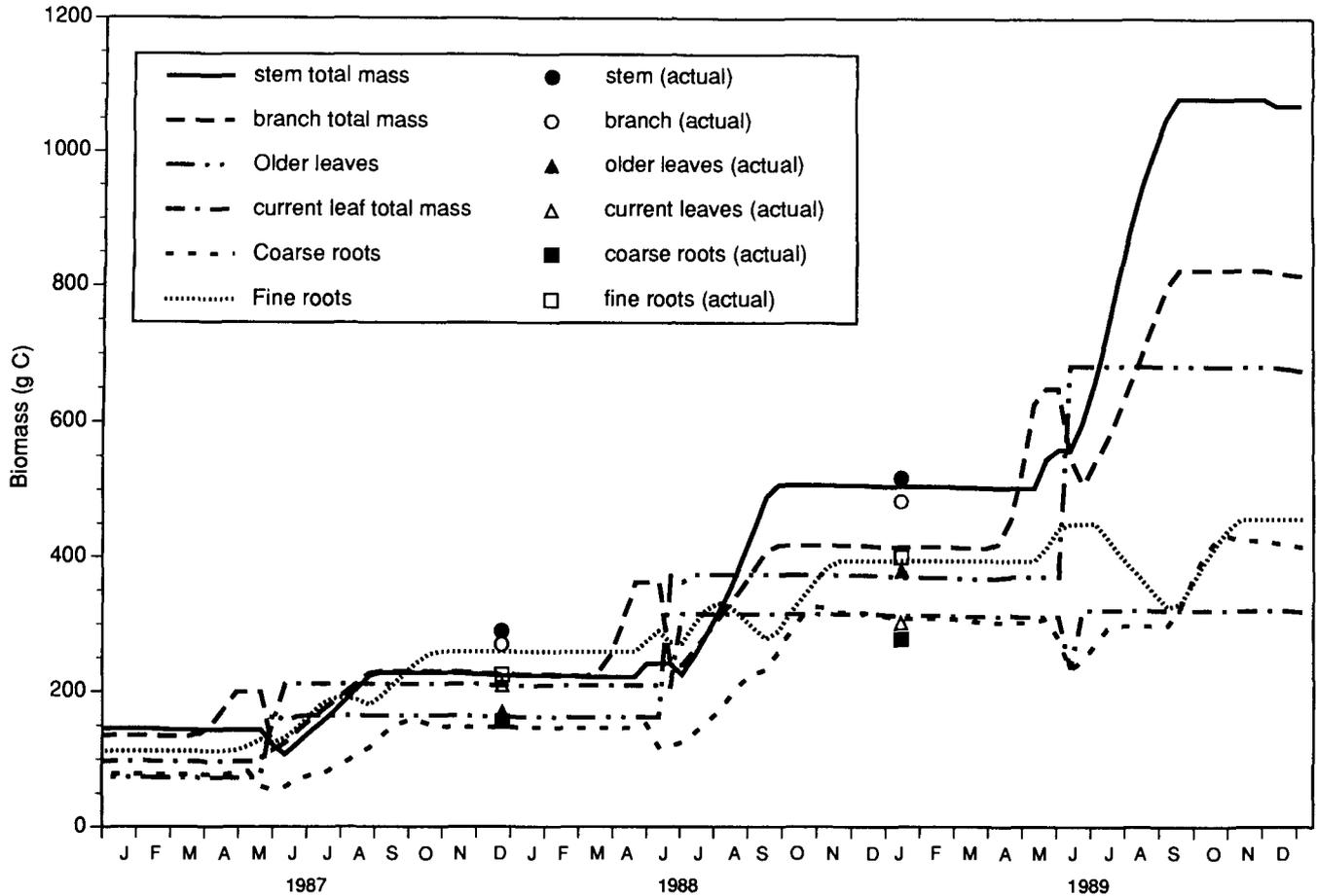
Fig. 2. Comparison of simulated growth of six major tissue types of a red spruce tree over 3 yr using TREGRO and the actual measured biomass of six tree components.

of the original mass after the ozone effect). If the stresses interacted in multiplicative reductions, a tree 45% as large as the unstressed one would be expected to result (80% of a tree already reduced to 56%). It is impossible, of course, to determine whether these are real differences with-

Fig. 3. Total biomass of a red spruce tree at the end of 3 yrs of simulation under different combinations of ozone and nutrient stress.

out error estimates made through a model error analysis. However, as a logical extension of our present understanding of the processes involved in stress response, the model suggested that stresses interact in neither an additive nor a multiplicative fashion.

