Low levels of ozone increase bean leaf maintenance respiration

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Pinto bean (Phaseolus vulgaris) plants were exposed to charcoal-filtered air with or without added low levels of ozone (90 nL· L–1). Dark respiration (CO2 efflux) by expanding primary leaves of the plants was measured and mathematically partitioned into growth and maintenance components. The growth respiration coefficient was unaffected by ozone, whereas the maintenance respiration coefficient increased 15%. Such a relative increase in maintenance respiration results in a diversion of energy and metabolic intermediates from growth processes.


Les plantes de haricot (Phaseolus vulgaris) sont exposées à de l’air filtré (charbon), additionné ou non d’ozone (90 nL· L–1). La respiration sombre (efflux de CO2) des feuilles primaires en expansion est mesuré et séparée mathématiquement entre les composantes croissance et maintien du métabolisme. Le coefficient de respiration de croissance n’est pas affecté par l’ozone alors que celui du maintien du métabolisme augmente de 15%. Une telle augmentation provient d’un détournement de l’énergie et des intermédiaires métaboliques des processus de croissance.

[Intaduit par la revue]

Introduction

Plant growth and productivity are inhibited by the air pollutant ozone at concentrations typically found in many areas of North America (Heck et al. 1984; Wang et al. 1986). Ozone may inhibit photosynthesis either indirectly via induced stomatal closure or directly by oxidative action on chloroplasts (Ormrod et al. 1981; Black et al. 1982; Reich and Amundson 1985). In addition to inhibition of apparent photosynthesis, ozone may increase the dark respiration rate (Dugger et al. 1966; Barnes 1972; Anderson and Taylor 1973; Pell and Brennan 1973; Reich 1983; Skarby et al. 1987), although effects of ozone on respiration have been studied less than effects on photosynthesis. Since net primary productivity is the balance of photosynthetic gains (minus photorespiratory losses) and respiratory losses, it is as important to understand effects of ozone on respiration as effects of ozone on photosynthesis.

Plant respiration is commonly considered in terms of two functional components: growth respiration and maintenance respiration (Thornley 1970; Lambers et al. 1983; Amthor 1984, 1986). The two respiratory components are not biochemically distinct. Growth respiration is associated with metabolism supplying energy and carbon skeletons for production of new phytomass. Maintenance respiration is associated with processes such as the turnover of labile molecules, the support of ion and metabolite gradients, and the acclimation to changing or stressful environments in existing phytomass. In the case of ozone stress, maintenance respiration includes the metabolic cost of repairing damaged cellular components (Sutton and Ting 1977).

Over the course of a growing season, approximately half the carbon gained during photosynthesis is lost to respiratory metabolism in many plants (Farrar 1985), and the respiratory loss is about evenly divided between growth and maintenance (Biscoe et al. 1975; Mogensen 1977; Hirota and Takeda 1978; Waring and Schlesinger 1985). Effects of air pollution, and in particular ozone, on these two functional components of respiration have not been previously examined. The purpose of the present study was to determine experimentally the effects of low levels of ozone on growth and maintenance respiration coefficients by plant leaves, using a functional model of respiration. This may partially explain observed reductions in plant growth due to ozone, provide information useful for strategies of selecting and breeding ozone-tolerant plants, and aid in modeling effects of ozone pollution on plant productivity.

Materials and methods

Measured CO2 efflux rates from expanding primary leaves of darkened pinto bean (Phaseolus vulgaris L.) plants were partitioned into growth and maintenance components with the following model.

\[ r = (g \cdot RGR) + m \]

where \( r \) is the leaf specific respiration rate (milligrams CO2 per gram dry weight per day), \( g \) is the growth coefficient (milligrams CO2 per gram dry weight), \( RGR \) is the leaf relative growth rate (change in grams dry weight per gram dry weight per day), and \( m \) is the maintenance coefficient (milligrams CO2 per gram dry weight per day). The coefficient \( g \) is a measure of the efficiency (not rate) with which assimilate is used during biosynthesis in growing tissues, whereas \( m \) represents the respiratory cost of maintaining existing phytomass. Estimates of \( g \) and \( m \) can be obtained by least-squares solutions of the model when \( r \) and RGR are experimentally measured. The reader is referred to Thornley (1970), Lambers et al. (1983), and Amthor (1984, 1986) for further details of this model.

Plants were grown from seed in pots containing a 1:1 mixture of peat and vermiculite with added macronutrients and micronutrients. These were housed in one of two controlled-environment growth chambers and irrigated daily with dionized water. Conditions within the chambers were as follows: 14 h light: 10 h dark; incident photosynthetic photon flux area density during the light period, 800—900 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) at plant height; air temperature, 24·19°C (light:dark); and relative humidity, 55—65%. Air entering the chambers was filtered through charcoal to remove ozone.

