

# A critical review and analysis of the use of exposure- and flux-based ozone indices for predicting vegetation effects

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## Abstract

Early studies of plant response to ozone (O<sub>3</sub>) utilized concentration-based metrics, primarily by summarizing the commonly monitored hourly average data sets. Research with the O<sub>3</sub> concentration parameter led to the recognition that both peak concentrations and cumulative effects are important when relating plant response to O<sub>3</sub>. The US and Canada currently use O<sub>3</sub> concentration-based (exposure-based) parameters for ambient air quality standards for protecting vegetation; the European countries use exposure-based critical levels to relate O<sub>3</sub> to vegetation response. Because plant response is thought to be more closely related to O<sub>3</sub> absorbed into leaf tissue, recent research has been focused on flux-based O<sub>3</sub> parameters. Even though flux-based indices may appear to be more biologically relevant than concentration-based indices, there are limitations associated with their use. The current set of flux-based indices assumes that the plant has no defense mechanism to detoxify O<sub>3</sub>. This is a serious limitation. In this paper, we review the literature on exposure- and flux-based indices for predicting plant response. Both exposure- and flux-based metrics may overestimate plant response. At this time, flux-based models that take into consideration detoxification mechanisms (referred to as effective flux) provide the best approach to relate O<sub>3</sub> to plant response. However, because there is considerable uncertainty in quantifying the various defense mechanisms, effective flux at this time is difficult to quantify. Without adequate effective-flux based models, exposure-based O<sub>3</sub> metrics appear to be the only practical measure for use in relating ambient air quality standards to vegetation response.

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## 1. Introduction

Surface ozone (O<sub>3</sub>) is an important air pollutant that affects both vegetation and human health (US EPA, 1996a). Ozone is routinely monitored throughout the world and data are mostly recorded as hourly or half-hourly averages. Over the last 30 years, hourly averaged data have been summarized in different ways for the purpose of assessing

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vegetation effects (US EPA, 1996a). The development of exposure indices to characterize plant exposure and to quantify the relationship between O<sub>3</sub> exposure and ensuing plant response has been, and continues to be, a challenge (US EPA, 1996a). Initially, concentration-based parameters such as the mean of the hourly concentrations over a defined period (e.g., daily 7-h average concentrations over a growing season) were used to relate O<sub>3</sub> to vegetation response. Subsequently, the hourly average concentration data were used in cumulative exposure indices, and more recently in flux models to predict vegetation effects.

In evaluating O<sub>3</sub> metrics for predicting vegetation effects, it is important to understand some of the general concepts of concentration, exposure, flux, and dose. The following definitions (in the mks system) are used:

“Concentration” is the molar density of a specific air pollutant or the number of moles per unit volume [mol m<sup>-3</sup>].

“Exposure” is the product of the concentration measured near the vegetation of interest and the length of time the vegetation is presumably exposed to the pollutant [commonly expressed in unit of mol m<sup>-3</sup>h or ppm-h]. Explicitly, exposure is the integral of the instantaneous concentration over the time period of interest. Although not necessarily considered exposure, seasonal average concentrations (e.g., 7- and 12-h daily average concentrations averaged over a growing season) have also been referred to as exposure indices (US EPA, 1996a).

“Stomatal flux” is a temporally dynamic measure of the rate of entry of the pollutant into the leaf [nmol m<sup>-2</sup> s<sup>-1</sup>].

“Surface flux” is the total flux to all surfaces, including stomatal and non-stomatal surfaces such as the cuticles, and non-plant surfaces such as soils [nmol m<sup>-2</sup> s<sup>-1</sup>].

“Effective flux” is the balance between stomatal flux and intra-leaf detoxification, because not all of the stomatal flux is associated with vegetation injury or damage [nmol m<sup>-2</sup> s<sup>-1</sup>].

“Dose” is the total amount of pollutant that actually is absorbed into the plant through the stomata over a period of time. Dose is the integral over time of the instantaneous stomatal flux [nmol m<sup>-2</sup>] (Fowler and Cape, 1982).