The stress interaction produced a complex set of effects in the simulations. Ozone exposure decreased the total C supply to the plant (25% less net allocatable C in the highest ozone treatment) (Fig. 4). This resulted in less available C with which to build new tissue, such as fine root structure. For example, the tree with the highest ozone treatment constructed 53% less fine root material. This result was expected since belowground tissues in TREGRO have a lower priority for newly fixed C than aboveground tissues. Since nutrient uptake rates in the model are proportional to the new fine root growth added daily, less new fine root growth and less nutrient uptake should have occurred with ozone exposure. Consequently, ozone exposure would be expected to have increased the impact of nutrient stress. In fact, however, the simulated tree in the lowest nutrient treatment grew only 7% slower with the highest level of ozone even though in the highest nutrient treatment ozone caused the tree to grow 25% slower.

Despite a 49% decrease in net allocatable C in the most nutrient limited simulation, fine root growth was reduced

Fig. 4. The effect of ozone and Mg concentration on the allocatable C, biomass, growth, and fine root growth of a red spruce tree after 3 simulation years, expressed as a percentage of the growth achieved in the simulation of an unstressed tree (0 times ambient ozone and 5×10^{-8} mol cm^{-3} Mg).

only 29%, reflecting the high priority of fine roots for C under nutrient limitation in the model. This shift of C away from leaf construction would have been expected to exacerbate the effect of ozone by limiting the carbon available to rebuild leaf tissue and photosynthetic capacity. However, in the lowest nutrient treatment there was only 6% less net allocatable C under high ozone than without ozone. Because of the high priority of aboveground tissues for newly fixed C, nutrient stress may not have increased the impact of ozone, provided nutrient supply remained sufficient to continue the production of new leaves. New leaves take some time to accumulate ozone damage, so that the production of new leaves tends to return the photosynthetic capacity of the plant to its previous levels. Therefore, it is not surprising that the simulated multiple stress effects were not found to follow the simple linear relationships of the individual stress effects.

Thresholds could have played a major part in determining whether the additional impact of a second stress was negligible or instead led to an exponential increase in damage. To produce the latter, ozone had to accumulate in each leaf class to a sufficient level to depress mesophyll conductance or hourly values had to be high enough to increase respiration. Nutrient uptake had to be low enough to restrict leaf growth. On the other hand, another set of thresholds existed where, over longer time periods, the trees could have adjusted through altered growth patterns to lessen continued injury. Leaves with a large accumulation of ozone damage could have been dropped and replaced by a new leaf class with no accumulated ozone exposure. Older leaves with less nutrient per gram of tissue and therefore less efficient photosynthetic rates could have been dropped and replaced by new leaves with higher nutrient concentrations and therefore more efficient nutrient usage and maintenance of photosynthetic potential. Ozone levels did not accumulate so long and nutrient availability did not drop so low so as to cause either of these phenomena to occur in the simulations. Nevertheless, the effects of a second stress were not as large as expected from the sum of the two stresses acting independently.

The cost of a stress in terms of reduced biomass accumulation is not constant with increasing stress nor with vary-

ing severity of a co-occurring stress. The cost of a stress, or the amount of additional reduction in biomass accumulation per unit increase in stress, varies with initial level of stress. This cost can be judged from Fig. 5. If the cost of each increment of stress were constant, the responses of biomass to increasing stress would be linear with both ozone and nutrient stresses. In fact, neither stress gave a perfectly linear response at any level of co-occurring stress. The additional damage caused by incremental nutrient stress was greatest in the trees with the least ozone exposure. This is another manifestation of the less-than-additive effects of the stresses on growth. The plant may have had only so much C to lose; additional stress could not reduce growth indefinitely.

