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Minireview

Biochemical and molecular basis for impairment of photosynthetic potential

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Abstract

Ozone induces reductions in net photosynthesis in a large number of plant species. A primary mechanism by which photosynthesis is reduced is through impact on carbon dioxide fixation. Ozone induces loss in Rubisco activity associated with loss in concentration of the protein. Evidence is presented that ozone may induce oxidative modification of Rubisco leading to subsequent proteolysis. In addition, plants exposed to ozone sustain reduction in *rbc*S, the mRNA for the small subunit of Rubisco. This loss in *rbc*S mRNA may lead to a reduced potential for synthesis of the protein. The regulation of O_3-induced loss of Rubisco, and implications of the decline in this protein in relation to accelerated senescence are discussed.

1. Introduction

The toxic effects of ozone (O_3) on the photosynthetic process were first described by Todd in 1958, the same year that the air pollutant was first defined as a phytotoxin (Richards et al. 1958). In Todd's study, Valencia orange plants were treated with 16 μL L^{-1} O_3 and subsequent reductions in photosynthesis were noted. Since then there have been many studies conducted with a plethora of plant species, under many different O_3 regimes, documenting reductions in this critical plant process. A review of that literature is beyond the scope or intent of this manuscript. Rather, we begin this paper under the premise that O_3 does impact photosynthesis, and set as our goal a consideration of the mechanisms by which the adverse effects occur.

In order for a plant to photosynthesize optimally, neither carbon dioxide (CO_2) nor light can be limiting. The first sites of photosynthetic limitation are the stomata which regulate the amount of CO_2 that enters the lamina. Hill and Littlefield (1969) showed that O_3 could induce a reduction in leaf conductance which could account for reduced photosynthesis. While others have shown that O_3 can cause stomatal closure, there are many reports which support the observation that O_3-induced reduction in net photosynthesis is an early event, independent of stomatal closure (Atkinson et al. 1988, Pell et al. 1992). In fact, it has been proposed that the closure of stomata may result from the build up of CO_2 which occurs when photosynthesis is impaired (Atkinson et al. 1988). The thrust of this paper is an examination of the evidence that O_3-induced reductions in net photosynthesis result from either impairment of elements of light harvesting and/or carbon dioxide fixation.

2. Evidence of O_3-induced impact on light harvesting

Several groups have reported that the light-saturated rate of CO_2 uptake decreases significantly when plants are exposed to elevated O_3 levels (Atkinson et al. 1988, Farage et al. 1991, Baker et al. 1994). Farage et al. (1991) explored the possibility that this decrease could be the result of reductions in pigment concentration and resultant absorption of light energy, or O_3-induced reduction in the efficiency of energy transduction of the photosynthetic membrane; such effects could lead to a loss of active Photosystem II. While long-term exposure of plants to O_3 eventually led to a loss in chlorophyll, this appeared to be a very late event and did not provide a satisfactory explanation for initial reduction in photosynthesis (Farage et al. 1991). Furthermore, in this study and several others, where the fluorescence ratio F_v/F_m was measured in O_3-treated foliage, either no impact was observed or the ratio was less sensitive than CO_2 fixation (see discussion below) (Farage et al. 1991, Godde and Buchhold 1992, Grandjean et al. 1992, Baker et al. 1994). Lack of responsiveness of Photosystem II to O_3 was further illustrated by Farage et al. (1991), as O_3 did not alter the ability of thylakoids of wheat chloroplasts to bind atrazine even though other responses were detected.

Many groups interested in photooxidation have studied the degradation and synthesis of the 32 kDa D1 protein located in the reaction center of Photosystem II. In studies in which wheat leaves received acute exposure to O_3 (Farage et al. 1991), and spruce trees received chronic exposures (Godde and Buchhold 1992), no changes in content of D1 protein were detected. However, Godde and Buchhold (1992) determined that the turnover rate of the D1 protein was significantly higher in O_3-treated spruce needles, suggesting that both degradation and synthesis of this protein increased. A similar response is evoked by high light intensity. The observation that D1 protein turns over more rapidly in the O_3-stressed spruce needles than in spruce needles of control plants is important, in that it is one of the few reports supporting the relative susceptibility of Photosystem II to O_3-induced damage. Their results suggest that the repair mechanisms are in place to neutralize any adverse effects to photosynthesis; however, the energy necessary for this repair should probably be factored into any cost analysis for maintenance of plants in an O_3-stressed environment.

3. Evidence of O_3-induced impact on carbon dioxide fixation

While the role of modified light harvesting as a primary explanation for O_3-induced reduction in net photosynthesis seems questionable, there is considerable evidence to support the importance of impaired carbon dioxide fixation in adverse effects of the pollutant. Below we consider the mechanisms which might account for this reduced carboxylation.