“Effective dose” is the integral over time of the “effective flux” [nmol m<sup>-2</sup>].

“Critical level” is the concentration, cumulative exposure, or cumulative stomatal flux of atmospheric pollutants above which direct adverse effects on sensitive vegetation may occur according to present knowledge (Mills, 2004, pII-1).

Others have called what we define as “dose”, the “effective dose” (Runeckles, 1974); and referred to what we define as “effective flux” as the “effective dose” (Dämmgen et al., 1993; Grünhage and Jäger, 1996; Grünhage and Haenel, 1997; Grünhage et al., 1999). Our use of “flux” and “dose” indicate the instantaneous action and that action integrated over time, respectively; and “effective” specifies the detoxification component.

In evaluating the potential plant or vegetation response to a pollutant, it is important to distinguish between “injury” and “damage”. The definitions here are derived from Guderian (1977):

“Injury” is leaf necrosis, premature leaf senescence, reduced photosynthesis, reduced carbohydrate production and allocation, reduced growth, and/or reduced plant vigor. Injury can be visible or invisible.

“Visible injury” is observable as oxidant stipple, chlorotic mottle, bronzing, or any other visual leaf necrotic symptom. It can also be premature leaf senescence. If reductions in growth are obvious to the observer, this is also considered visible injury.

“Invisible injury” (sometimes referred to as “hidden injury”) is that which is not visible to an observer, such as changes in photosynthesis, carbohydrate production and allocation, or plant vigor.

“Damage” is a reduction in the intended value or use of the plant. Included in this definition are reductions in economic, ecologic, or aesthetic value.

Injury can be damage, or the consequences of injury can become damage if the injury can cause a subsequent impairment of the intended use of the plant. For example, injury resulting from reduced carbohydrate production and allocation can lead to reduced plant vigor and reduced growth. Reduced growth can lead to damage by reducing crop yield. Damage to vegetation from pollutants can occur without visible symptoms of injury, but damage cannot occur without some form of injury preceding damage.

For forest and agricultural crops, damage can be expressed as a yield loss or a crop loss.

“Yield loss” is a reduction in quantity or quality of the harvestable portion of a plant.

“Crop loss” is a reduction in the economic or monetary value of a forest or agricultural crop.

Yield loss does not always result in crop loss. For example, yield loss occurring over a region could cause a decreased supply and result in increased economic value of the crop.

## 2. The use of biologically relevant exposure indices for assessing vegetation effects

For over 80 years air pollution specialists have explored mathematical approaches for summarizing ambient air quality information in biologically meaningful forms that can relate O<sub>3</sub> exposure to vegetation effects (O’Gara, 1922 as cited in Olsen, 1922). Considerable discussion has occurred regarding how well exposure indices perform in predicting vegetation effects (US EPA, 1986, 1996a; Krupa et al., 1994; Grünhage et al., 1994; Grünhage and Jäger, 1994; Legge et al., 1995; Grünhage and Jäger, 1996; Grünhage et al., 1997; Grünhage and Haenel, 1997; Lefohn et al., 1997; Musselman and Massman, 1999; Musselman and Minnick, 2000; Massman et al., 2000; Panek et al., 2002; Kurpius et al., 2002; Massman, 2004; Uddling et al., 2004). One of the main concerns in using exposure as an index is that O<sub>3</sub> uptake may be decoupled from the time period when the highest O<sub>3</sub> concentrations occur. For example, Krupa et al. (1998) indicate that daily peak (highest) hourly O<sub>3</sub> values ( $\geq 90$  ppb) do not necessarily occur simultaneously with conditions that promote atmospheric conductivity (O<sub>3</sub> deposition) and plant uptake (O<sub>3</sub> absorption). In Section 3, we discuss the synchronization of deposition, uptake, and defense mechanisms. Grünhage et al. (1999) likewise cautioned that the use of exposure indices may provide an overestimate of vegetation effects and concluded that what is needed to better predict vegetation effects is a metric that is more sensitive to plant and ecosystem phenology.

