

OZONE-INDUCED ACCELERATED FOLIAR SENESCENCE: IMPLICATIONS FOR TOXICITY AND COMPENSATION

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Abstract: Two-year-old seedlings of black cherry, *Prunus serotina* Ehrh., northern red oak, *Quercus rubra* L. and sugar maple, *Acer saccharum* Marsh., and ramets of hybrid poplar, *Populus maximowizii* x *trichocarpa*, clone 245 were grown in eight charcoal-filtered open-top chambers per species. Half the chambers, per species, received 0.08 $\mu\text{L L}^{-1}$ O_3 from 1000 to 1800 h each day of the growing season. Accelerated foliar senescence and associated O_3 -induced loss in Rubisco were observed in older foliage of hybrid poplar and black cherry. Younger leaves were less responsive to O_3 , and in the case of hybrid poplar actually exhibited signs of compensation to the stress. Sugar maple and northern red oak were less responsive and exhibited no signs of accelerated senescence. The relevance of the latter response is considered in the context of the indeterminate and determinate growth habits of these two groups of plant species.

INTRODUCTION

Ozone (O_3) has been associated with the induction of accelerated foliar senescence in many plant species (Reich & Lassoie, 1985; Pell, Eckardt & Enyedi, 1992). During normal leaf development, expansion of the lamina is associated with an increase in the concentration of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). Once the leaf has reached full expansion, synthesis of Rubisco becomes of less consequence and the protein levels decline until senescence (Dalling, 1987). Because Rubisco is the protein responsible for fixing CO_2 during the Calvin Cycle, decline in this enzyme is linked with a reduction in net photosynthesis. We have shown that O_3 -stressed plants exhibit a more rapid loss in Rubisco protein coupled with a more rapid decline in net photosynthesis (Pell, Eckardt & Glick, 1994); ultimately the leaf becomes chlorotic and abscises earlier than non stressed counterparts. Previously, we demonstrated that the accelerated loss in Rubisco can be attributed, in part, to enhanced degradation of the protein (Eckardt & Pell, 1994). In addition, Reddy et al. (1993) have shown that O_3 induces a reduction in mRNA for the large and small subunits of Rubisco; how this reduction in transcript relates to possible reduction in synthesis of Rubisco remains to be determined.

The accelerated loss of photosynthetic tissue has inherently negative implications for the plant. However, plants possess compensatory mechanisms to minimize the adverse effects of stress. We have associated O_3 -accelerated reduction in net photosynthesis and Rubisco content of older leaves of trembling aspen (*Populus tremuloides*) with increases in these parameters, above the level of control tissue, in younger leaves (Pell et al., 1994). The ability of plants to compensate for injury by accelerated foliar senescence as just described, is dependent on the capacity for plants to initiate new foliage. Thus, indeterminate species would have options unavailable to species that exhibit determinate or fixed growth habit. In this study we explored the ability of O_3 to induce accelerated senescence in plants with different rates of growth and with different growth habits viz. hybrid poplar, and black cherry, both indeterminate as young plants; and northern red oak, and sugar maple, relatively determinate as seedlings.

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METHODS

Cuttings of hybrid poplar clone 245, and two-year-old seedlings of black cherry, northern red oak and sugar maple were cultured as described elsewhere (Pell et al., 1995); plants received a nutrient supplement of 3.53 g l⁻¹ Osmocote (N:P:K 14:14:14; Sierra Chemical Co., Milpitas, CA) at planting. All plants were grown in open-top chambers receiving charcoal-filtered air (Pell et al., 1993); in 1993 and 1994 O₃ concentrations in these chambers averaged 0.04 µl l⁻¹ during the exposure period. For each species, four replicate chambers served as controls while an additional four chambers received supplemental O₃ from 1000 to 1800 h each day resulting in a summer average of 0.08 µl l⁻¹. Experiments were conducted in 1993 and 1994. Most of the data reported herein was collected in 1993 with the results in 1994 supporting those of 1993. In addition, in 1992 a baseline study was conducted using only plants growing in charcoal-filtered air. Ozone exposures were conducted from June 24 - September 22, 1993 and June 24 - September 27, 1994.

When hybrid poplar and black cherry plants were approximately 18 cm in height, and when the second flush of sugar maple and northern red oak was initiated, a newly emergent leaf on each plant in every chamber was tagged. Once every two weeks throughout the growing season, leaves were sampled from two plants per chamber. In the case of hybrid poplar and black cherry, a second leaf 17 and 25 leaves above the first leaf tagged, respectively, were also tagged at emergence. Subsequently these leaves were sampled along with the leaf positioned lower in the canopy.

