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Setting ozone critical levels for annual Mediterranean pasture species: Combined analysis of open-top chamber experiments

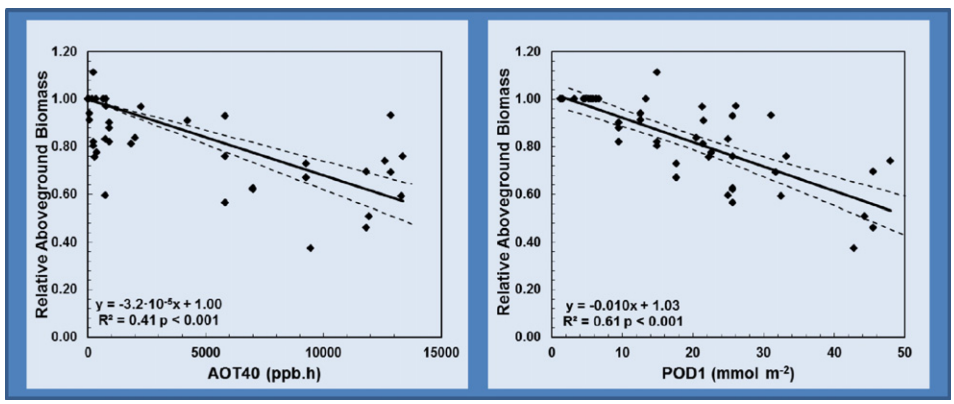
J. Sanz [a,*], I. González-Fernández [a], S. Elvira [a], R. Muntifering [b], R. Alonso [a], V. Bermejo-Bermejo [a]

[a] Ecotoxicology of Atmospheric Pollutants, CIEMAT, Avda. Complutense 40, 28040, Madrid, Spain
[b] Department of Animal Sciences, Auburn University, Auburn, AL 36849, USA

HIGHLIGHTS

- O_3 critical levels are developed for annual Mediterranean pasture species.
- Functions available for aboveground biomass, consumable food value and seed biomass.
- Functions were derived only for sensitive species.
- O_3 flux indices performed better than the AOT40.
- Functions valid only for risk assessment not suitable for quantification of effects

GRAPHICAL ABSTRACT



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ABSTRACT

Annual Dehesa-type pastures comprise semi-natural vegetation communities dominated by annual species characteristic of the Mediterranean basin areas of Southern Europe. This study analyses all the datasets available on the effects of ozone (O_3) on annual pasture species in order to review and propose new exposure- and flux-based O_3 critical levels (CLes) following the methodology of the Convention on Long-Range Transboundary Air Pollution (CLRTAP). Based on the potential effect on pastures main ecosystem services, the availability of data and the statistical significance of the regressions, three variables have been selected for establishing CLes: total above-ground biomass, consumable food value (CFV), as a nutritional quality index, and reproductive capacity based on flower and seed production. New CLes proposed for a 10% loss (with 95% confidence intervals between brackets) of above-ground biomass and reproductive capacity were, respectively, AOT40 = 3.1 (2.6, 3.8) and 2.0 (1.5, 2.8) ppm h and POD1 = 12.2 (8.9, 15.5) and 7.2 (1.1, 13.3) mmol m^{-2}. The provisional AOT40- and POD1-based CLes for CFV were 2.3 (1.6, 4.0) ppm h and 4.6 (2.7, 6.5) mmol m^{-2} respectively. By using only O_3-sensitive species for the exposure and dose-response functions, the proposed CLes should be used for risk assessments. Their use for quantifying O_3 damage may lead to an overestimation of the effects.

1. Introduction

Gas emissions from human activities into the atmosphere have substantially changed its composition, causing increased concentrations of tropospheric ozone (O_3) (Vingarzan, 2004). This pollutant is a powerful

* Corresponding author.
E-mail addresses: j.sanz@ciemat.es (J. Sanz), ignacio.gonzalez@ciemat.es (I. González-Fernández), susana.elvira@ciemat.es (S. Elvira), muntirb@auburn.edu (R. Muntifering), rocio.alonso@ciemat.es (R. Alonso), victoria.bermejo@ciemat.es (V. Bermejo-Bermejo).

