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*Technical communication*

# Sensitivity of infrared water vapor analyzers to oxygen concentration and errors in stomatal conductance

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## Abstract

Use of infrared analyzers to measure water vapor concentrations in photosynthesis systems is becoming common. It is known that sensitivity of infrared carbon dioxide and water vapor analyzers is affected by the oxygen concentration in the background gas, particularly for absolute analyzers, but the potential for large errors in estimates of stomatal conductance due to effects of oxygen concentration on the sensitivity of infrared water vapor analyzers is not widely recognized. This work tested three types of infrared water vapor analyzers for changes in sensitivity of infrared water vapor analyzers depending on the oxygen content of the background gas. It was found that changing from either 0 or 2% to 21% oxygen in nitrogen decreased the sensitivity to water vapor for all three types of infrared water vapor analyzers by about 4%. The change in sensitivity was linear with oxygen mole fraction. The resulting error in calculated stomatal conductance would depend strongly on the leaf to air vapor pressure difference and leaf temperature, and also on whether leaf temperature was directly measured or calculated from energy balance. Examples of measurements of gas exchange on soybean leaves under glasshouse conditions indicated that changing from 21% to 2% oxygen produced an artifactual apparent increase in stomatal conductance which averaged about 30%. Similar errors occurred for 'conductances' of wet filter paper. Such errors could affect inferences about the carbon dioxide dependence of the sensitivity of photosynthesis to oxygen.

## Introduction

Change in the sensitivity of infrared carbon dioxide analyzers depending on the oxygen concentration of the background gas has long been recognized (Gale et al. 1975), and should be taken into account for accurate estimates of the oxygen dependence of photosynthetic carbon dioxide fixation. Absolute analyzers are more influenced by oxygen concentration than are differential analyzers (Gale et al. 1975). Some of the newer portable photosynthesis systems utilize matched absolute infrared analyzers (e.g. LiCor-6400, LiCor Inc., Lincoln Nebraska, and CIRAS-1, PP Systems, Hitchin, UK), and thus are more subject to this error than systems using differential analyzers. These newer portable photosynthesis systems also use infrared analyzers for measurements of water vapor concentration for the calculation of transpiration rates and stomatal conductances. The sensitivity of infrared analyzers to water vapor also depends on the oxygen concentration in the background gas (Gale et al. 1975). It seems not to be widely recognized, however, that changes in oxygen concentration potentially produce much larger errors in estimates of stomatal conductance than in estimates of photosynthetic rates. Errors in estimates of stomatal conductance resulting from changing oxygen concentrations could affect inferences about the carbon dioxide dependence of the oxygen sensitivity of carbon dioxide fixation, which are used, for example, in studies of the limitation of photosynthesis by triose phosphate utilization rate (Sage et al. 1989; Socias et al. 1993) and for *in vivo* estimates of Rubisco carboxylation/oxygenation kinetics (e.g. Bernacchi et al. 2001). Here we report that three different infrared water vapor analyzers were sensitive to the oxygen concentration in the background gas, to the extent that large errors occurred in stomatal conductances when oxygen concentration was changed, if the change in analyzer sensitivity was not taken into account.

## Materials and methods

Three different infrared water vapor analyzers were tested, LI - 6262, LI - 6400 (LiCor, Inc., Lincoln, Nebraska), and CIRAS-1 (PP Systems, Haverhill, Massachusetts). The LI - 6262 is a stand-alone carbon dioxide and water vapor analyzer, while the LI -6400 and the CIRAS-1 are photosynthesis systems incorporating matched absolute carbon dioxide and water vapor analyzers.

For the LI-6262, the instrument was configured as an absolute analyzer, and calibrated using air dried by passing through a column filled with magnesium perchlorate to set the 'zero' value, and the 'span' adjusted using air pumped through a dew point generator (LI-610). The Li-6400 and the CIRAS-1 systems were factory calibrated and configured as standard open systems, and provided with gas from the dew point generator at their inlet ports. The performance of the dew point generator was checked by passing the gas stream over an optical condensation dew point hygrometer before entering the infrared analyzers. In one set of tests, the effect of oxygen mole fraction on the output of the infrared analyzers was tested by comparing air and nitrogen as the sources of gas pumped through the dew point generator. This test was performed over a range of dew point temperatures. In another test, the analyzer outputs were compared over the range of 0–1 mole fraction of oxygen in nitrogen, with the mixing ratio controlled by a gas blending system using mass flow meters calibrated for nitrogen and oxygen.