Loss in Rubisco activity

There is both in situ and in vitro evidence that O_3 can induce reductions in the activity of ribulose-1,5-bisphosphate carboxylase (Rubisco). We have used gas exchange analysis to construct carbon dioxide response curves (A vs. c_i) for leaves of hybrid poplar plants which received 0.10 μL L^{-1} O_3 4 h day^{-1} throughout a 56-day interval (Pell et al. 1992). As the leaf matured, the CO_2-fixing capability of the leaf declined and the decline occurred more rapidly in the O_3-stressed leaf. Reduction in leaf conductance could not fully account for this in situ indication of reduced carboxylation as declines in A preceded reductions in leaf conductance. A more likely explanation, therefore, resides with the activity of Rubisco.

It is well known that Rubisco accounts for as much as 50–70% of the soluble leaf protein, but the percentage of the pool of enzyme which is active varies with environment and genotype (Miller and Huffaker 1982). When activity is measured in vitro, the enzyme may be extracted and activated with CO_2 and magnesium prior to analysis. In this case the resulting measure of carboxylation is a reflection of the potential of the existing pool to function fully (heretofore referred to as total activity). Alternatively, the enzyme can be extracted in a CO_2 free environ-

ment with subsequent measure of activity presumably only reflecting the endogenous Rubisco activity. Ozone-induced reductions in total Rubisco activity have been reported for hybrid poplar, potato, radish, rice and wheat (Nakamura and Saka 1978, Lehnherr et al. 1987, Dann and Pell 1989, Enyedi et al. 1992, Pell et al. 1992, Landry and Pell 1993). In experiments with potato (Dann and Pell 1989) and with radish (unpublished data) we could detect differences in the magnitude of endogenous (initial) and total Rubisco activities, and in both cases O_3-induced reductions were observed; however, no effects of O_3 on the percent activation of the enzyme were detected. There are several reports suggesting that Rubisco extracted from O_3-treated plants does exhibit an increase in percent activation (Lehnherr et al. 1987, 1988). These observations were made on the flag leaf of wheat plants at a single time point. Activation was incomplete rendering total activity lower than initial activity. It is conceivable that at certain times during leaf development O_3-treated plants could exhibit increased percent activation of Rubisco; this would provide a mechanism for prolonging the maximum CO_2 fixing capability of a leaf. Ultimately, however, there is concurrence that carboxylation capacity of leaves is diminished by O_3.

Loss in Rubisco quantity

Loss in activity of Rubisco has been frequently associated with a reduction in the quantity of the protein (Dann and Pell 1989, Pell et al. 1992, Baker et al. 1994). Ozone-induced reductions in Rubisco concentrations were first observed for rice and alfalfa plants exposed to short term, relatively high doses of O_3 exposures (Nakamura and Saka 1978, Pell and Pearson 1983). Since then, it has been reported that longer term relatively low doses of O_3 resulted in reductions in the concentration of Rubisco in potato, radish, aspen, hybrid poplar, and wheat (Dann and Pell 1989, Pell et al. 1992, 1994b, Baker et al. 1994).

A major focus of our laboratory has been to explain the mechanism by which the loss in Rubisco protein is induced by O_3. There are two ways in which this may occur; either O_3 enhances degradation or this oxidizing air pollutant reduces synthesis of the protein. The notion that O_3 might induce enhanced degradation was derived from a hypothesis which Dalling (1987) proposed to explain the loss of Rubisco from normally senescing foliage. Dalling (1987) suggested that while proteases necessary for Rubisco degradation may reside in the chloroplast, the protein would have to undergo some modification before proteolysis could proceed. While this proposal was developed to explain the degradation of Rubisco during normal aging, we believed that it could be logically extended for an O_3 stressed plant. When Rubisco is oxidized in vitro by O_3, hydrogen peroxide, copper sulfate or dimethylsulfoxide, chymotrypsin-induced proteolysis is enhanced (Pell et al. 1990, Peñarrubia and Moreno 1990).

In vitro experiments are always useful in establishing the potential of a hypothesis. The greater challenge, however, is to determine whether the phenomenon of interest occurs in vivo. Initially, we exposed plants to O_3 and examined protein extracts by SDS–PAGE gels and western blot immunoblotting with antibody for Rubisco. While we were able to detect loss in Rubisco large and small subunits, we did not detect increases in degradation products (Landry and Pell 1993). This led again to the bifurcated path to explain O_3-induced loss in Rubisco protein, either by reduced synthesis or very rapid turnover of the protein to fragments too small to be detected by SDS-PAGE.