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oxidant that induces damage to living organisms (Weschler, 2006; Lenka and Lenka, 2012). During the late fifties in California, in areas with air masses polluted by atmospheric oxidants, small, brown to black, discrete, punctate lesions appearing on the upper part of vine leaves (Richards et al., 1958) and flecking on tobacco leaves (Heggestad and Middleton, 1959) were identified. This was the first evidence of the toxic nature of O_3 on vegetation. Since then visible damage has been detected in a large number of plant species distributed through a sizeable number of countries (Mills et al., 2011a), serving to verify the importance of the toxic effects of O_3 on vegetation. The effects of O_3 on agricultural crops and forest trees have been extensively studied and documented, however, on herbaceous vegetation they are less known (CLRTAP, 2010) due to the number of species and the complexity of their communities (Caballero et al., 2009).

The Dehesa, a traditional agro-silvopastoral system comprising an open oak forest with annual pasture understory, occupies $>6\cdot10^6$ ha in the Iberian peninsula. Its ecological functioning is conditioned by two fundamental characteristics, the Mediterranean climate and a low soil fertility (Olea and San Miguel-Ayanz, 2006). Today it is protected by the Habitat Directive 92/43/EEC (Annex 1 habitat 6310) and included in the Natura 2000 network, as an example of the sustainable management of natural resources. Dehesas hold different herbaceous communities, mainly composed by annual species providing fodder for wildlife and livestock. Their great annual and seasonal variability, in terms of production and nutritional quality, is related with the variability of the climatic conditions of the area (Vazquez-de-Aldana et al., 2008). Besides, these pastures present a high floristic diversity which ranges 102–135 species per 0.1 ha^{-1}, with the most prevalent families being *Gramineae*, *Leguminosae* and *Compositae* (Marañon, 1985; García del Barrio et al., 2014). Inter-annual meteorological variability characteristic of the Mediterranean climate can bring early droughts leading to plants dying even before producing seeds, but in more humid years the life span can extend until early summer. Therefore, annuals have evolved developing transient and persistent seed banks. Seed germination is diversified depending on species, soil moisture, temperature and light and it can even be partially delayed until more favourable years (Peco et al., 2009). This leads to a characteristic inter-annual change in the species composition of the pasture (Vazquez-de-Aldana et al., 2008).

In the past decade, several studies have been conducted to evaluate the O_3 effects in Mediterranean herbaceous communities within the dehesas. Results indicated some legume genus like *Trifolium* among the most O_3 sensitive but others tolerant like *Ornithopus* or *Anthyllis* (Gimeno et al., 2004a), showing not only a high variability between but also within families. Therefore, the heterogeneous response of the species to the pollutant might change species competitiveness affecting the biodiversity and nutritional quality of the pastures (Calvete-Sogo et al., 2016). Some of the O_3 effects described for sensitive annuals include: the appearance of visible characteristic symptoms (Chaudhary and Agrawal, 2013); decreasing growth rate (Gimeno et al., 2004a); senescence acceleration (Bermejo et al., 2003); altered assimilated carbon allocation (Sanz et al., 2005); reduced seed production (Gimeno et al., 2004b; Sanz et al., 2007); changes in the structure of cell walls, in particular the increase in the lignin content (Sanz et al., 2014); loss of forage quality (Sanz et al., 2014); and modification of the phenological cycle usually shortening the growing season (Sanz et al., 2011).

