Environmental Pollution xxx (2013) 1–7

Contents lists available at SciVerse ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Perchlorate content of plant foliage reflects a wide range of species-dependent accumulation but not ozone-induced biosynthesis☆,☆☆

D.A. Grantz [a,*], K.O. Burkey [b], W.A. Jackson [c], H.-B. Vu [a], M.T. McGrath [d], G. Harvey [e]

[a] Department of Botany and Plant Sciences, University of California at Riverside, Kearney Agricultural Center, 9240 South Riverbend Avenue, Parlier, CA 93648, USA
[b] USDA/Agricultural Research Service, Plant Sciences Research Unit, 3127 Ligon Street, Raleigh, NC 27607, USA
[c] Texas Tech University, Lubbock, TX, USA
[d] Cornell University, Riverhead, NY, USA
[e] U.S. Air Force, Wright Patterson AFB, OH, USA

ARTICLE INFO

Article history:
Received 13 February 2013
Received in revised form
23 March 2013
Accepted 25 March 2013

Keywords:
ClO_4^-
Perchlorate
Gas exchange
Air pollution
Oxidants
Crop contamination
Ozone
O_3

ABSTRACT

Perchlorate (ClO_4^-) interferes with uptake of iodide in humans. Emission inventories do not explain observed distributions. Ozone (O_3) is implicated in the natural origin of ClO_4^-, and has increased since pre-industrial times. O_3 produces ClO_4^- *in vitro* from Cl^-, and plant tissues contain Cl^- and redox reactions. We hypothesize that O_3 exposure may induce plant synthesis of ClO_4^-. We exposed contrasting crop species to environmentally relevant O_3 concentrations. In the absence of O_3 exposure, species exhibited a large range of ClO_4^- accumulation but there was no relationship between leaf ClO_4^- and O_3, whether expressed as exposure or cumulative flux (dose). Older, senescing leaves accumulated more ClO_4^- than younger leaves. O_3 exposed vegetation is not a source of environmental ClO_4^-. There was evidence of enhanced ClO_4^- content in the soil surface at the highest O_3 exposure, which could be a significant contributor to environmental ClO_4^-.

1. Introduction

Perchlorate (ClO_4^-) salts are detected across large areas of the environment. The ClO_4^- anion is a tetrahedral cluster of oxygen with a central chlorine, exhibiting similar ionic radius and charge density to iodide. It therefore disrupts thyroid metabolism in mammals, including humans, by interference with uptake of iodide. In plants ClO_4^- competes for uptake with other anions such as NO_3^-.

ClO_4^- is highly soluble in water and does not adsorb substantially to mineral or organic constituents of soils. There appear to be atmospheric sources of naturally occurring ClO_4^-, derived from atmospheric oxidation of Cl^- and other Cl species, potentially mediated by lightning, ultraviolet radiation, or tropospheric O_3 (Michalski et al., 2004; Dasgupta et al., 2006; Rajagopalan et al., 2006; Jaegle et al., 1996; Kang et al., 2006; Rao et al., 2007, 2012). In areas with significant rainfall it is readily leached out of surface strata into ground and surface waters (Urbansky and Brown, 2003). In arid regions it accumulates at the surface and considerable concentrations have been detected (Rao et al., 2007; Jackson et al., 2010). If other areas are equally prolific sources of ClO_4^-, but lack the concentrating mechanism, this would imply widespread, multi-media dispersal of ClO_4^- across the environment. ClO_4^- occurrence at significant concentration in surface waters such as the Colorado River, and in ground waters such as the Ogalalla Aquifer in the Midwestern U.S. emphasize the breadth of the problem (Dasgupta et al., 2006; Rajagopalan et al., 2006).

In addition to natural atmospheric sources, a number of anthropogenic point sources have been identified. These include industrial and military application as a rocket fuel, in munitions, in consumer products such as fireworks and highway flares, and in Chilean nitrate fertilizer which contains high natural concentrations of ClO_4^-. Evidence of ClO_4^- at significant levels in other fertilizers, some of which may contain Chilean nitrate, has been inconsistent (e.g. Susarla et al., 1999, 2000b; Urbansky et al., 2000b; Vogt and Jackson, 2010). The current ambient distribution of ClO_4^-

☆ An evaluation of the role of tropospheric ozone in phytoaccumulation of perchlorate reveals large interspecific differences in accumulation but no relationship with ozone exposure or absorbed ozone dose.
☆☆ The authors declare no competing financial interest.

* Corresponding author.
E-mail address: dagrantz@ucanr.edu (D.A. Grantz).

0269-7491/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.envpol.2013.03.048

does not correspond with the abundance of known point sources, suggesting that additional area sources may remain to be identified.