Net photosynthesis and leaf conductance were measured by nondestructive gas exchange analysis with a Li-Cor 6200 closed-loop photosynthesis system (Li-Cor, Inc., Lincoln, NE) as described by Pell et al. (1992). After the analysis samples were harvested in the field, frozen in liquid nitrogen and stored at -80°C. Rubisco quantity was determined as described by Eckardt and Pell (1994).

Statistics

Data for each species were analyzed separately by analysis of variance (ANOVA) and significance was accepted at the P ≤ 0.05 level (SAS Institute Inc., 1985).

RESULTS

In 1992 we followed the performance of foliage of the four species in the absence of O₃. Ozone concentration from 1000 to 1800 h averaged 0.03 µl l⁻¹ for the growing season. As we have previously reported (Pell et al., 1994), as the leaves of the indeterminate species (hybrid poplar and black cherry) aged, there was a rapid increase in concentration of Rubisco followed by a precipitous decline. In contrast, northern red oak and sugar maple foliage exhibited an increase in concentration of Rubisco followed by a prolonged plateau. Gas exchange data profiles were similar in all four cases (data not shown).

The first (older) leaf of hybrid poplar to be sampled, exhibited a significant O₃-induced decline in net photosynthesis that paralleled the decline in Rubisco concentration and preceded a reduction in stomatal conductance (Fig. 1 A-C). The second (younger) leaf to be sampled, exhibited an initial increase in net photosynthesis, stomatal conductance and Rubisco content in response to O₃, followed by a decline (Fig. 1 D-F).

The older leaf of black cherry sampled exhibited a significant decline in net photosynthesis, stomatal conductance and Rubisco quantity in response to O₃. Accelerated senescence was observed in these leaves (Fig. 2 A-C). The younger leaf also sustained a reduction in net photosynthesis and stomatal conductance, but these responses were observed after a far longer O₃ exposure than was necessary to elicit a similar response in older leaves (Fig. 2 D & E). Accelerated senescence was not observed in these younger leaves, and a significant reduction in Rubisco concentration was observed only at the last sampling point (Fig. 2F).

Northern red oak did exhibit a significant reduction in net photosynthesis coupled with a drop in stomatal conductance in response to O₃ stress (Fig. 3 A & B). Neither accelerated foliar senescence nor significant changes in Rubisco content were detected (Fig. 3C).