### 2.1. Response of vegetation to high ozone concentrations

Key research experiments that evaluate the importance of the higher O<sub>3</sub> concentrations in plant response have been performed under (1) controlled

conditions in the laboratory and in the field and (2) uncontrolled conditions in the San Bernardino National Forest. These studies provide a framework from which one can develop relevant exposure–response models that provide a consistent relationship between O<sub>3</sub> conditions and vegetation biological endpoints.

It has long been recognized that peak O<sub>3</sub> concentrations are an important factor when examining exposure indices and plant injury (Heck et al., 1966). Stan and Schicker (1982) reported that plants exposed to a series of successive short periods with high concentrations suffered more injury than did those plants that received a continuously uniform exposure, but at a lower concentration, with all plants receiving equal total exposure.

In 1996, the EPA proposed the use of a cumulative exposure index which weighted the higher hourly average concentrations greater than the mid and lower values as a secondary O<sub>3</sub> standard to protect vegetation from damage (US EPA, 1997). The EPA (US EPA, 1996b) considered two specific concentration-weighted indices: the threshold-based SUM06 (the sum of all hourly average concentrations  $\geq 0.06$  ppm) and the sigmoidally weighted W126 exposure index (Lefohn and Runeckles, 1987; Lefohn et al., 1988). After reviewing the literature based on controlled fumigation experiments that investigated yield reduction of agricultural crops, the Agency concluded that both indices performed similarly as exposure measures to predict the exposure–response relationships observed in the National Crop Loss Assessment Network (NCLAN) crop studies (US EPA, 1996b; Heck and Cowling, 1997) and initially recommended the threshold-based SUM06 exposure index for a proposed secondary standard. In its final recommendation, the Agency concluded that the new more stringent concentration-based primary (i.e., human health) standard would be sufficient to protect vegetation and decided not to establish a separate exposure-based form and level for the secondary standard (US EPA, 1997).

When yield or growth is considered, O<sub>3</sub> concentration and duration of exposure are both important; but the diurnal dynamics of the exposure are also significant (US EPA, 1986). Controlled fumigation experimental results (Musselman et al., 1983, 1986, 1994; Hogsett et al., 1985) have been cited by the EPA (US EPA, 1986, 1992, 1996a) as the experimental basis for emphasizing the importance of episodic peak exposures. More recent research by

Nussbaum et al. (1995), Yun and Laurence (1999a), Lee and Hogsett (1999), Oksanen and Holopainen (2001), and Köllner and Krause (2003) provide additional support for this concept. Using data from controlled experimental studies, Lee et al. (1987, 1988), Lefohn et al. (1988), Musselman et al. (1988), Tingey et al. (1989), and US EPA (1996a) concluded that the cumulative effects of peak hourly  $O_3$  concentrations were of greater importance than seasonal (i.e., long term) mean exposures in predicting vegetation damage.

Yet, concern has been expressed that the experiments reporting the importance of the higher hourly average concentrations have been performed under controlled fumigation conditions not representative of actual field conditions and the results obtained from these experiments may not provide realistic results that are applicable for developing predictive models for assessing vegetation effects in natural environments (Krupa et al., 1995; Grünhage and Jäger, 1994, 2003; Grünhage et al., 1997, 2002; Krupa et al., 2001). However, comparisons of chamber results with field results have shown that  $O_3$  uptake in chambers may be similar to that experienced under field conditions (Unsworth et al., 1984a, b; Karlsson et al., 2004; Skärby et al., 2004), thereby alleviating some of these concerns. Unsworth et al. (1984a) reported that although deposition of pollutants would be greater in chambers than in ambient air, there would be little difference on rate of pollutant uptake. Canopy resistance to  $O_3$  uptake in chambers was similar to those measured in the field (Unsworth et al., 1984b). Karlsson et al. (2004) reported that chambers versus open-release exposure systems had no influence on dose–response relationships. Skarby et al. (2004) examined Norway spruce response to  $O_3$  using pooled analysis of several chamber and field experiments. They concluded that removing the chamber studies from the analysis resulted in nearly the same regression equation, and indicated that data from chambers can be used in risk assessment.