Additionally, the CIRAS-1 system was used to measure stomatal conductance of soybean leaves under midday conditions in a glasshouse (air temperature 25–32 °C, inlet water vapor pressure 1–2 kPa, and a PPFD of 1700 lmol $m^{-2}$ $s^{-1}$ from a lamp. A 'broad leaf' cuvette with automatic temperature and light control was used. The temperature was set to track that of ambient air, and the boundary layer conductance to water vapor was 2.9 mol $m^{-2}$ $s^{-1}$ for each surface. The air flow rate was 400 ml $min^{-1}$. Leaves were first equilibrated in air until stomatal conductance and photosynthetic rates were constant, then the input gas stream was changed to 0.02 mole fraction of oxygen in nitrogen and stomatal conductance recorded when photosynthetic rates had re-stabilized, about 2 min later. Leaf temperature was estimated from standard energy balance equations in the system software. Stomatal conductances were calculated by the system software using standard gas exchange equations (Von Caemmerer and Farquhar 1981). This test was conducted on five days, with six leaves measured each day. On one of the measurement days, the effect of changing between air and 0.02 mole fraction of oxygen in nitrogen on the apparent stomatal conductance of a piece of wet filter paper partially covering the cuvette window was also determined. The wet filter paper covered about 20% of the cuvette window, producing approximately the same 'conductance' as leaves.

## Results

For all three infrared water vapor analyzers, switching from air to nitrogen as the background gas produced an apparent increase in the partial pressure of water vapor. The relative increase in apparent water vapor partial pressure with nitrogen was independent of the absolute water vapor partial pressure. The optical condensation dew point meter indicated that no change in dew point occurred when switching between air and nitrogen as the gas pumped through the dew point generator. For the LI-6262, the relative increase in apparent water vapor partial pressure averaged 3.9%, with a standard deviation of 0.9%, for $n = 6$ determinations. For the LI-6400, the mean increase was 4.0%, with a standard deviation of 1.2%, for $n = 8$. For the CIRAS-1 the mean increase was 4.1%, with a standard deviation of 1.0%, for $n = 10$.

The relative sensitivity of all of the analyzers to water vapor decreased approximately linearly with increasing oxygen in nitrogen over the whole range from mole fractions of 0 to 1 (Figure 1). The overall linear regression predicted that changing from air to nitrogen would increase the sensitivity by 3.9%.

Switching from air to 0.02 mole fraction of oxygen in nitrogen also increased the apparent water vapor partial pressure for both the reference and sample analyzers of the CIRAS-1 system. The magnitude (4.1%) was indistinguishable from that cause by switching between air and nitrogen. The increase in analyzer

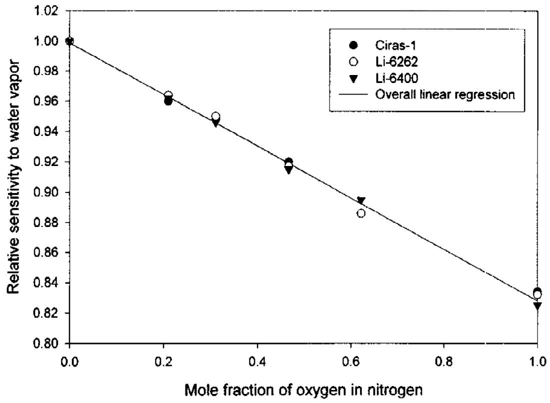


*Figure 1.* The sensitivity of three types of infrared analysers to water vapor as a function of the mole fraction of oxygen in nitrogen in the background gas. Values are expressed relative to the analyser sensitivities in nitrogen. The overall regression equation was: relative sensitivity = 0.999–0.171 (mole fraction), with $r^2 = 0.99$.

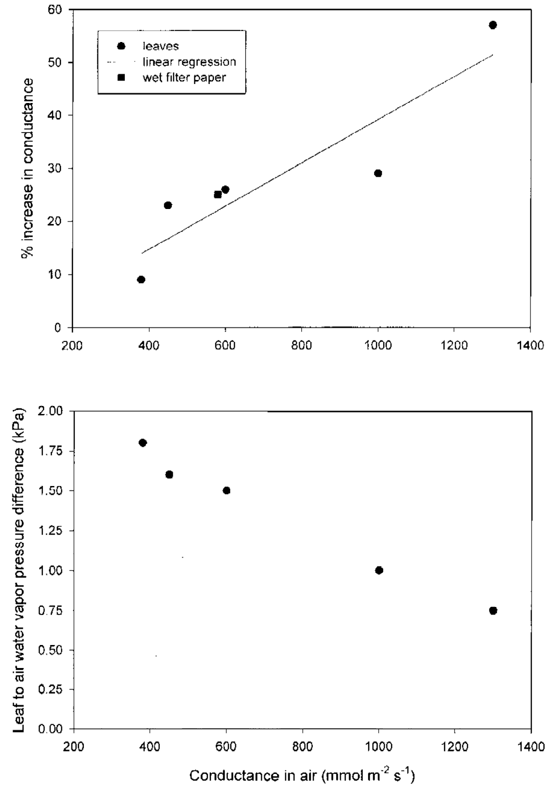


*Figure 2.* The relative increase in stomatal conductance caused by changing from air to 0.02 mole fraction of oxygen in nitrogen as the background gas, and the leaf to air difference in water vapor pressure during the measurement, as a function of the mean stomatal conductance in air. Measurements were made on five days under glasshouse conditions. Each point represents mean values for six leaves (or pieces of wet filter paper) on a given measurement day. The line indicates a linear regression. The apparent increase in conductance is an artifact of the change in sensitivity of the infrared analyzer to water vapor depending on the oxygen content of the background gas.