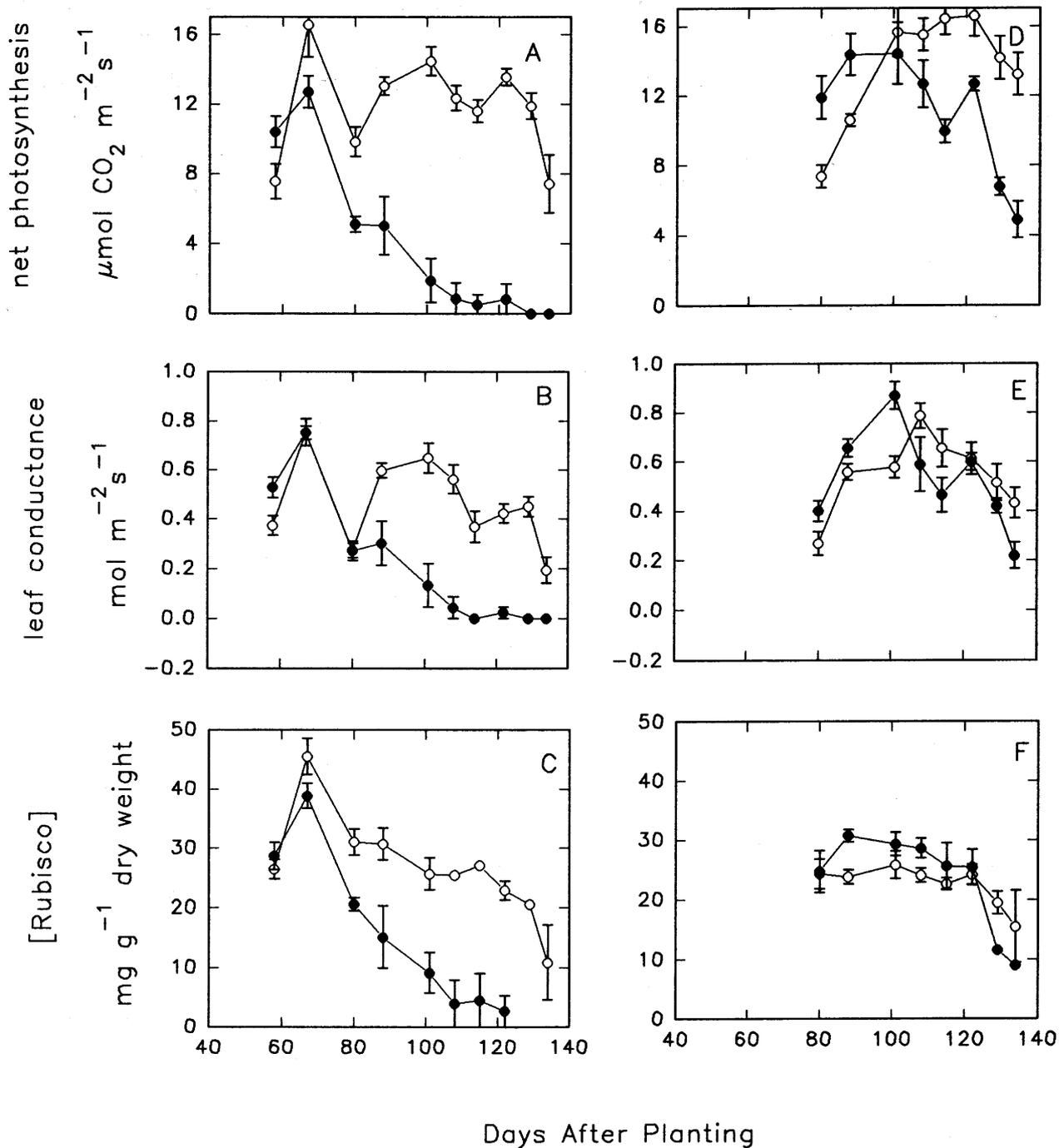


Figure 1. Effect of O_3 on net photosynthesis, leaf conductance and Rubisco content of hybrid poplar foliage, from emergence to senescence. (A-C) All samples derive from the two leaves that emerged when the plants were 18 cm tall. (D-F) All samples derive from the two leaves that emerged at a position 17 leaves above the first leaf sampled. (○) Plants grown in charcoal-filtered open-top chambers. (●) Plants grown in open-top chambers supplemented with $0.08 \mu\text{L L}^{-1} O_3$ for 8h per day. Each value is the mean of eight and four observations \pm standard error of the mean, for gas exchange and Rubisco measurements, respectively.