Complementing the controlled fumigation results are findings from the conifer forest ecosystem of the San Bernardino National Forest in California. These findings provide additional evidence for the greater importance of the higher hourly average concentrations than the mid and low values. For the period 1973–1992, a population sample of 219 ponderosa pines in the conifer forest ecosystem of the San Bernardino National Forest showed that 84% had no change or an improvement in needle

whorl retention (where abscission was due to  $O_3$ ) (Miller and Rechel, 1999), while peak  $O_3$  concentrations decreased during this time period. A wider area of the San Bernardino National Forest examined between 1974 and 1988, using a broader index of injury (Forest Pest Management (FPM) method), also indicate an improvement of crown condition coincident with an improvement of  $O_3$  air quality (Miller and Rechel, 1999). Tingey et al. (2004) reported that reductions in  $O_3$  in the San Bernardino Mountains during the time period 1963–1999 benefited growth of Ponderosa pine.

During the period 1950–1980, extremely high  $O_3$  concentrations impacted the San Bernardino National Forest (US EPA, 1996a). However, over the past 25 years, significant reductions in the  $O_3$  concentrations have occurred in this area (Lloyd et al., 1989; Davidson, 1993; Lefohn and Shadwick, 2000; Lee et al., 2003, Tingey et al., 2004). Upon examination of the reduction in the hourly average concentrations over the period 1980–2003, several interesting patterns emerge. Fig. 1a shows that from 1989 to 2003 the 24-h cumulative W126 and SUM06 exposure indices have decreased. Over a 24-h April–October period, Fig. 1b illustrates a decreasing trend in the number of hourly average concentrations greater than or equal to 80, 120, and 150 ppb. For the same period of time, the number of hourly average concentrations between 50 and 89 ppb increased or remained stable in the most recent years compared to the early 1980s (Fig. 1c). Thus, for the period 1980–2003, the reductions of  $O_3$  in the San Bernardino, California area appear to be associated with reductions in the higher hourly average concentrations, while those in the range of 50–89 ppb appear to be either stable or increasing. Other researchers have reported similar observations for other locations in the United States (Lefohn et al., 1998). Reynolds et al. (2003, 2004) have described in detail reasons for the disproportionate reduction in the higher hourly average concentrations compared to the lower values.

While other factors may be involved in this improvement of forest growth in the San Bernardino National Forest, including removal of  $O_3$  sensitive genotypes and increased growth from nitrogen deposition, conifers at high pollution sites still show severe leaf necrosis from  $O_3$ , and the number of whorls retained remains low (Grulke and Balduman, 1999). Research has suggested that nitrogen fertilization from deposition has increased some tree growth and changed species composition