The role of tropospheric O_3 in the origin of non-anthropogenic ClO_4^- remains unclear. Tropospheric O_3 has increased in concentration since pre-industrial times (Vingarzan, 2004; Stevenson et al., 2006). Current levels of ambient O_3 are injurious to crop species and to native vegetation (Avnery et al., 2011; Booker et al., 2009; USEPA, 2013). High concentrations of O_3 have been shown experimentally to produce ClO_4^- from Cl^- in both aqueous solution and in dry systems (Dasgupta et al., 2005; Kang et al., 2006, 2008; Rao et al., 2010). Stable isotopic composition of some indigenous ClO_4^- in the US and Chile exhibits a significant $\Delta^{17}O$ anomaly, suggesting some production of natural ClO_4^- through O_3 mediated oxidation reactions. However, other sources appear to have a small O_3 mediated contribution (Bohlke et al., 2005; Jackson et al., 2010). Our preliminary evidence (Burkey et al., unpublished) shows that O_3-sensitive and O_3-tolerant genotypes of snap bean (*Phaseolus vulgaris*) accumulate foliar ClO_4^- under field conditions, and the role of contrasting O_3 environments is currently being evaluated. Through abscission and litter turnover this would represent an unaccounted source of ClO_4^- in the environment.

Plants, particularly in arid environments, may contain abundant chloride in their tissues; display a vast array of hydrated internal and external reaction surfaces; and catalyze a multitude of redox reactions that could be involved in biosynthesis of ClO_4^-. These factors, the ubiquitous distribution of plants, and the post-industrial increase in O_3 exposure are consistent with the possibility that tropospheric O_3 may induce biosynthesis of ClO_4^- from chloride in plants. This would represent a novel source of ClO_4^- in the environment.

We present the results of a series of experimental exposures to environmentally relevant concentrations of O_3 of a broad range of contrasting food, feed and fiber species under controlled conditions. We test the hypotheses that (1) exposure of plants to O_3 leads to foliar biosynthesis of ClO_4^- in young, physiologically active leaves, that (2) such exposure leads to accumulation of ClO_4^- in older, senescing leaves, and that (3) contrasting plant species exhibit little foliar ClO_4^- at low O_3 exposure.

2. Materials and methods

2.1. Plant material

We have chosen a range of plants of economic importance, used for human consumption, animal feed, or fiber. These species represent diverse classes of crop species, leafy green vegetable row crops and extensively cultivated grain and forage crops, warm season and cool season crops, and both C_3 and C_4 species.

The C_3 species were spinach (*Spinacia oleracea* cv. Bloomsdale Long Standing; Ferry Morse Seed Co., Fulton KY), lettuce (*Lactuca sativa* cv. Romaine, Parris Island Cos; Ferry Morse Seed Co., Fulton KY), broccoli (*Brassica oleracea* cv. De Cicco, Ferry Morse Seed Co., Fulton KY), soybean (*Glycine max* cv. Disoy; Ferry Morse Seed Co., Fulton KY), Pima cotton (*Gossypium barbadense* cv. Phytogen 800, Dow AgroScience, Indianapolis IN and cv. S-6, J.G. Boswell Company, Corcoran CA; foundation seed stock), and bush bean (*Phaseolus vulgaris* cv. Bush Blue Lake 156; Ferry Morse Seed Co., Fulton KY), The C_4 species were sorghum (*Sorghum bicolor* cv. 4662, Pioneer Seed Co., Johnston IA), sugarcane (*Saccharum oficinarum* × *S. spontaneum* hybrid cv. Elephant; Grantz and Vu, 2009; Grantz et al., 2012), and maize (*Zea mays* cv. Golden Cross Bantam (hybrid); Ferry Morse Seed Co., Fulton KY).

Seed (stalk cuttings in the case of sugarcane) were planted in moist commercial potting mix (Earthgro Potting Soil; Scotts Company, Marysville, OH) in 10 cm square pots. After emergence, pots were thinned to 1 plant per pot. Plants were grown in a research greenhouse at Kearney Research and Extension Center (103 msl; 36.598 N 119.503 W). Irrigation was provided daily through a drip emitter in each pot. A complete (N–P–K; 24-8-16) fertilizer solution was administered twice weekly (2.9 g L^{-1}, Miracle Gro, Scotts Miracle-Gro Products Inc., Port Washington, NY) through the same emitters. Both irrigation and fertilizer were applied until substantial drainage through the potting medium occurred, to avoid accumulation of salts or fertilizer in the soil (Grantz et al., 2010). Pots retained 68.9 mL of solution against drainage.

Plants were grown from germination until harvest in one of nine continuously stirred, Teflon lined tank reactors (CSTRs; Heck et al., 1978; Grantz et al., 2010) located in the greenhouse. Growth temperature was 15–30 °C, illuminated with natural sunlight (approximately 300 μmol m^{-2} s^{-1} PPFD; 400–700 nm at plant level) near solar noon.