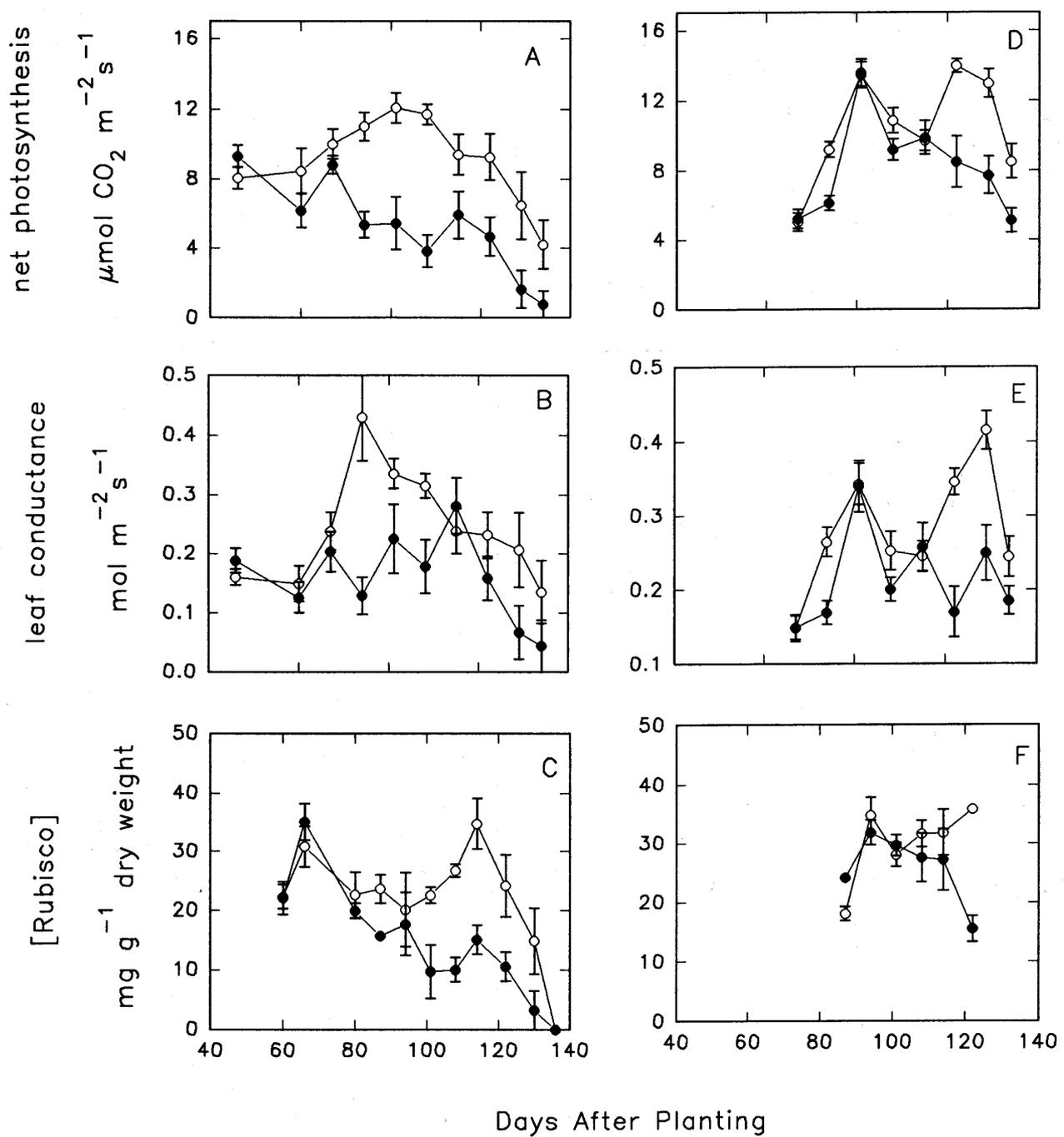


Figure 2. Effect of O_3 on net photosynthesis, leaf conductance and Rubisco content of black cherry foliage, from emergence to senescence. (A-C) All samples derive from the two leaves that emerged when the plants were 18 cm tall. (D-F) All samples derive from the two leaves that emerged at a position 17 leaves above the first leaf sampled. (O) Plants grown in charcoal-filtered open-top chambers. (●) Plants grown in open top chambers supplemented with $0.08 \mu\text{L L}^{-1} \text{ O}_3$ for 8h per day. Each value is the mean of eight and four observations + standard error of the mean, for gas exchange and Rubisco measurements, respectively.