We have since produced several avenues of support for the notion that O_3 does cause modification of the Rubisco protein, potentially leading to its degradation. Landry and Pell (1993) reported that when crude leaf extracts of hybrid poplar were treated with O_3, changes occurred in enzyme activity and CABP binding before any changes in either total soluble leaf protein or concentrations of the large subunit of Rubisco could be detected. Since both enzyme activity and CABP binding require structural integrity, these experiments supported the possibility that O_3 somehow modified the structure of the enzyme. When isolated chloroplasts of hybrid poplar and potato were treated with O_3, there was a loss in the 55 kD Rubisco large subunit while several aggregates could be detected at 65, 110

and > 205 kD (Landry and Pell 1993, Eckardt 1993). These aggregates were recognized by antibody raised against LSU and SSU of Rubisco (Landry and Pell 1993, Eckardt 1993). Since minimal lower molecular weight breakdown products were detected, we concluded that if Rubisco were oxidized during an O_3 exposure the outcome would be either a rapid degradation of the enzyme to fragments too small for detection by SDS–PAGE, or the aggregation of structurally destabilized subunits of the protein. Mehta et al. (1992) reported that copper sulfate treatment of wheat plants or isolated chloroplasts led to aggregation of Rubisco at the chloroplast membrane. Once located at the membrane, the protein was degraded by membrane-bound proteases. However, in experiments in which isolated hybrid poplar chloroplasts were treated with O_3, Landry (1992) found no shift in protein from the stroma to the thylakoid.

One of the conundrums of these studies was our inability to detect the aggregates from extracts of leaves taken from whole plants exposed to O_3. It was possible that the O_3-induced aggregates of Rubisco were short-lived and difficult to detect due to rapid proteolysis. Eckardt (1993) treated potato plants with O_3 and at the conclusion of the exposure removed leaf discs and incubated them in the dark at either 0 °C or 30 °C. At 30 °C, a temperature which enhances proteolysis, Rubisco concentration decreased measurably and no aggregate was observed. In contrast at 0 °C, when proteolysis was inhibited, the primary observation was the appearance of aggregate which bound positively to antibody for Rubisco. It is possible that the aggregate is formed in situ and serves as a transient intermediate in proteolysis. Alternatively, it seems equally plausible that O_3 induces an oxidatively modified, unstable form of Rubisco. This form of Rubisco may be rapidly degraded in situ where all the necessary proteases are available. However, in systems where proteolysis is inhibited (e.g., during a cold temperature incubation) the modified Rubisco may not degrade and aggregation will ensue. If the latter scenario is correct, then detection of the aggregate is on one hand an artifact, and on the other an indication that oxidative modification of Rubisco may be occurring in vivo.

The mechanism of O_3-induced oxidation of Rubisco represents another intriguing issue. As was suggested by Laisk et al. (1989), it seems highly unlikely that O_3 per se would penetrate to the chloroplast. The interaction of O_3 with the plasma membrane may lead to the production of a variety of free radical species, including superoxide, singlet oxygen, the hydroxyl radical, organic free radicals, and hydrogen peroxide (Mustafa 1990). Over time, this cascade of free radical formation might lead to an increase in the oxidative potential throughout the cell, including the chloroplast. As a result, certain amino acid residues of Rubisco may be subjected to noncatalytic oxidation. Through studies in which purified Rubisco was treated with O_3 in vitro, we have demonstrated the oxidation of sulfhydryl groups and formation of carbonyl derivatives (Eckardt 1993, Pell et al. 1994a). However, when whole plants were exposed to O_3 and Rubisco purified after the treatment, neither sulfhydryl oxidation nor formation of carbonyl derivatives could be detected (Eckardt 1993). Whether oxidation of Rubisco in situ does not occur, or the modified protein cannot be recovered during subsequent purification remains unclear.

While there is no evidence that O_3-stressed plants sustain noncatalytic oxidation of Rubisco, it may be postulated that changes in the enzyme could occur via a catalytic process. Protein oxidation by metal-catalyzed oxidation (MCO) systems is well-documented as a marker for proteolytic degradation in animal and bacterial systems (Levine et al. 1981, Rivett 1986, Stadtman 1988), but has not been studied as much with plant systems. MCO systems active in vivo include the enzymes cytochrome P-450 reductase, NADH oxidase, putidaredoxin reductase, and xanthine oxidase, together with their respective substrates (Fucci et al. 1983). Induction of MCO systems usually occurs under conditions of oxidative stress, such as during aging, disease, or abnormally high O_2 pressures (Starke et al. 1987). Many of the enzymes sensitive to MCO-mediated inactivation play a central role in metabolism and have a nucleotide binding site and/or a histidine residue in the active site.

Rubisco shares many of the characteristics of enzymes inactivated by MCO systems. It is a key enzyme in photosynthesis and is a branch point

in the competing pathways of photosynthesis and photorespiration. Rubisco purified from spinach and *Rhodospirillum* (Cook et al. 1988) and *Euglena gracilis* (García-Ferris and Moreno 1993) was susceptible to inactivation by the ascorbate/Fe MCO system.

A few reports have provided evidence that Rubisco in both bacteria and higher plants may be susceptible to inactivation via the induction of an MCO system under varying degrees of oxidative stress. In the purple non-sulfur bacterium *Rhodospirillum rubrum*, Rubisco activity and CO_2 fixation cease rapidly upon transfer of the organism from an anaerobic to an aerobic environment (Tabita 1988). The switch to an aerobic environment in the *R. rubrum* system seems to be associated with the appearance of a heat-labile, dialyzable factor capable of causing oxidative inactivation of Rubisco in vitro (Cook and Tabita 1988). In the aquatic dicot *Lemna minor*, osmotic stress has been found to produce an oxidase system which catalyzes the inactivation of Rubisco (Ferreira and Davies 1989).

