Available online at www.sciencedirect.com
ScienceDirect
Environmental and Experimental Botany 58 (2006) 169–179
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Physiological responses to cumulative ozone uptake in two white clover (*Trifolium repens* L. cv. Regal) clones with different ozone sensitivity

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Received 5 May 2005; received in revised form 26 June 2005; accepted 26 July 2005

Abstract

Critical ozone levels must be determined to assess ozone damage on plants, but the cumulative ozone exposure concept of 'AOT40' fails to consider actual ozone uptake via the stomata. While the use of ozone fluxes to assess ozone-induced plant responses is mechanistically appropriate, few studies have examined the relationship between cumulative ozone fluxes and physiological dysfunction. Physiological differences between a widely used ozone-sensitive (NC-S) and ozone-resistant (NC-R) bio-monitor clone of white clover (*Trifolium repens* L. cv. Regal) were studied and related to cumulative ozone fluxes (CUO_3). Generally, no physiological effect of ozone uptake was detected in the NC-R clone, whereas there were negative responses in the NC-S clone for most leaf gas exchange parameters, including net photosynthesis (A_{sat}) and carboxylation capacity (V_{cmax}). Stomatal conductance (g_s) was not significantly different between clones in ozone-free conditions, but g_s decreased significantly for the NC-S clone during ozone exposure. The NC-S clone showed higher electron transport rates but lower non-photochemical quenching under high photon flux densities and elevated ozone compared to NC-R clone. Our results suggest that avoiding ozone-induced damage depends on the ability of different genotypes to reduce O_3 uptake through stomatal closure and on the capacity for non-photochemical quenching to scavenge reactive oxygen. Relating key physiological parameters to cumulative ozone fluxes contributes to refining flux-based ozone uptake models used in setting critical ozone levels to alleviate the detrimental impact of O_3 exposure on vegetation.

Keywords: Cumulative ozone; Ozone flux; Non-photochemical quenching; Photosynthesis; *Trifolium repens*; Clones; Electron transport rates

1. Introduction

Tropospheric ozone is considered to be the most widespread atmospheric pollutant and can have a major impact on plant performance and growth (Reich, 1987; Musselman and Massman, 1999). Critical levels of ozone that are commonly used for evaluating impacts on crops and natural vegetation are based on the AOT40 concept (Fuhrer et al., 1997; Fuhrer, 2002), as most recently used in the United Nations-Economic Commission for Europe (UN-ECE) Gothenburg protocol. Although other cumulative ozone exposure indices are used, such as SUM06 (Lefohn, 1992), AOT40 represents the accumulated ozone exposure over a threshold of 40 ppb during daylight hours, and was accepted in Europe for setting critical levels to enable broad-scale, quantitative assessements of ozone impact on crops (Level I assessment; Fuhrer, 2000). However, it is generally accepted that the ozone impact on plants is more accurately determined by the amount of ozone taken up by the leaf via the stomata than by concentration-based exposure levels like the AOT40 (Reich, 1987; Fuhrer, 2002; Karlsson et al., 2004b; Pleijel et al., 2004).

Assessing ozone impacts on plants thus requires understanding ozone fluxes to the plant and depends on the quantitative relationships between the cumulative amount of ozone absorbed by the leaves, the capacity of the internal ozone detoxification, and the plant response to the effective dose (Fuhrer et al., 1997; Meyer et al., 1997). Since ozone uptake into plants is strongly mediated by stomata (Fredericksen

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doi:10.1016/j.envexpbot.2005.07.007

et al., 1996), ozone flux considers both the external uptake of ozone as well as the internal physiology (termed Level II assessment by UN-ECE; Fuhrer et al., 1997). Hence, ozone flux rates are considered to provide a more detailed and physiologically meaningful relationship of plant damage and yield loss (Emberson et al., 2000). However, utilizing them requires more complicated modeling and extensive measurements than the AOT40 concept, because many factors influence the actual ozone uptake by the plant. These factors include among others stomatal conductance, which itself integrates environmental conditions like temperature, vapor pressure deficit and soil moisture (Weber et al., 1993; Emberson et al., 2000; Karlsson et al., 2004; Wieser and Emberson, 2004).