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Plants were exposed to charcoal-filtered air with or without additions of ozone. Ozone was generated by energetic excitation of oxygen (Ozone Research and Equipment Corp., model 03V5-0, Phoenix, AZ), and metered into one of the two growth chambers to maintain a level of 90 ± 9 nL (ozone)·L⁻¹ (air) during the middle 6 h of each photoperiod. The other growth chamber was a control treatment. A Monitor Labs (San Diego, CA) model 8410 ozone analyzer was used to measure ozone concentration in the chambers. The ozone concentration within the control chamber (and within the fumigation chamber the remaining 18 h of each day) varied during the day and from day to day but did not exceed 15 nL (ozone)·L⁻¹ (air). Fumigations began 8 days after planting and continued, for 4–9 days, until respiration was measured. Exposure duration varied in order to obtain plants with a range of primary leaf RGR while maintaining the initiation of fumigations at the same developmental stage. After half the data were collected, the control chamber was used for fumigations and the fumigation chamber became the control. This allowed the estimation of effects of the chambers per se on CO₂ efflux rates.

At the end of the normal photoperiod on the day of a respiration measurement (12–17 days after planting), plants were moved to the laboratory and placed in the dark. Respiration measurements were made during the second and third hour of the normal dark period. Primary leaf blades were cut from the petiole with a sharp razor blade under very dim light, gently rolled, and placed entirely within a closed 0.34-L system containing an infrared gas analyzer (LI-COR, model LI-6000, Lincoln, NE). This allowed us to maximize the ratio of leaf tissue to cuvette airspace and hence increase the reliability of the measurements. Preliminary experiments showed that no statistically significant effect of cutting the petiole on respiration rate existed under the protocol used. The rate of CO₂ efflux in the dark was measured for a 40-s period, which was preceded by an approximately 15-s equilibration period. During the 40-s measurement period CO₂ content within the 0.34-L system typically increased 6–12 μL. All respiration rates were normalized to a temperature of 24°C, which was near the mean value for the laboratory at the time of the measurements (minimum, 21.6°C; maximum, 25.9°C), by assuming a Q₁₀ of 2. After this measurement the leaf blade was dried at 80°C and weighed. The dry mass of the leaf blade and rate of CO₂ efflux were used to calculate r.

Leaf-blade RGR was estimated by measuring the relative increase in leaf-blade area during the 25–27 h prior to respiration measurement. Area increases were proportional to dry mass increases and the measures of RGR were accurate to about ±3%. During the study, r and RGR were estimated for leaves of 201 plants: 103 control and 98 ozone fumigated. The range in r was 101 to 204 mg CO₂·g dry weight⁻¹·day⁻¹ and the range in RGR was 0.00 to 0.16 per day.

Analysis of covariance was conducted to determine effects of ozone on g and m. The ozone treatment and growth chamber used were qualitative variables. The RGR was a continuous covariate, and r was the dependent variable.

**Results and discussion**

The data are illustrated in Fig. 1, where leaf-blade specific respiration rate, r, is plotted as a function of leaf-blade relative growth rate, RGR. The ordinate intercept is an estimate of m and the slope is an estimate of g. ○, leaves from plants grown in charcoal-filtered air; +, leaves from plants exposed to the ozone treatment. The broken lines are determined by the least-squares solution for each ozone treatment as given in the text.

![Fig. 1. Plot of leaf-blade specific respiration rate, r, as a function of leaf-blade relative growth rate, RGR.](image)

**Table 1. Analysis of covariance.** The ozone treatment and growth chamber are qualitative variables, while the relative growth rate is a quantitative covariate. The dependent variable is leaf specific respiration rate, r, which has been normalized to a temperature of 24°C.

<table>
<thead>
<tr>
<th>Factor</th>
<th>F-value</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone</td>
<td>8.90</td>
<td>0.0032</td>
</tr>
<tr>
<td>Growth chamber</td>
<td>1.78</td>
<td>0.18</td>
</tr>
<tr>
<td>Ozone × growth chamber</td>
<td>0.58</td>
<td>0.45</td>
</tr>
<tr>
<td>RGR</td>
<td>454.26</td>
<td>0.0001</td>
</tr>
<tr>
<td>RGR × ozone</td>
<td>0.20</td>
<td>0.66</td>
</tr>
<tr>
<td>RGR × growth chamber</td>
<td>0.69</td>
<td>0.41</td>
</tr>
<tr>
<td>RGR × ozone × growth chamber</td>
<td>0.01</td>
<td>0.97</td>
</tr>
</tbody>
</table>

*NOTE: Factors including RGR represent effects on g. Terms not including RGR represent effects on m. This analysis is associated with the partial sums of squares, i.e., the complete least-squares analysis. Each effect (main and interaction) is adjusted for all other effects. Probability of obtaining this value of F or a larger one by chance alone based on one degree of freedom for each factor.*
growth processes. In the context of a plant’s carbon balance, an increase in respiration is equivalent to a decrease in photosynthesis. Indeed, previous reports of inhibition of apparent photosynthesis by ozone may have been partially due to increased rates of respiration and not solely due to direct effects of ozone on stomatal physiology or photosynthetic function.

It has been previously suggested that plant maintenance expenditures are enhanced by exposure to ozone (Reich 1983), but this is the first published report to our knowledge providing quantitative data in support of this hypothesis. We feel it is simplest to attribute the increased value of $m$ to increased costs of repair of damaged cellular constituents due to ozone fumigation. This increase in $m$ will result in an altered plant carbon budget, which in turn is likely to lead to altered patterns of productivity in managed and natural ecosystems. We suggest that future experiments designed to develop dose–response functions of ozone effects on $m$ are needed.

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