The hypotheses explored in the previous several paragraphs all rest on the assumption that Rubisco modification through oxidation is necessary for proteolysis. This perspective is consistent with the view of Stadtman (1988) that specific proteases are designed to recognize oxidized enzymes. As stated earlier, Dalling (1987) has suggested that the proteases necessary for Rubisco degradation reside in the chloroplast and that it is the oxidation of the enzyme which leads to proteolysis. Landry and Pell (1993) exposed hybrid poplar plants to O_3 at levels which reduced total activity of Rubisco and occasionally quantity, as well. Crude leaf extracts of these plants were analyzed to determine the proteolytic potential to hydrolyze exogenously added Rubisco; free amine release was either unaffected by the treatment or actually reduced. This observation supports Dalling's notion that degradation of Rubisco is triggered by oxidation rather than a change in protease activity. It remains possible that some specific protease in the chloroplast is induced during senescence, and that measurement of protease activity in crude extracts simply masks detection of the activity of a newly induced enzyme. Recently, Hensel et al. (1993) have reported that a cysteine protease was induced during the senescence of *Arabidopsis* leaves.

Effect of O_3 on synthesis of Rubisco

Earlier we suggested that a second explanation for O_3-induced loss in Rubisco protein might be a decrease in the synthesis of the protein. When potato plants were treated with O_3 there was a rapid loss of the mRNA for both the large and small subunits of Rubisco, *rbc*L and *rbc*S, respectively (Reddy et al. 1993, Pell et al. 1993). Recently, we completed experiments in which we demonstrated that chronic exposure of potato plants to O_3 levels of 0.08 μL L^{-1} for 4 hours/day resulted in a rapid decrease in the levels of *rbc*S mRNA with only minimal loss in *rbc*L mRNA (Glick and Pell, unpublished data). A decrease in the steady-state level of *rbc*S mRNA may be an indication that the potential for Rubisco synthesis is decreased in plants exposed to O_3. Currently we are conducting protein synthesis experiments to test directly the impact of O_3 on Rubisco synthesis.

A loss in mRNA could be attributed to regulation at the transcriptional level. We have recently conducted nuclear run-on experiments to determine whether the loss in *rbc*S mRNA detected by northern blot analysis could be explained by reduced levels of transcription. There is a decrease in the levels of transcription of *rbc*S in nuclei isolated from O_3-treated plants in comparison to nuclei isolated from plants grown in charcoal filtered air; however, the O_3-induced decrease in the level of steady-state *rbc*S mRNA detected by northern blot analysis is greater than the detected loss in transcription (Glick and Pell, unpublished data). Whether the loss in transcription is sufficient to account for the loss in *rbcS* mRNA remains to be determined.

Post-transcriptional processes (e.g., mRNA turnover) may be as important as transcriptional processes in regulating the levels of mRNA (Brawerman 1987, Shapiro et al. 1987). There is evidence that stage of plant development and environmental cues, e.g., light, influence the degradation of *rbc*S mRNA in soybean foliage (Shirley and Meagher 1990, Thompson and Meagher 1990). Very little is known about ribonucleases in plants. However, three ribonuclease activities have been identified in wheat

leaves where the activities of the ribonucleases increased during senescence (Blank and McKeon 1991a,b), and a senescence associated ribonuclease has been described in *Arabidopsis* (Taylor et al. 1993). Since O_3 causes accelerated senescence, it is possible that the loss of *rbc*S mRNA may be a consequence of increased degradation of the transcript.

Our work with *rbc*S mRNA was performed with young foliage which had not yet fully expanded, as well as, with tissue which had reached full expansion at the time of O_3 exposure (Reddy et al. 1993). Interestingly, both tissues exhibit losses of *rbc*S and *rbc*L mRNAs for Rubisco transcript messages (Reddy et al. 1993). It is well known, however, that visual symptoms and accelerated senescence are characteristic O_3 responses of mature leaf tissue. Using potato, Eckardt (1993) attempted to determine the importance of O_3-induced degradation of Rubisco, loss in *rbc*S mRNA, and the induction of accelerated senescence. Potato plants were treated with 0.30 μL L^{-1} O_3 for six hours. Immediately after the exposure, the *rbc*S transcript level decreased in immature (unexpanded), and mature (fully expanded) leaves in response to O_3. The level of *rbc*S mRNA was considerably higher in immature leaves and the reduction due to O_3 was only significant in that tissue. There were no significant effects on the levels of *rbc*L mRNA level. These results supported previous observations that O_3 could induce a reduction in *rbc*S mRNA, thus potentially influencing the capacity of the leaf to synthesize Rubisco. As a second phase of this experiment, a set of plants was placed in the dark immediately after the O_3 exposure. The *rbc*S mRNA rapidly declined in all plants placed in the dark, thereby preventing any further synthesis of the protein. After 48 hours in the dark there was a 12% drop in the quantity of Rubisco in immature leaves of both O_3-treated plants and control plants. In mature leaves the O_3 treatment resulted in a 53% decrease in Rubisco quantity, while control leaves only exhibited a drop of 13% (Eckardt 1993).