The total 3-yr allocation of C to respiration, senescence, TNC, and growth of leaves, fine roots, and other tissue structure (stem, branch, and coarse roots) under each combination of stresses is shown in the stacked bars of Fig. 5. Respiration and senescence changed relatively little with increasing stress, despite the fact that gross photosynthesis (the total height of the bars) changed dramatically. As a result, respiration and senescence made up a greater fraction of C use in the more severe stress scenarios.

Stored nonstructural C plays a role as a buffer against stress damage; this C used up under stress conditions and makes the plant more susceptible to additional stress because damaged tissue or tissue whose construction has been delayed cannot be quickly replaced or manufactured. Clearly, both stresses reduced this pool significantly. Since plant growth must have been limited by lack of TNC in the more stressed trees, we can speculate that recovery and, consequently, total biomass levels achieved were regulated by this lack of TNC. It remains uncertain whether trees with large storage capacities and pools of TNC would be expected to show less loss of biomass under stress.

Fine root mass was more constant under nutrient stress than any other compartment; fine roots in TREGRO have first priority on using nutrients for growth, and therefore suffered the least growth reduction. Ozone stress, however, caused a reduction in fine root mass at all levels of nutrient stress. The root/shoot ratio is shown in Fig. 6. While increasing levels of ozone stress caused a decrease in the
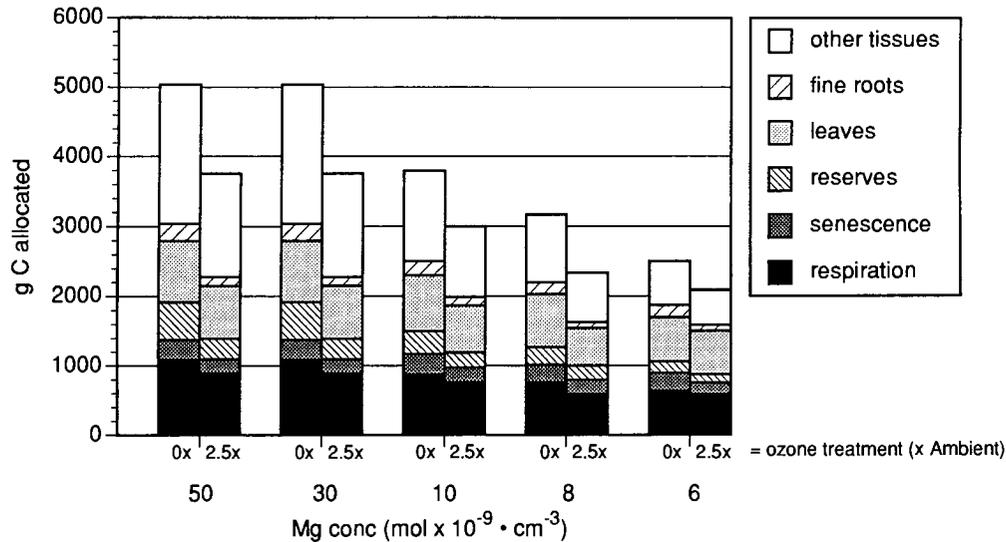
Fig. 5. Comparison of the simulated allocation in different tissues of two red spruce trees, one with no ozone exposure and one exposed to 2.5 × ambient ozone. The first stack in each pair at a given Mg concentration is 0.0 ozone, the second stack is 2.5 × ambient ozone.

