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Using *Pinus uncinata* to monitor tropospheric ozone in the Pyrenees

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ABSTRACT

Field metrics were investigated using the conifer species *Pinus uncinata* for the biomonitoring of tropospheric ozone in the Pyrenees of Catalonia, Spain. The Ozone Injury Index (OII) was investigated piecewise for improvement as a biomonitoring field metric for using sensitive conifer species to monitor tropospheric ozone across variable environmental conditions. The OII employs a weighted average of visual chlorotic mottling (VI), needle whorl retention (RET), needle length (LGT), and crown death (CD). Of note, VI includes subcomponents VI-Amount (% of symptomatic needles) and VI-Severity (% of chlorotic mottling on symptomatic needles) and RET includes the FWHORL subcomponent (average fraction of needles retained per whorl). All components and subcomponents of the OII correlated better to multiple year ozone exposure compared to single year ozone exposure measurements. VI-Severity and FWHORL modeled over half the variability of the three year average of ambient ozone concentrations ($P < 0.0001$, $R^2 = 0.53$, RMSE = 2.73). Combining the biomonitoring metrics with GIS models related to landscape-scale variability in plant water relations resulted in considerable improvement in the ozone exposure model explanatory power ($P < 0.0001$, $R^2 = 0.90$, RMSE = 1.35) including the parameters VI-Amount, VI-Severity, elevation, slope and topographic curvature.

1. Introduction

Biological indicator species are often used in biomonitoring the ambient concentrations and long-term impacts of ozone due to their cost effectiveness in terms of supplies and time. For example, in much of Europe, cultivated tobacco plants with different sensitivities to ozone and other pollutants have been used for biomonitoring efforts (Ribas et al., 1998; Ribas and Peñuelas, 2003; www.eurobionet.org). Meanwhile, in the USA sensitive native species such as *Pinus ponderosa* and *Pinus jeffreyi* have long been used as bio-indicator species for nationwide monitoring of air pollution (Duriscoe et al., 1996; www.fiaozone.net). However, using cultivated species outside of their normal distributions where sufficient environmental hardening may not be possible, such as in montane environments, can lead to significant errors and even plant death thus making them poor indicators of ozone conditions. Also, observational studies of native bioindicator species suffer from the effects of environmental variability, particularly in Mediterranean climates where there can be a large seasonal disconnect between ambient ozone mixing ratios (O_3 ppb) and stomatal uptake of ozone (Matyssek et al., 2007; Panek, 2004; Peñuelas et al., 1999). As such, efforts to understand the potential for biomonitoring of ambient ozone must consider not only the physiological injury of ozone to bioindicator species, but also incorporate some direct or indirect measure of ozone stomatal conductance, especially in highly drought stressed environments (Manning, 2005; Matyssek et al., 2007). Therefore, many recent studies (Elvira et al., 2007; Filella et al., 2005; Gimeno et al., 1996; Inclan et al., 1999; Novak et al., 2003; Peñuelas et al., 1999; Ribas et al., 2005a) have shifted toward identifying native bioindicator species that are sensitive to tropospheric ozone and ways to account for environmental conditions that cause variability in ozone stomatal uptake.

If the environmental variability in plant water relations can be directly or indirectly assessed, then it could be used to better understand the interrelationship between ozone exposure, uptake, and injury. Using GIS to indirectly measure plant water availability and potential usage (Hofierka and Suri, 2002; Iverson et al., 1997; Urban et al., 2000) in combination with field studies of ozone injury takes advantage of natural gradients which affect ozone uptake and can provide a means to integrate environmental conditions

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with physiological measures of ozone injury. Many environmental parameters indirectly related to plant water availability, drought stress, evapotranspiration and thus ozone stomatal conductance are readily modeled using satellite-based digital elevation models (DEMs) in a GIS, such as elevation, slope, aspect, topographic curvature (Zeverbergen and Thorne, 1987), topographic convergence (Beven and Kirkby, 1979) solar radiation (Hofierka and Suri, 2002) and distance to water sources (Watson and Philip, 1985). Each GIS variable estimates environmental factors indirectly related to plant water usage (elevation, aspect, and solar irradiance) and availability (distance to water sources, slope, topographic curvature, and topographic convergence). By including proxies related to both plant water usage and availability, an improved approximation of ozone stomatal conductance can be obtained *via* combinations of GIS variables.

As a secondary, sun-catalyzed, pollutant, tropospheric ozone is inherently trans-boundary (Finlayson-Pitts and Pitts, 1997; van Pul et al., 1998), commonly found in higher concentrations some distance from its precursor sources, and frequently surpassing recommended air quality standards for human and environmental health (Hayhoe et al., 2004; Skarby et al., 1998; Smith et al., 2003). The accumulation of tropospheric ozone increasingly affects Mediterranean-type climate areas, with implications to high population densities and highly productive agriculture and silviculture (Brunekreef and Holgate, 2002; Inclan et al., 1999; Paoletti, 2006). Ozone affects plant health through stomatal uptake (Díaz-de-Quijano et al., 2011a; Matyssek et al., 2007; Panek, 2004), causing chlorosis (Arbaugh et al., 1998; Ribas et al., 2005b; Vollenweider et al., 2003), accelerated leaf senescence (Pronos et al., 1978; Ribas et al., 2005b), growth reduction (Panek and Goldstein, 2001), changes in carbon allocation (Díaz-de-Quijano et al., 2011b; Grulke and Balduman, 1999; Grulke et al., 2002), and forest composition (Miller, 1973, 1993; Miller et al., 1995).