Tropospheric O_3 is currently considered as the most phytotoxic air pollutant given the large number of sensitive plant species and the geographic extent of the problem. Accordingly, this pollutant has been included in international policies and programmes to develop environmental management strategies which control air pollution like the UNECE Convention on Long-Range Transboundary Air Pollution (CLRTAP). The CLRTAP developed the methodology of Critical Levels to establish environmental criteria based on the sensitivity of vegetation to O_3 and other gaseous pollutants to negotiate protocols for reducing atmospheric emissions.

Critical levels (CLe) of O_3 were first established in 1996 and have been revised periodically based on new knowledge (Mills et al., 2011b). Today CLe for vegetation are defined as: "concentration, cumulative exposure or cumulative stomatal flux of atmospheric pollutants above which direct adverse effects on sensitive vegetation may occur according to present knowledge". The CLe are calculated using exposure and dose-response relationships using O_3 concentration-based indices like the AOT40, which is the exposure index currently used by the European legislation on air quality (2008/50/EC), or O_3 flux-based indices (POD_y) more recently developed under the frame of the CLRTAP. However, some important limitations and uncertainties have been recognized when AOT40 is used. Primarily, because the impact depends on the amount of O_3 absorbed by the leaves, while this index considers the O_3 concentration in the air at canopy height. As a result, current studies focus on developing stomatal flux-based CLe (CLe_f), as plant responses are generally more closely related to the O_3 dose absorbed through the stomata than to O_3 exposure (Mills et al., 2011b; González-Fernandez et al., 2014).

This study analyses the available data sets from studies conducted on Mediterranean Dehesa annual herbaceous species following a similar experimental protocol, open-top chamber (OTC) experiments and plants growing in pots, with the aim of reviewing and proposing new O_3 CLe for this type of vegetation under the framework of the CLRTAP (2010).

2. Materials and methods

2.1. Open-top chamber experiments

Data were analysed from five independent experiments in an OTC experimental field located in a rural area in the northeast of the Iberian Peninsula (Tarragona, 40° 41′ N, 0° 47′ E), away from local sources of air pollution. For a detailed description of the experimental facility see Alonso et al. (2001). The experiments were conducted over four campaigns from 2000 to 2003. The therophyte species assayed belonged to the *Leguminoseae* (10 species) and *Gramineae* (9 species) families, two of the most prevalent families in Mediterranean grasslands of high grazing and ecological value (Vazquez-de-Aldana et al., 2008). All studies followed a similar protocol: plants were grown in pots on similar artificial substrate and were kept at field water capacity. The O_3 exposure length was similar, 45 days on average. Filtered air treatments (CFA) were used on all experiments and considered as the control treatment. In some experiments the plants were exposed to other factors such as soil N availability and plant-plant competition in order to observe possible interactions with the O_3. However, interactions between factors are not discussed herein. Ozone effects were analysed considering the average effect across the levels of the non-O_3 factors. Further details regarding the species, exposure levels and dates, parameters measured, factor assayed and related references are indicated in Table 1.

2.2. Response parameters and ozone indices

Response parameters studied can be classified into three categories: biomass, grazing quality and reproductive capacity. Biomass parameters studied were: aerial green, senescent, total aerial (sum of green and senescent biomass), subterranean and total biomass (sum of aboveground and subterranean biomass). The ratios senescent/green biomass and total aerial/subterranean biomass were also calculated. Nutritional quality parameters from the sequential separation of cell wall constituents according to the method of Van Soest et al. (1991) were studied: the Neutral Detergent Fibre (NDF) fraction containing insoluble constituents of the cell wall (hemicellulose, cellulose, lignin and other recalcitrant materials); the Acid Detergent Fibre (ADF) fraction, consisting of lignocellulosic material and other recalcitrant materials; and the Acid Detergent Lignin (ADL) fraction, consisting of lignin and other recalcitrant materials. Two indices of nutritional quality were also calculated considering these parameters: The relative food value (RFV), which integrates the NDF and ADF (Linn and Martin, 1989), and the consumable food value (CFV), which integrates NDF and ADF with the green biomass loss (González-Fernández et al., 2008). Given the strong correlation