ratio of root structure to shoot structure at all concentrations of soil Mg, the ratio did not always increase with decreases in soil Mg, as was expected. The pattern of change in shoot/root ratio with decreases in soil Mg was consistent across ozone concentrations, with a peculiar increase and recovery occurring in the intermediate nutrient stress levels. With ozone stress pushing the plant toward an decrease in this ratio (through the C starvation of the root system) and nutrient stress pushing toward an increase (through nutrient starvation of the aboveground portions), it is clear that the absolute levels of each stress played a major role in determining which pathways of response would be followed. The lower ratio developed under intermediate soil Mg treatments must be attributable to the specifics of C and nutrient allocation open to the plant under each of these scenarios, specifics that we could not have predicted because of the complexity of the allocation processes.
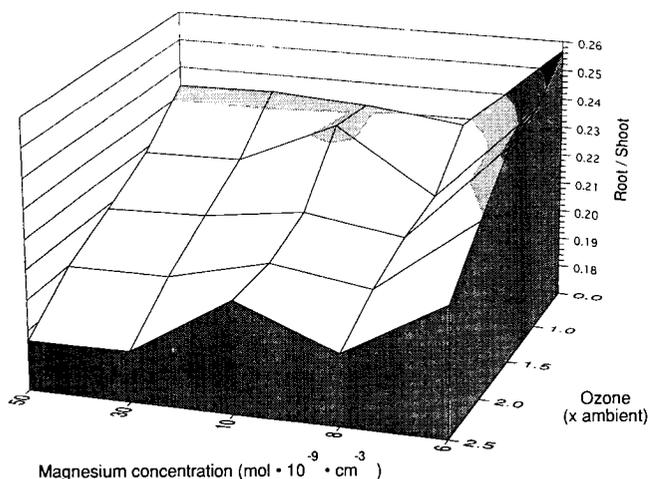
Fig. 6. Ratio of root structure to shoot structure at the end of 3 yr of simulation under different combinations of ozone and nutrient stress.

Simulated Effects of Growth Strategy on Multiple Stress Response. A ponderosa pine tree of similar size was simulated under varying treatments of ozone and nutrient deficiency to examine the effect of growth strategy on the response to multiple stresses (Fig. 7). Higher maximum potential growth rates in the TREGRO ponderosa pine parameter set caused this species to grow new tissue preferentially during the simulation when C was available instead of storing this C in reserves, as occurred more frequently in the red spruce simulation in the lowest stress treatments. A comparison with Fig. 2 shows that much of the growth in the simulated ponderosa pine occurred in leaf and fine root development, while the simulated red spruce grew mostly in added stem increment. The model mimics the observed growth strategy behavior of these two species in which ponderosa pine adds resource gathering structure as quickly as possible and red spruce adds storage material first while slowing increasing leaf and root mass. The tendency of the simulated ponderosa pine to use C to grow leaves and fine roots led it to show no increase in coarse root material over the 3 yr (although it did begin the simulation with a much larger coarse root mass than red spruce).

In Fig. 8 the differences in allocation patterns between the two species during the 3 yr of simulated growth are directly compared. Ponderosa pine allocated more C to leaves and almost twice the amount to roots than red spruce. In contrast, red spruce used almost twice the C for building storage and structural organs, such as branches, stems, and coarse roots, summarized in this figure as *other tissues.* Storage organs were constructed at the additional cost of higher maintenance respiration, and caused the total respiration expenditure of red spruce to be significantly higher than respiration of ponderosa pine. Senescence losses were also higher for red spruce. The relative values shown for the two species' reserves in this figure are deceptive. They represent the net amount that the original storage pool of C was increased during the simulations. Although
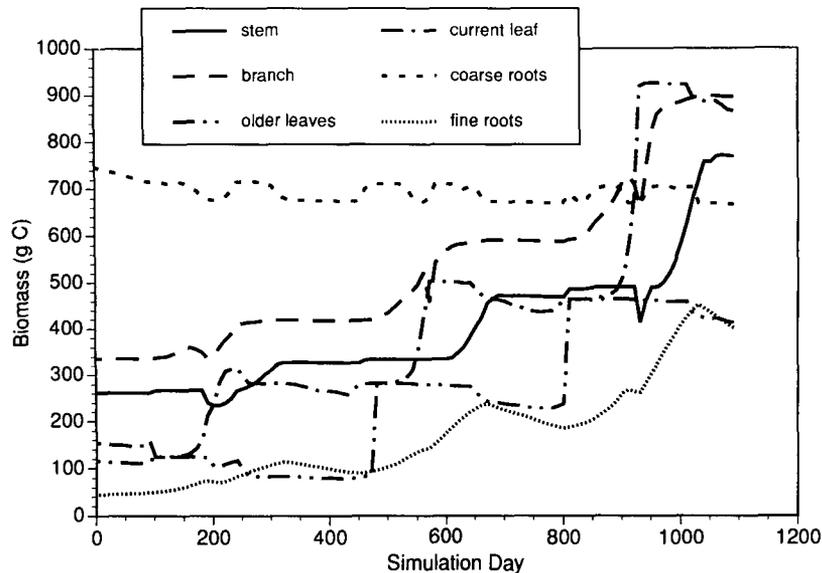
Fig. 7. The simulated growth of six major tissue types of a ponderosa pine tree over 3 yr using TREGRO.

red spruce did not increase this pool as much as ponderosa pine, it maintained a higher pool throughout the simulation, allocating more to storage than did ponderosa pine. The relative total allocation to storage between the two species is shown in Fig. 8.