2.2. Ozone exposure

Plants were exposed to environmentally relevant O_3 concentrations (12 h means nominally 4, 59, and 114 ppb; 8 h means of 4, 75 and 150 ppb, and daily maxima near solar noon of 4, 89 and 163 ppb) from emergence in the CSTRs. Exposures were imposed as daily half-sine wave, 7 days per week. O_3 was provided to the CSTRs by corona discharge (Model SGC-11, Pacific O_3 Technology, Brentwood, CA) from purified oxygen (Series ATF-15, Model 1242, SeQual Technologies Inc., San Diego CA). Feedback for the O_3 generator was provided by the exit stream of a single exposure chamber, monitored with an ultraviolet O_3 monitor (ThermoElectron Model 41C), with other CSTRs controlled by ratio of O_3 flow rate (Grantz et al., 2010). Each CSTR was monitored every 15 min, independently of the control system, with a separate ThermoElectron Model 41C. All monitors were calibrated against a factory certified calibration unit (Model 306; 2B Technologies; Boulder CO). Air with the desired O_3 concentration was introduced at one complete air exchange per minute.

2.3. Perchlorate determination

Plants were harvested at about 9 weeks after germination. Species varied with their specific rate of development, but all runs within a species were harvested at precisely the same plant age. Roots were washed in cool water to remove the potting medium. Leaves, roots and stems were separated and immediately frozen at −20 °C. Older leaves, senescing or recently abscised, were gathered separately and treated similarly.

Samples of unused planting media and fertilizer were collected and stored at −20 °C in zip-lock polyethylene bags. The surface 1 cm of soil was sampled following plant harvest and treated similarly. Irrigation water was sampled directly from the emitters of the drip irrigation system into plastic, screw-top vials and immediately frozen at −20 °C. Samples were shipped on dry ice to the analytical laboratory for ClO_4^- analysis.

Soil samples were extracted using Milli-Q water at a 2:1 mass ratio (water:soil) by shaking for 24 h. The samples were centrifuged for 10 min and the supernatant decanted and filtered through a 0.2 micron Nylon membrane (ion chromatography (IC)-certified Acrodisc syringe filter). All extraction sets were accompanied by an extraction duplicate, an extraction spike (soil + known amount of added ClO_4^-), and an extraction blank (DDI water only). The moisture content of parallel samples was determined by drying at 105 °C for 24 h. The final filtered extract was analyzed for major anions and ClO_4^-.

Plant leaf samples were pre-dried (105 °C for 12 h) and approximately 1 g placed in a 45-mL capacity centrifuge tube to which 25 mL of Milli-Q water was added. The centrifuge tubes, containing the samples, were boiled for 1 h (water bath temperature ~ 99 °C) and centrifuged at 5000 rpm for 5 min. A 2 mL aliquot of the supernatant was gently transferred into a plastic bottle containing 1.0 ± 0.1 g of activated alumina. The alumina-extract mixture was diluted by adding 18 mL of DDI water, capped, and held at 3 °C for 8 h. The suspension was then re-centrifuged at 5000 rpm for 5 min, and the final supernatant filtered (0.2 micron) and passed through a pre-cleaned and activated OnGuard® RP cartridge (Dionex Corporation). The extraction procedure was repeated for the extraction duplicate, spike and blank (DDI). The final solution was then diluted and analyzed for ClO_4^-.

Perchlorate in the resulting solutions was quantified by loading through a 25 μL pre-concentrating loop into an ion chromatograph-tandem mass spectrometer (IC-MS/MS with GP50 pump, CD25 conductivity detector, AS40 automated sampler and Dionex IonPac AS16 (250 × 2 mm) analytical column). This was coupled with an Applied Biosystems – MDS SCIEX API 2000® triple quadrupole mass spectrometer with a Turbo-IonSpray™ source. A 45 mM hydroxide (NaOH) eluent at 0.3 ml min^{-1} was followed by 90% acetonitrile (0.3 ml min^{-1}) as a post-column solvent. To overcome matrix effects all samples were spiked with an oxygen-isotope (^{18}O) labeled ClO_4^- internal standard. The method detection limit (MDL) for ClO_4^- was 0.01 μM.

ClO_4^- content of tissue, potting medium, and fertilizer is reported as (μg (kg dry wt)$^{-1}$). ClO_4^- content of irrigation water and fertilizer solution is reported as (μg L^{-1}).

2.4. Ozone flux

Stomatal conductance of young, healthy, fully expanded leaves (leaf 0 and leaf 2) was determined with a porometer (LI 1600; LiCor Inc., Lincoln NE USA or AP4; Delta T Devices, Cambridge UK). Measurements were determined on both classes of leaves at 2 h intervals throughout the day, and on 2 occasions at 14 day intervals. Values were averaged from these 4 leaves as an estimate of stomatal conductance over time, developmental age, and leaf position. Conductance was converted from water vapor to O_3 (Massman and Grantz, 1995) and multiplied by mean O_3 concentration over the surrounding 2 h period. The products were summed diurnally over daylight hours and over the lifespan (germination to harvest) of each species to yield cumulative flux, or dose.

Please cite this article in press as: Grantz, D.A., et al., Perchlorate content of plant foliage reflects a wide range of species-dependent accumulation but not ozone-induced biosynthesis, Environmental Pollution (2013), http://dx.doi.org/10.1016/j.envpol.2013.03.048