From these results we conclude that O_3 can influence synthesis and proteolysis of Rubisco. The importance of each of these responses is age-dependent. In mature leaves of potato plants the capacity to synthesize Rubisco diminishes with age, as reflected by the decline in *rbc*S mRNA (Reddy et al. 1993). If the protein is compromised by O_3-induced oxidation, as we proposed above, proteolysis would be favored and total quantity of Rubisco would decrease. If an immature leaf is stressed by O_3, immediate loss in Rubisco is not apparent. Since Rubisco is located in the chloroplast, which is also the site of an array of antioxidants, it is possible that in young leaf tissue protein oxidation may not present a major problem. However, we speculate that a loss in *rbc*S mRNA occurring early in the life history of a leaf may result in effects later in the life cycle. Because Rubisco has a long half life of five to six days (Simpson 1981) losses in *rbc*S mRNA in the immature leaf would not be of any initial consequence to the concentration of protein in the leaf. However, as the leaf ages, the diminished levels of *rbc*S mRNA will become important since the leaf loses its ability to produce new transcript with age. We propose that an immature leaf stressed by O_3 may enter maturity with a more rapid loss in the ability to synthesize new protein. Put simply, O_3 may not induce any immediately obvious effects on young leaves, but this oxidant may predispose the leaf to early onset of senescence.

The model described above was developed based largely on research with potato. Leaf development for this annual herbaceous species is a rapid process. It is possible that the picture could be somewhat different if a more long-lived species were considered. Harkov and Brennan (1979) proposed that faster growing tree species were more sensitive to O_3 than slower growing species. Davis and Wilhour (1976) demonstrated that tree species with a fixed-growth habit, including northern red oak and sugar maple, were more tolerant to O_3-induced visual injury than species like hybrid poplar with a free-growing habit.

The longer a leaf persists the more likely it is to have prolonged potential to synthesize Rubisco. In Fig. 1 we illustrate the Rubisco content of leaves of black cherry and hybrid poplar, free-growing species, and northern red oak and sugar maple, species with a fixed-growth habit, from emergence in the spring of 1993 until September 1993 (Pell and Sinn, unpublished

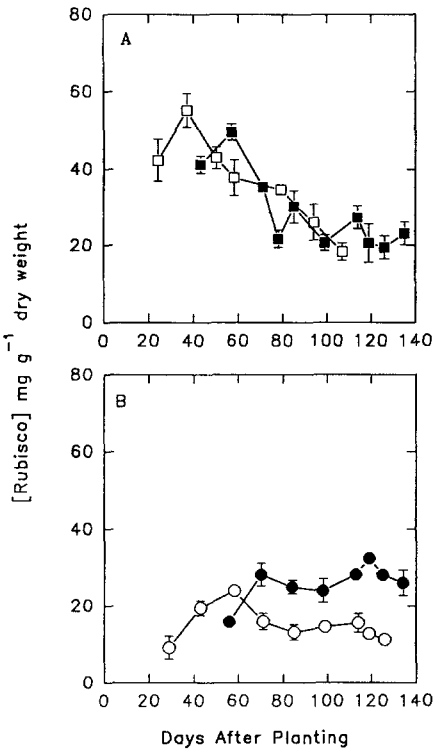

Fig. 1. Concentration of Rubisco, determined as previously described (Pell et al. 1992), for (A) hybrid poplar, *Populus maximowizii x trichocarpa*, clone 245 (□) and black cherry, *Prunus serotina* Ehrh. (■), and (B) northern red oak *Quercus rubra* L. (○) and sugar maple *Acer saccharum* Marsh. (●). The youngest newly emerged leaves of poplar cuttings and first year seedlings of black cherry were identified when single terminal shoots were 20 cm tall. The third emerging leaf of the first and second flushes of northern red oak and sugar maple, respectively, were identified for these genotypes. Periodically for 78 to 97 days, thereafter, the leaves were sampled for analysis. All plants were grown in charcoal filtered air. Each value is the mean of four observations ± standard error of the mean.