The difference in growth strategy produced a difference in response to the multiple stresses. The simulated ponderosa pine tree was more sensitive to ozone and less sensitive to nutrient deficiencies, so the amounts of ozone and Mg concentrations used in the simulated treatments had to be decreased to produce effects of similar magnitude as those in the red spruce response. For the purposes of
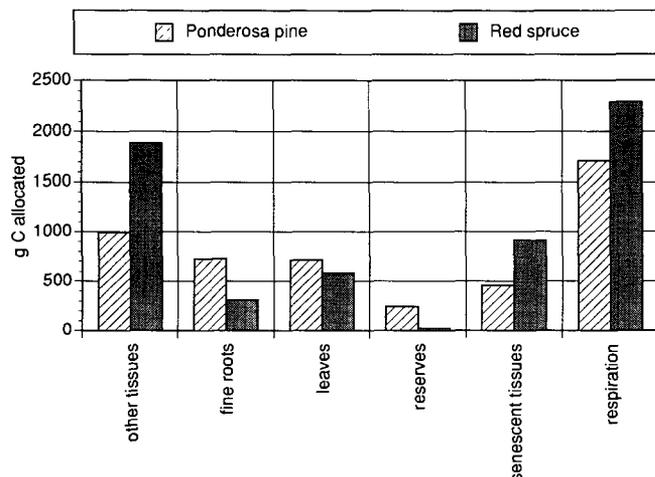
Fig. 8. Comparison of the simulated patterns of C accumulation in six C flow pathways in an unstressed ponderosa pine tree and an unstressed red spruce tree. The C values for other tissues, fine roots, leaves, and reserves were calculated as the difference between the final mass after 3 yr and the initial mass. More C than that shown here may have been allocated to produce these final masses. The C values for senescent tissues and for respiration are accumulated totals over the 3 yr.

this investigation it was more important that the amount of injury each stress induced in each species was comparable than their relative response to the same level of ozone or Mg. With equivalent levels of growth reduction the differences in the pattern of allocation response to the stresses causing that growth reduction could be examined. We were interested in whether the growth strategy altered the influence of a given level of stress on the tree's sensitivity to each increment of a second stress.

Figure 9 shows the response of ponderosa pine to higher ozone levels and to Mg deficiency. As in the red spruce responses, the ponderosa pine tree showed large growth decreases in response to the lowest Mg treatment, and smaller but substantial growth decreases in the high ozone treatment (only the highest ozone level is shown here for simplicity). Unlike red spruce, however, the response of ponderosa pine to ozone did not decrease substantially in the lower Mg level treatments. As in the red spruce simulations fine root allocation was maintained, perhaps as the basic pattern of amelioration of low nutrient availability.

Both species greatly reduced their allocation to other tissues, these being the lowest priority sinks and therefore the sinks most likely to be deprived of C when supplies are low. However, in ponderosa pine the allocation to leaves declined dramatically as both stresses increased, and allocation to storage reserves became negligible. Red spruce, on the other hand, was able to maintain the flow of C for leaf construction, possibly because its reserves were sufficient to supplement photosynthesis in meeting the modest demand for leaf growth, where as ponderosa pine maintained little reserves and had a high demand.