By carefully combining a suite of the various measurable physiological injury symptoms of tropospheric ozone to plant health, a variety of indexes have been developed. One of the most thoroughly developed and applied is the Ozone Injury Index (OII), which is specific to conifer species (Arbaugh et al., 1998; Duriscoe et al., 1996; Miller et al., 1995) was developed specifically for ponderosa (*P. ponderosa*) and Jeffrey pines (*P. jeffreyi*) in the United States, but offers great potential for biomonitoring tropospheric ozone using other conifer species. In particular, the mountain pine (*Pinus uncinata* Ram.) in the Catalan Pyrenees exhibits specific visual symptoms related to foliar ozone injury which have been verified microscopically (Díaz-de-Quijano et al., 2011a; according to Vollenweider et al., 2003), suggesting the mountain pine as a species particularly sensitive to elevated tropospheric ozone. Similar tree symptoms, including chlorotic mottling (Díaz-de-Quijano et al., 2012; Kefauver et al., 2012), accelerated needle senescence (Kefauver et al., 2012), decreased root biomass (Díaz-de-Quijano et al., 2012); and increased tree mortality (Díaz-de-Quijano et al., 2011b) have been observed in *P. uncinata* in controlled studies and *in situ* in the Catalan Pyrenees. The OII represents over 20 years of ozone biomonitoring research development in the Sierra Nevada Mountains, CA, USA (Duriscoe et al., 1996), and is expected to prove similarly useful in conversion and application the ozone-sensitive mountain in the Pyrenees Mountains in terms of its measurable ozone-induced physiological changes at the needle, whorl and tree levels.

The objective of this research is to test the utility of *P. uncinata*, the mountain pine, as a bioindicator for monitoring ambient tropospheric ozone concentrations. Various ozone-induced physiological injury symptoms using multiple years of conifer needles, taken from the Ozone Injury Index, were measured to assess cumulative exposure to tropospheric ozone. Emphasis was placed on finding an optimal combination of OII components and subcomponents, as well as observation period in the transfer of the OII to new species. This study was conducted in a remote Mediterranean montane environment where climate patterns and topographical features produce substantial ozone stomatal uptake variability. The use of GIS spatially explicit variables related to plant water usage and availability to account for stomatal conductance variability in Mediterranean montane habitats is proposed. GIS models that approximate environmental gradients related to variable air pollution uptake at the landscape scale were combined with optimized field biometrics of ozone injury to provide the necessary link between a measured ozone exposure and variable ozone stomatal uptake in a Mediterranean climate.

2. Materials and methods

2.1. Study site characteristics

The transects of “Guils” and “Meranges” in Catalonia, Spain include similar elevation ranges as California pine bioindicator species, crossing at 2100 m and extending down to 1100 m. The East–West facing transect of Guils with its generally North facing aspect provides a good contrast to the North–South oriented transect of Meranges with its generally South facing aspect. The two transects include similar elevation ranges, crossing at 2100 m at one shared point (Fig. 1, made with QGIS, http://qgis.osgeo.org). This part of the Catalan Pyrenees is classified as Mediterranean montane and also features large glacially carved granitic valleys. The vegetation is dominated by mountain pine mixed with, Pyrenean Broom (*Cytisus purgans*), common juniper (*Juniperus communis* subsp. *alpina* var. *alpina*), and alpenrose (*Rhododendron ferrugineum L.*). The North aspect of the Guils transect provides a more mesic contrast to the more xeric South facing aspect Meranges. Gradients of ozone concentration in the Catalonia follow air flow patterns off the Mediterranean Sea up from the coastal urban centers such as Barcelona into the Central Catalan Pyrenees to the remote regions of Meranges and Guils de la Cerdanya (Díaz-de-Quijano et al., 2009; Ribas and Peñuelas, 2004). The Central Catalan Pyrenees in this region experiences a prolonged summer drought and moderate winter snowpack. The dominant soil parent material for the study site is granitic with a highly variable soil depth.

2.2. Passive monitoring of air pollution

The air pollution passive monitoring sampling included ozone, mono-nitrogen oxides (NOx), and volatile organic compound (VOCs) ambient concentrations, and temperature and humidity monitoring at ten stations along altitudinal gradients (1100–2300 m) of two cross-mountain transects conducted by the CREAF lab at the Autonomous University of Barcelona. The passive sensors were developed by Radiello (www.sigma-aldrich.com/radiello). Temperature and humidity monitors were used in the processing of the passive sensor absorption rates for calculation of the exposure levels. Ten passive sensor sites were collocated with two active sensors (at 1100 m and 2100 m) at 1300, 1500, 1800, 2100, 2200, and 2300 m along each transect. Along these transects, air pollution sampling was conducted at ten sites distributed at altitudinal intervals. Thus, the sampling distribution is even in regards to altitude, but not geometric distance as a result of local topography. Passive sensor placement was at a height of 2 m in open to semi-open areas (not within dense forest) with three-sided protective coverings. Ozone concentration data was collected monthly November to March and biweekly April to October from 2004 to 2007 (see Díaz-de-Quijano et al., 2009; Ribas and Peñuelas, 2006 for details).