data). Rubisco content of black cherry and hybrid poplar leaves follow profiles which are similar to those previously reported for other free-growing species like potato, radish and hybrid poplar (Dann and Pell 1989, Pell et al. 1992). Rubisco content of leaves of northern red oak and sugar maple show a much lower concentration of the protein, but the level is maintained throughout the life of the leaf. We propose that the capacity for continued synthesis allows these species to maintain Rubisco for a prolonged period of time. Preliminary results suggest that mRNA levels for Rubisco in these genotypes follow a pattern similar to that observed for the protein (Sinn, Brendley and Pell, unpublished data). We propose that when black cherry and hybrid poplar are stressed with O_3, responses will be similar to those observed for potato. In contrast we predict that no detectable effects will be observed with northern red oak or sugar maple. It is still possible that Rubisco protein and or mRNA coding for Rubisco will be compromised and subsequently degraded due to O_3-induced oxidation in northern red oak and sugar maple; but the continued capacity to synthesize both the protein and the mRNA for Rubisco will mask any detectable losses in the protein. The impact of O_3 on Rubisco in these fixed-growth species, and impact on turnover rates, are currently being studied in our laboratory. Godde and Buchhold (1992) demonstrated that while spruce needles exhibited no sustained loss in D1 protein following O_3 exposure, the turnover of the protein was greater in treated plants. Spruce is also a slow growing species with a fixed-growth habit; as such the needles on these trees must maintain the capacity to synthesize these photosynthetic proteins for multiple years. It appears possible that species classified as O_3-tolerant based on measurement of net responses viz. visual symptoms, net photosynthesis, etc. are in fact responsive. However, the mechanisms of repair preclude detection of the transient effects. As stated earlier, increased turnover of protein and/or mRNA to maintain photosynthetic rate, will occur at a cost to the plant.

Regulation of loss of Rubisco

Above we proposed two mechanisms by which O_3 could induce loss of Rubisco, viz. through oxidation of protein and through loss of the *rbc*S transcript for the small subunit of Rubisco. Oxidation could occur through random oxidation of vulnerable sites resulting in compromised protein with increased susceptibility to proteolysis. Alternatively, metal catalyzed oxidation could be promoted by O_3 exposure. The regulation of these events could occur indirectly through the ability of cells to scavenge free radicals (Alscher et al. 1991). It is well known

that plants contain constitutive levels of antioxidants and exhibit induction of many others during O_3 exposures. The ability of O_3 to reduce Rubisco concentration through the oxidation process will depend upon the status of antioxidants within the cell.

The alternative mechanism by which O_3 might induce loss in Rubisco is by reducing the mRNA levels for *rbc*S, thereby affecting synthesis of the protein. We have established that this response is regulated at the transcriptional level and/or by inducing an increase in specific RNases. Since Rubisco declines in non-stressed foliage during senescence, it is possible that O_3-induced reductions in *rbc*S mRNA are regulated indirectly as this pollutant induces accelerated senescence. Ethylene, a well-known hormone of senescence, is induced in many plant species after O_3 exposure (Tingey et al. 1976); although the mechanism by which this emission might induce toxic effects is unclear. It has been shown that when inhibitors of ethylene biosynthesis, aminooxyacetic acid or aminoethoxyvinyl glycine, are provided to leaf tissue toxic effects of O_3 on net photosynthesis or *rbc*S mRNA are minimized (Taylor et al. 1988, Pell et al. 1994a).

While it has been suggested that ethylene emission is directly associated with visible injury, we were surprised to find significant levels of ethylene emitted by immature potato leaves which were not vulnerable to development of foliar injury (Reddy et al. 1993). These emissions correlated with the suppression of *rbc*S mRNA levels. There has been recent work associating ethylene emission with induction of pathogenesis related (PR) protein accumulation (Raz and Fluhr 1993). When inhibitors of protein kinases were added, ethylene induction of PR proteins was blocked. Whether putative phosphorylated intermediates are produced after leaves produce O_3-induced ethylene, and whether these intermediates might influence the loss in *rbc*S mRNA, is an area worthy of exploration.

4. Conclusion

Over the last three decades O_3-induced reduction in photosynthesis has been well documented with many plant species. Based on the studies reviewed in this paper, we propose that CO_2 fixation is more sensitive to O_3 exposures than are processes associated with light harvesting. Because of the high reactivity of the gas, O_3 per seis unlikely to be directly toxic to the chloroplast. Rather the loss in quantity and associated activity of Rubisco are probably the indirect result of increases in oxidative stress brought about by uptake of the pollutant. One of the central questions remaining is whether the loss in Rubisco is a consequence of induction of senescence through an unrelated set of O_3-induced reactions, or if the O_3-induced loss of Rubisco is responsible for promoting accelerated senescence.

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References

Alscher RG, Madamanchi NR and Cramer CL (1991) Protective mechanisms in the chloroplast stroma. In: Pell EJ and Steffen KL (eds) Active Oxygen/Oxidative Stress in Plant Metabolism, pp 145–155. American Society of Plant Physiologists, Rockville, MD

Atkinson CJ, Robe SV and Winner WE (1988) The relationship between changes in photosynthesis and growth for radish plants fumigated with SO_2 and O_3. New Phytol 110: 173–184

Baker NR, Nie G and Tomasevic M (1994) Responses of photosynthetic light-use efficiency and chloroplast development on exposure of leaves to ozone. In: Alscher RG and Wellburn AR (eds) Plant Responses to the Gaseous Environment. Molecular, Metabolic and Physiological Aspects, pp 219–238. Chapman and Hall, London, UK

Blank A and McKeon TA (1991a) Three RNases in senescent and non-senescent wheat leaves. Plant Physiol 97: 1402–1408