Another difference in response was the lack of change in fine root in ponderosa pine in the ozone treatments. Red spruce showed marked reductions in allocation to fine roots in high ozone treatments at all nutrient levels. Ponderosa pine showed no change in C passed to fine roots. It is possible that ponderosa pine was much more responsive to
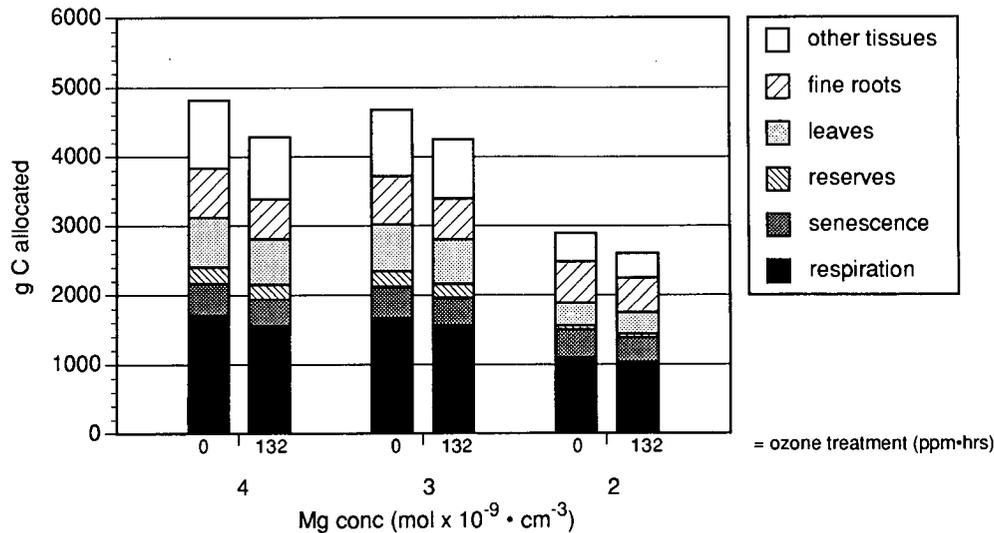
Fig. 9. Comparison of the simulated allocation in different tissues of two trees, one with no ozone exposure and one exposed to 132 μg L^{-1} (ppm) × h of ozone. The first stack in each pair at a given Mg concentration is 0.0 ozone, the second stack is 132 μg L^{-1} (ppm) × h of ozone.

low nutrient levels in terms of its ability to maintain fine root growth and uptake. However, this responsiveness cost the pine tree significantly with respect to its ability to maintain leaf mass. Possibly a species must sacrifice one resource gathering organ or another, and even the greater storage reserves of red spruce did not prevent it from losing fine root mass and uptake capacity.

Figure 10 directly compares the patterns of allocation for each of these species in the most severe treatments. Though red spruce still had a tendency for greater development of its storage tissues (other tissues), it had far less

Fig. 10. Comparison of the patterns of C accumulation in six C flow pathways in a ponderosa pine tree and a red spruce tree, each simulated under the highest respective treatments of ozone and lowest respective treatment of Mg. The C values for other tissues, fine roots, leaves, and reserves were calculated as the difference between the final mass after 3 yr and the initial mass. More C than that shown here may have been allocated to produce these final masses. The C values for senescent tissues and for respiration are accumulated totals over the 3 yr.

allocation to fine roots and far more to leaves. Spruce was able to maintain a higher flow of C into reserves, perhaps because much more of the C fixed by ponderosa pine was expended in senescent tissues and in respiration.

DISCUSSION

Comparative simulations of red spruce and ponderosa pine suggested two very different strategies in face of multiple stresses. Red spruce, the species with lower maximum potential growth rates and tendencies to build C reserves, maintained leaf mass, reducing the impact of ozone. This occurred at the cost of construction of root tissue and the potential exacerbation of the nutrient deficiency. Ponderosa pine, the fast growing species tending toward rapid utilization of fixed C into construction of resource gathering organs, continued production of fine root mass, minimizing the impact of the nutrient deficiency. However, this development pattern occurred at the continued cost of leaf tissue and the enhancement of the influence of ozone on growth reduction. Since ponderosa pine fared worse in terms of experiencing large reductions from both low Mg levels and from high ozone exposures in the multiple stress simulation, one might conclude that the allocation pattern the pine tree demonstrated was not an effective response. Red spruce was more effective in reducing the impact of the multiple stresses by maintaining leaf tissue mass at the cost of reserves and root mass. However, in the simulations of neither species were large growth decreases avoided by the patterns of allocation.