A complimentary approach for assessing ozone impacts among different geographical areas uses visible or physiological damage in a common species to monitor ozone impacts across a wide region. One so-called bio-monitor is white clover (*Trifolium repens* L.), a widespread species in crop and native ecosystems. The clover clone system has been a useful tool for bio-monitoring ozone impacts in the USA and Europe (Heagle et al., 1994, 1995; Fumagalli et al., 2003; Karlsson et al., 2003) and relies on two clones of white clover (*T. repens* cv. Regal) first identified by Heagle et al. (1994): an ozone-resistant clone (NC-R) and an ozone-sensitive clone (NC-S) that clearly differ in their visible injury responses to ozone exposure. Despite the observed differences in ozone responses between these two clones, such as differences in biomass and the appearance of necrotic lesions, relatively little work has identified physiological differences underlying these different responses (Heagle et al., 1995; Heagle and Stefanski, 2000; Postiglione et al., 2000) especially as a function of ozone fluxes. Combined measurements of gas exchange and chlorophyll fluorescence could generate an image of the physiological performance of these clones and their differences in ozone sensitivity.

In this study, we examine physiological differences between the two commonly used white clover clones (referred to as NC-S and NC-R; Heagle et al., 1994). We hypothesized that the sensitive clone will differ from the resistant clone in net photosynthesis (A_{net}), stomatal conductance (g_s) and photosynthetic characteristics (V_{cmax} and J_{max}) under ozone exposure. We also relate these physiological responses to cumulative ozone fluxes (CUO_3) as opposed to ozone concentrations. Since ozone uptake and high light intensities are two stress factors that often co-occur in nature, we studied the response of both clones to ozone under different photon flux densities. Hence, we hypothesized that if oxidative stress is enhanced at high photon flux densities, the NC-S clone shows more damage. This last mentioned hypothesis was related to electron transport and quenching parameters because ozone damage could alter the efficiency of light capturing processes. These relationships can be used towards a more detailed and physiologically more meaningful Level II assessment of ozone effects (Fuhrer et al., 1997) and possibly help to confirm an ozone critical level based on ozone fluxes rather than concentrations.

2. Materials and methods

Two independent fumigation experiments (Experiments 1 and 2) were conducted, each with a duration of 5 days (Table 1). The first experiment aimed to test the effects of cumulative ozone flux on leaf gas exchange characteristics of white clover (*T. repens* L. cv. Regal) clones with contrasting sensitivity to ozone (NC-S and NC-R), while the second experiment focused on the effects of cumulative ozone on electron transport and quenching characteristics of these clones elucidated via chlorophyll fluorescence measurements (Table 1). During both experiments, day/night-time conditions in the environmentally controlled growth chambers were kept constant with air temperature of 24/18 °C, relative atmospheric humidity of 60/71% and photosynthetic photon flux density (PPFD) of 200 μmol m^{-2} s^{-1} at plant level and day-length of 13 h. All physiological measurements were performed on the third fully expanded leaf from the end of the stolon because the third and fourth leaves of clover have highest photosynthetic rates and stomatal aperture (Werner et al., 1988; Heagle et al., 1993).

2.1. Plant material and fumigation treatments

Seven weeks prior to each experiment, 36 cuttings of each white clover clone were planted, an ozone-resistant and an ozone-sensitive one, originating from North Carolina State University (Raleigh, NC, USA). Each plant was grown in 4.6 l pots filled with an artificial medium of peat soil, vermiculite and osmocote slow-release fertilizer (4 g l^{-1}) and intermediately cut, 4 weeks after planting. Shortly before the beginning of each experiment, 36 pots of each clone were equally distributed among four environmentally controlled

Table 1
Summary of the experimental conditions and measurements during the first and second ozone fumigation experiments (Experiments 1 and 2)

Parameter	Experiment 1	Experiment 2
Ozone exposure regime	7 h/day, 5 days duration	7 h/day, 5 days duration
Average ozone concentration (each chamber)	0, 40, 80, 110 ppb	0, 40, 65, 95 ppb
Clones	NC-R, NC-S; 9 plants/clone/treatment	NC-R, NC-S; 9 plants/clone/treatment
Physiological measurements	Gas exchange (A–C_i) on days 2, 4 and 9	Chlorophyll fluorescence quenching on days 1, 3 and 5
Conductance measurements	Exposure days 1, 3 and 5	Exposure days 2 and 4

Both experiments were conducted in July 2000, 2 weeks apart from each other.