Blank A and McKeon TA (1991b) Expression of three RNase activities during natural and dark-induced senescence of wheat leaves. Plant Physiol 97: 1409–1413

Brawerman G (1987) Determinants of messenger RNA stability. Cell 48: 5–6

Cook LS and Tabita FR (1988) Oxygen regulation of ribulose 1,5-bisphosphate carboxylase activity in *Rhodospirillum rubrum*. J Bact 170: 5468–5472

Cook LS, Im H and Tabita FR (1988) Oxygen-dependent inactivation of ribulose 1,5-bisphosphate carboxylase/oxygenase in crude extracts of *Rhodospirillum rubrum* and establishment of a model inactivation system with purified enzyme. J Bact 170: 5473–5478

Dalling MJ (1987) Proteolytic enzymes and leaf senescence. In: Thomson WW, Nothnagel EA and Huffaker RC (eds) Plant Senescence: Its Biochemistry and Physiology, pp 54–70. American Society of Plant Physiologists, Rockville, MD

Dann MS and Pell EJ (1989) Decline of activity and quantity of ribulose bisphosphate carboxylase/oxygenase and net photosynthesis in ozone-treated potato foliage. Plant Physiol 91: 427–432

Davis DD and Wilhour RG (1976) Susceptibility of Woody Plants to Sulfur Dioxide and Photochemical Oxidants. Ecological Research Series. EPA-600/3-76-102. Corvallis, Oregon

Eckardt NE (1993) Ozone-Induced Structural Modification and Enhanced Degradation of Rubisco in Potato Foliage. PhD Thesis. The Pennsylvania State University, University Park, Pennsylvania

Enyedi AJ, Eckardt NA and Pell EJ (1992) Activity of ribulose bisphosphate carboxylase/oxygenase from potato cultivars with differential response to ozone stress. New Phytol 122: 493–500

Farage PK, Long SP, Lechner EG and Baker NR (1991) The sequence of change within the photosynthetic apparatus of wheat following short-term exposure to ozone. Plant Physiol 95: 529–535

Ferreira RB and Davies DD (1989) Conversion of ribulose-1,5-bisphosphate carboxylase to an acidic and catalytically inactive form by extracts of osmotically-stressed *Lemna minor* fronds. Planta 179: 448–455

Fucci L, Oliver CN, Coon MJ and Stadtman ER (1983) Inactivation of key metabolic enzymes by mixed-function oxidation reactions: Possible implication in protein turnover and ageing. Proc Natl Acad Sci USA 80: 1521–1525

García-Ferris C and Moreno J (1993) Redox regulation of enzymatic activity and proteolytic susceptibility of ribulose-1,5-bisphosphate carboxylase/oxygenase from *Euglena gracilis*. Photosynth Res 35: 55–66

Godde D and Buchhold J (1992) Effect of long term fumigation with ozone on the turnover of the D-1 reaction center polypeptide of Photosystem II in spruce (*Picea abies*). Physiol Plant 86: 568–574

Grandjean A, Grimm A and Fuhrer J (1992) The response of spring wheat (*Triticum aestivum* L.) to ozone at higher elevations. III. Responses of leaf and canopy gas exchange, and chlorophyll fluorescence to ozone flux. New Phytol 122: 321–328

Harkov R and Brennan E (1979) An ecophysiological analysis of the response of trees to oxidant pollution. J Air Pollut Control Assoc 29: 157–161

Hensel LL, Vojislava G, Baumgarten DA and Bleecker AB (1993) Developmental and age-related processes that influence the longevity and senescence of photosynthetic tissues in Arabidopsis. Plant Cell 5: 553–564

Hill AC and Littlefield (1969) Ozone: Effect on apparent photosynthesis, rate of transpiration, and stomatal closure in plants. Environ Sci Technol 3: 52–56

Laisk A, Kull O and Moldau H (1989) Ozone concentration in leaf intercellular air spaces is close to zero. Plant Physiol 90: 1163–1167

Landry LG (1992) The Role of Proteolysis in Ozone-Induced Rubisco Decline in Hybrid Poplar. PhD Thesis. The Pennsylvania State University, University Park, Pennsylvania

Landry LG and Pell EJ (1993) Modification of Rubisco and altered proteolytic activity in O_3-stressed hybrid poplar (*Populus maximowizii x trichocarpa*). Plant Physiol 101: 1355–1362

Lehnherr B, Grandjean A, Mächler F and Fuhrer J (1987) The effect of ozone in ambient air on ribulosebisphosphate carboxylase/oxygenase activity decreases photosynthesis and grain yield in wheat. J Plant Physiol 130: 189–200

Lehnherr B, Mächler F, Grandjean A and Fuhrer J (1988) The regulation of photosynthesis in leaves of field-grown spring wheat (*Triticum aestivum* L. cv Albis) at different levels of ozone in ambient air. Plant Physiol 88: 1115–1119

Levine RL, Oliver CN, Fulks RM and Stadtman ER (1981) Turnover of bacterial glutamine synthetase: Oxidative inactivation precedes proteolysis. Proc Natl Acad Sci USA 78: 2120–2124