It is a more stable strategy in the face of multiple co-occurring stresses to maintain growth rates significantly below demand, permitting C reserves to be sequestered (Fig. 11). A tree with a growth strategy of moderate maximum potential growth rates will easily saturate the C demanded by those rates and have high rates of accumulation of C in storage reserves. Low growth rates may limit the amount of C that will be allocated to new root growth, even under conditions of nutrient deficiency. This will cer-

Growth Stategy:
High rates of storage in reserves.

Ozone damage

Leaf growth

| Old leaves damaged by ozone are replaced by new leaves. | New leaves grown despite low nutrient supplies instead of roots. | Carbon reserves are used to replace injured leaves. |

Large storage pool

Carbon fixation   Tree growth

| Nutrient uptake is low and fixation is reduced. |

Root growth

| Tree growth is decreased by nutrient deficiency because root allocation.is not increased. |

| Limited carbon used for leaf growth instead of roots despite nutrient deficiency. |

| Tree growth decreases by ozone are lessened because of replacement of damaged leaves. |

Nutrient deficiency

Fig. 11. Schematic of the major responses to high ozone levels combined with nutrient deficiencies in a tree with a growth strategy of high rates of C storage in reserves.

tainly be true when ozone stress also occurs, since C will be reserved for growth of new leaves. Even though reduced root allocation under conditions of nutrient deficiency will not improve uptake rates and will likely lead to reductions in C fixation, a net benefit is gained by using C instead to replace injured leaves. This benefit is even more pronounced in a plant with an ample C reserve to draw upon for leaf replacement. These processes are likely to result

in a tree reduced in growth because of nutrient deficiency and low uptake, but a tree in which growth decreases by ozone are lessened because of the replacement of damaged leaves.

Large maximum potential growth rates can prevent a tree from sequestering C into storage reserves (Fig. 12). In a tree with these large rates rapid allocation of C to the most immediate C sink can result in high rates of root

Growth Stategy:
High rates of maximum
potential tissue growth

Ozone damage

Leaf growth

| High ozone damages old leaves which are not replaced by new leaves. | Low nutrient limits allocation for new leaf growth. | Little carbon is available from reserve pool for new leaf growth. |

Small carbon reserve pool

Carbon fixation   Tree growth

| Despite increased allocation to roots, nutrient uptake is low and fixation is reduced. |

Root growth

| Tree growth is decreased by nutrient deficiency despite increased root allocation and replacement. |

| Low nutrient increases allocation for root growth |

| Tree growth is also decreased by ozone because of insufficient replacement of damaged leaves. |

Nutrient deficiency

Fig. 12. Schematic of the major responses to high ozone levels combined with nutrient deficiencies in a tree with a growth strategy of high rates of maximum potential tissue growth.

growth under nutrient deficient conditions,. Despite increased allocation to roots, nutrient uptake is likely to remain low causing fixation to be reduced. In addition, allocation to roots is likely to occur at the expense of allocation to leaves. If ozone begins to damage leaf photosynthetic capacity, adding a second stress to the nutrient deficiency, little C is available from either fixation or from reserves to grow replacement leaves. The result can be that nutrient deficiency produces a decrease in tree growth despite the increase in allocation to root growth. By allocating C rapidly for root growth, the tree may be committing itself to a course of action that no longer gives a high rate of return on its investment under multiple stress conditions. Little adjustment of morphology is possible without significant reserves.

It is possible that different results would be obtained from different combinations of stresses. Further, it is clear that the condition of the tree at the beginning of each simulation strongly influences the nature of the multistress response. In addition, differences due to different species tested (red spruce vs. ponderosa pine) could have influenced the results, although these species span the gamut from a slow growing conservative growth strategy (red spruce) to a fast growing opportunistic growth strategy (ponderosa pine) with clear differences in ozone and nutrient tolerances between them.

This study shows the great advantages of modeling as a tool to understand responses to simultaneous stresses. Situations that could not be treated in an experimental setting were tested more completely and responses dissected more thoroughly than is conceivable in most research. With this model we can fill in the gaps in understanding necessary to produce a comprehensive theory of tree response to multiple stresses.

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