sensitivity to water vapor partial pressure with 0.02 mole fraction of oxygen in nitrogen compared with air increased the stomatal conductance calculated by the system software by 29% when averaged over days. The relative increase in conductance ranged from 9% to 57% on the different days, and was larger on days with higher average conductance and lower average LAVPD (Figure 2). The apparent conductance of wet filter paper also increased upon changing from air to 0.02 mole fraction of oxygen, to the same relative extent as for leaves (Figure 2).

## Discussion

The change in sensitivity of infrared analyzers to carbon dioxide and water vapor with the oxygen mole fraction of the background gas is thought to result from a change in the absorption spectrum of the gas being analyzed depending on the composition of the background gas (T. Doyle, PP Systems, personal communication). The error in analyzer output produced depends on the characteristics of the detector system. For absolute carbon dioxide analyzers, the change in sensitivity from air to nitrogen is typically about 3%. This potentially causes an error of about 3% in carbon dioxide flux rates, and also in the absolute value of carbon dioxide concentration outside the leaf. While these errors are not large, they are systematic and the corrections are simple. However, even though the change in sensitivity to water vapor is only slightly larger than that for carbon dioxide, the potential errors in stomatal conductance are much larger and depend strongly on the measurement conditions.

A change in sensitivity to water vapor of 4% with 0 or 0.02 vs. 0.21 mole fraction of oxygen in nitrogen would change transpiration rate about 4%. However, larger and variable errors in stomatal conductance would occur because of errors introduced into the estimate of the leaf to air water vapor pressure difference (LAVPD). At constant leaf temperature, the relative error in LAVPD caused by changing oxygen mole fraction would decrease with increasing LAVPD. For example, with a saturated vapor pressure (based on leaf temperature) of 3 kPa, and a vapor pressure in the air around the leaf of 2 kPa (i.e. LAVPD = 1.0

kPa), a 4% overestimate of the vapor pressure in the air around the leaf would produce an estimated LAVPD of 0.88 kPa for an error of 14%. If the vapor pressure in the air around the leaf had been 2.5 kPa (i.e.. LAVPD = 0.5 kPa), the erroneous estimate of LAVPD would be 0.4 kPa for an error of 25%. At constant LAVPD, the relative error in calculated LAVPD would increase with increasing leaf temperature. For example, with a saturated vapor pressure (based on leaf temperature) of 3.0 kPa and a LAVPD of 1.0 kPa, a 4% overestimate of the vapor pressure in the air around the leaf would produce a new LAVPD of 0.92 kPa, for an error of 9%, but if the saturated vapor pressure had been 4.0 kPa with a LAVPD of 1.0 kPa, the new estimate of LAVPD would be 0.88 kPa, for an error of 14%. Additional errors in LAVPD would be introduced if leaf temperature were calculated from leaf energy balance, as recommended in some systems. In our measurements of soybean leaves with the CIRAS-1 system, switching from air to 0.02 mole fraction of oxygen in nitrogen reduced calculated leaf temperatures on average about 0.3 °C. At a leaf temperature of 30 °C, a change of 0.3 °C would change LAVPD by about 0.07 kPa. Errors in transpiration rate caused by change in the sensitivity of infrared analyzers to water vapor with change in oxygen concentration are multiplicative with the errors in the estimates of LAVPD, sometimes producing large errors in leaf conductance. At high conductances, the separation of leaf conductance into boundary layer and stomatal conductance further increases the relative error in stomatal conductance, because the system software attributes all of the apparent change in leaf conductance to a change in stomatal conductance.

The increase in the oxygen-dependent error in stomatal conductance with increased stomatal conductance in our measurements with soybean leaves in the glasshouse primarily resulted from lower measurement LAVPD when conductance was high, although leaf temperature and reference water vapor pressure also varied from day to day. While the cause of the variation in stomatal conductances from day to day was the LAVPD in the glasshouse, high stomatal conductances by themselves lowered the LAVPD in the measurement cuvette.

While the sometimes large errors in stomatal conductance caused by changes in the oxygen content of the background gas have a complex relationship to the measurement conditions, they all propagate from the change in the sensitivity to water vapor of the infrared analyzer depending on the oxygen content of the background gas. A purely physical cause of the errors was confirmed by the use of wet filter paper in place of leaves. The change in sensitivity of the analysers is easily measured, and adjusting the analyzer sensitivity would eliminate the errors in stomatal conductance.

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