Mehta RA, Fawcett TW, Porath D and Mattoo AK (1992) Oxidative stress causes rapid membrane translocation and in vivo degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase. J Biol Chem 267: 2810–2816

Miller BL and Huffaker RC (1982) Hydrolysis of ribulose-1,5-bisphosphate carboxylase by endoproteinases from senescing barley leaves. Plant Physiol 69: 58–62

Mustafa MG (1990) Biochemical basis of ozone toxicity. Free Rad Biol Med 9: 245–267

Nakamura H and Saka H (1978) Photochemical oxidants injury in rice plants. Jpn J Crop Sci 47: 704–714

Pell EJ and Pearson NS (1983) Ozone-induced reduction in quantity of ribulose-1,5-bisphosphate carboxylase in alfalfa foliage. Plant Physiol 73: 185–187

Pell EJ, Eckardt N and Enyedi AJ (1992) Timing of ozone stress and resulting status of ribulose bisphosphate carboxylase/oxygenase and associated net photosynthesis. New Phytol 120: 397–405

Pell EJ, Enyedi A, Eckardt N and Landry L(1990) Ozone-induced alterations in quantity and activity of rubisco: Implications for foliar senescence. In: Reddy CC, Hamilton GA and Madyastha KM (eds) Biological Oxidation Systems, Vol 1, pp 389–403. Academic Press, New York

Pell EJ, Landry LG, Eckardt NA and Glick RE (1994a) Effects of gaseous air pollutants on ribulose bisphosphate carboxylase/oxygenase: effects and implications. In: Alscher RG and Wellburn AR (eds) Plant Responses to the Gaseous Environment. Molecular, Metabolic and Physiological Aspects, pp 239–254. Chapman and Hall, London, UK

Pell EJ, Temple PJ, Friend AL, Mooney HA and Winner

WE (1994b) Compensation as a plant response to ozone and associated stresses: an analysis of ROPIS experiments. J Environ Qual 23 (in press)

Peñarrubia L and Moreno J (1990) Increased susceptibility of ribulose-1,5-bisphosphate carboxylase/oxygenase to proteolytic degradation caused by oxidative treatments. Arch Biochem Biophys 281: 319–323

Raz V and Fluhr R (1993) Ethylene signal is transduced via protein phosphorylation events in plants. Plant Cell 5: 523–530

Reddy G, Arteca RN, Dai Y-R, Flores HE, Negm FB and Pell EJ (1993) Changes in ethylene and polyamines in relation to mRNA levels of the large and small subunits of ribulose bisphosphate carboxylase/oxygenase in ozone-stressed potato foliage. Plant Cell Environ 16: 819–826

Richards BL, Middleton JT, and Hewitt WB (1958) Air pollution with relation to agronomic crops. V. Oxidant stipple of grape. Agron J 50: 559–561

Rivett JA (1986) Regulation of intracellular protein turnover: Covalent modification as a mechanism of marking proteins for degradation. Curr Top Cell Reg 28: 291–337

Shapiro DJ, Blume JE and Nielsen DA (1987) Regulation of messenger RNA stability in eukaryotic cells. BioEssays 6: 221–226

Shirley BW and Meagher RB (1990) A potential role for RNA turnover in the light regulation of plant gene expression: ribulose-1,5-bisphosphate carboxylase small subunit in soybean. Nucleic Acids Res 18: 3377–3385

Simpson E (1981) Measurement of protein degradation in leaves of *Zea mays* using [^{3}H]acetic anhydride and tritiated water. Plant Physiol 67: 1214–1219

Stadtman ER (1988) Biochemical markers of aging. Exp Geront 23: 327–347

Starke PE, Oliver CN and Stadtman ER (1987) Modification of hepatic proteins in rats exposed to high oxygen concentration. FASEB 1: 36–39

Tabita FR (1988) Molecular and cellular regulation of autotrophic carbon dioxide fixation in microorganisms. Microbiol Rev 52: 155–189

Taylor CB, Bariola PA, DelCardayré SB, Raines RT and Green PJ (1993) RNS2: A senescence-associated RNase of *Arabidopsis* that diverged from the S-RNases before speciation. Proc Natl Acad Sci USA 90: 5118–5122

Taylor GE Jr, Ross-Todd BM and Gunderson CA (1988) Action of ozone on foliar gas exchange in *Glycine max* L. Merr: A potential role for endogenous stress ethylene. New Phytol 110: 301–307

Thompson DM and Meagher RB (1990) Transcriptional and post-transcriptional processes regulate expression of RNA encoding the small subunit of ribulose-1,5-bisphosphate carboxylase differently in petunia and in soybean. Nucleic Acids Res 18: 3621–3629

Tingey DT, Standley C and Field RW (1976) Stress ethylene evolution: A measure of ozone effects on plants. Atmos Environ 10: 969–974

Todd GW (1958) Effect of ozone and ozonated 1-hexene on respiration and photosynthesis of leaves. Plant Physiol 33: 416–420