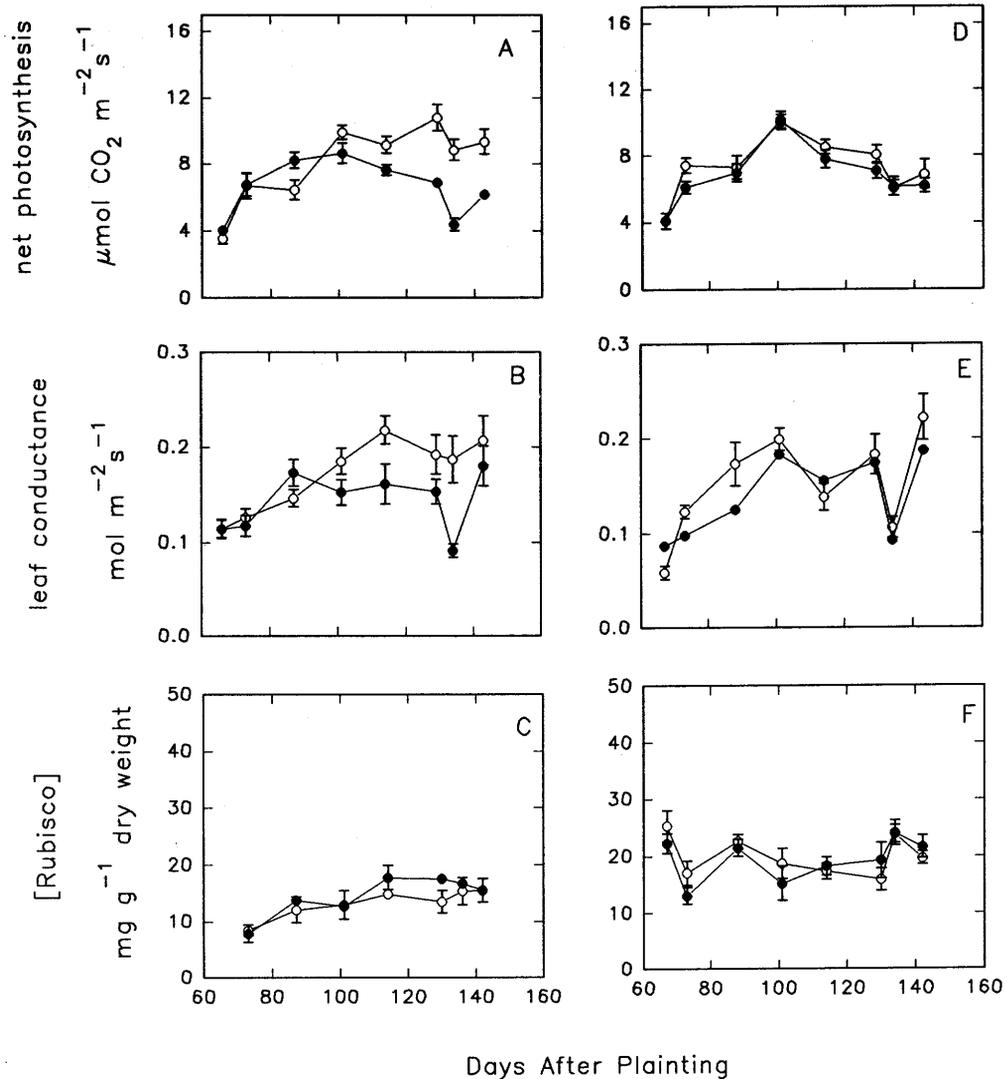


Figure 3. Effect of O_3 on net photosynthesis, leaf conductance and Rubisco content of foliage from the second flush of growth of northern red oak (A-C) and sugar maple (D-F). The leaf was analyzed from emergence to senescence. (○) Plants grown in charcoal-filtered open-top chambers. (●) Plants grown in open top chambers supplemented with $0.08 \mu\text{L L}^{-1} \text{ O}_3$ for 8h per day. Each value is the mean of observations \pm standard error of the mean, for gas exchange and Rubisco measurements, respectively.

When sugar maple seedlings were stressed by O_3 , we detected no significant effects on net photosynthesis, stomatal conductance or Rubisco content (Fig. 3 D-F). Accelerated senescence of foliage was not detected.

DISCUSSION

The resource allocation strategies of the indeterminate species were clearly different from the more determinate species examined in this study. Hybrid poplar and black cherry leaves demonstrated rapid synthesis of protein as exhibited by the brief residence time of the Rubisco peak (Figs. 1 & 2). Both species exhibited less responsive younger foliage. In fact younger leaves of hybrid poplar actually performed better in plants under O_3 stress. These

data are supported in a previous study with trembling aspen (Pell et al., 1994). Elsewhere Brendley et al. (1994) have reported that in O₃-stressed plants, younger foliage actually synthesized higher rates of Rubisco than were observed in non stressed plants. Hybrid poplar may be more effective in compensating for O₃ than is black cherry because of the higher rate of growth of the former species. Rapid rate of growth may lead to the greatest rate of senescence; it will also allow for nitrogen to be recycled most rapidly as Rubisco degrades. Thus, re utilization of nitrogen for a compensatory function in younger leaves will occur most readily in the plants with the most rapid growth rates.

Neither northern red oak nor sugar maple exhibited accelerated senescence or changes in Rubisco content following O₃ exposure (Fig. 3). Northern red oak did show a reduction in net photosynthesis; this response seemed to be closely associated with a reduction in stomatal conductance (Fig. 3).

We conclude that O₃-induced reduction in net photosynthesis may be regulated in part by a reduction in Rubisco content as shown for hybrid poplar, but it can occur without changes in this protein. Accelerated senescence seems to be tightly linked to the reduction in Rubisco content. The latter response seems to be associated with the growth habit of plants. In indeterminate species where nitrogen re utilization is a possible strategy for survival, accelerated senescence and loss of Rubisco provide a viable mechanism of compensation. For more determinate species this type of nitrogen recycling is not possible. Therefore, for plants with a determinate growth habit, accelerated senescence may be a less likely response to stress. Whether recycling of nitrogen within a leaf occurs as a result of the stress has not been determined.

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