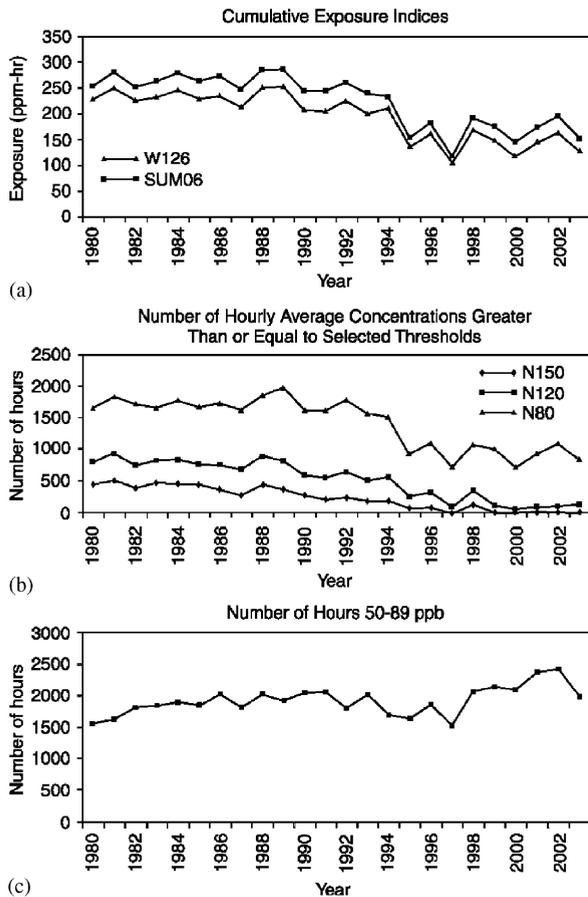


Fig. 1. Trend in the ozone (a) 24-h cumulative W126 and SUM06 exposure indices, (b) number of hourly average concentrations greater than or equal to 80, 120, and 150 ppb for the period 1980–2003, and (c) number of hourly average concentrations between 50 and 89 ppb for the Crestline, San Bernardino, CA monitoring site, April–October, 0100–2359 h. *Source:* US EPA AQS database.

in mixed conifer forests (Gulke and Balduman, 1999; Takemoto et al., 2001; Bytnerowicz, 2002; Fenn et al., 2003a, b).

Conversely, over the last 20 years, there has been a fairly steady decline in  $\text{NO}_2$  concentrations in the California South Coast Air Basin, with the maximum peak 1-h indicator for  $\text{NO}_2$  in 2001 nearly half what it was in 1982 (CARB, 2003). A National Acid Deposition Program (NADP) monitoring site at Tanbark Flat (NADP, 2004), near the San Bernardino National Forest, shows that between 1982 and 2002 the annual nitrate wet deposition at the site did not appear to increase (Fig. 2). Nitrate components of  $\text{PM}_{2.5}$  and  $\text{PM}_{10}$ , and  $\text{NO}_x$  have decreased in California's South Coast Air Basin (Motallebi et al., 2003). Since the early 1980s, for 12

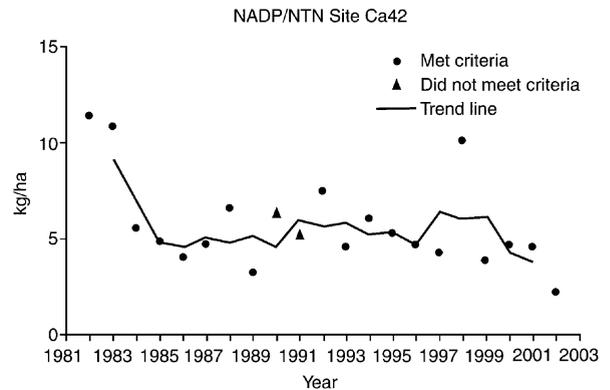


Fig. 2. Trend in annual  $\text{NO}_3$  wet deposition for the period 1982–2002 for the NADP site at Tanbark Flat, California. The plotted line is the moving 3-year mean value centered over the middle year. *Source:* NADP (2004).

sites in the South Coast Air Basin of southern California, annual  $\text{NO}_x$  and  $\text{PM}_{10}$ -nitrate averages of the 24-h average concentrations show a downward trend (Fig. 3a). Similar trends are observed for urban sites closest to the San Bernardino National Forest, San Bernardino and Riverside (Figs. 3b and c). Thus,  $\text{NO}_2$  concentrations, wet annual nitrate deposition, and  $\text{PM}_{10}$  nitrate appear to be declining. Nitrogen saturation in the San Bernardino National Forest (Fenn et al., 2003a, b) has likely been present much longer than the recent reduction in  $\text{O}_3$  concentrations; thus, most of the changes in vegetation in the conifer forest ecosystem of the San Bernardino National Forest in California appear to be linked with changes in  $\text{O}_3$  exposure associated with the higher hourly average concentrations.

## 2.2. The role of plant moisture status in vegetation response

Water is a strong driving force for plant growth and health. As the water content of the plant declines due to high use or low availability, the stomata tend to close. Thus, either a high vapor pressure deficit (VPD), which increases water use, or low soil water content can be used for an indication of stomatal conductance. Efforts were made to combine exposure indices and soil moisture measurements to predict vegetation effects. Lefohn et al. (1997) presented an approach for combining exposure information with potential soil moisture considerations. The authors interpolated the  $\text{O}_3$  exposures in many areas but cautioned that the

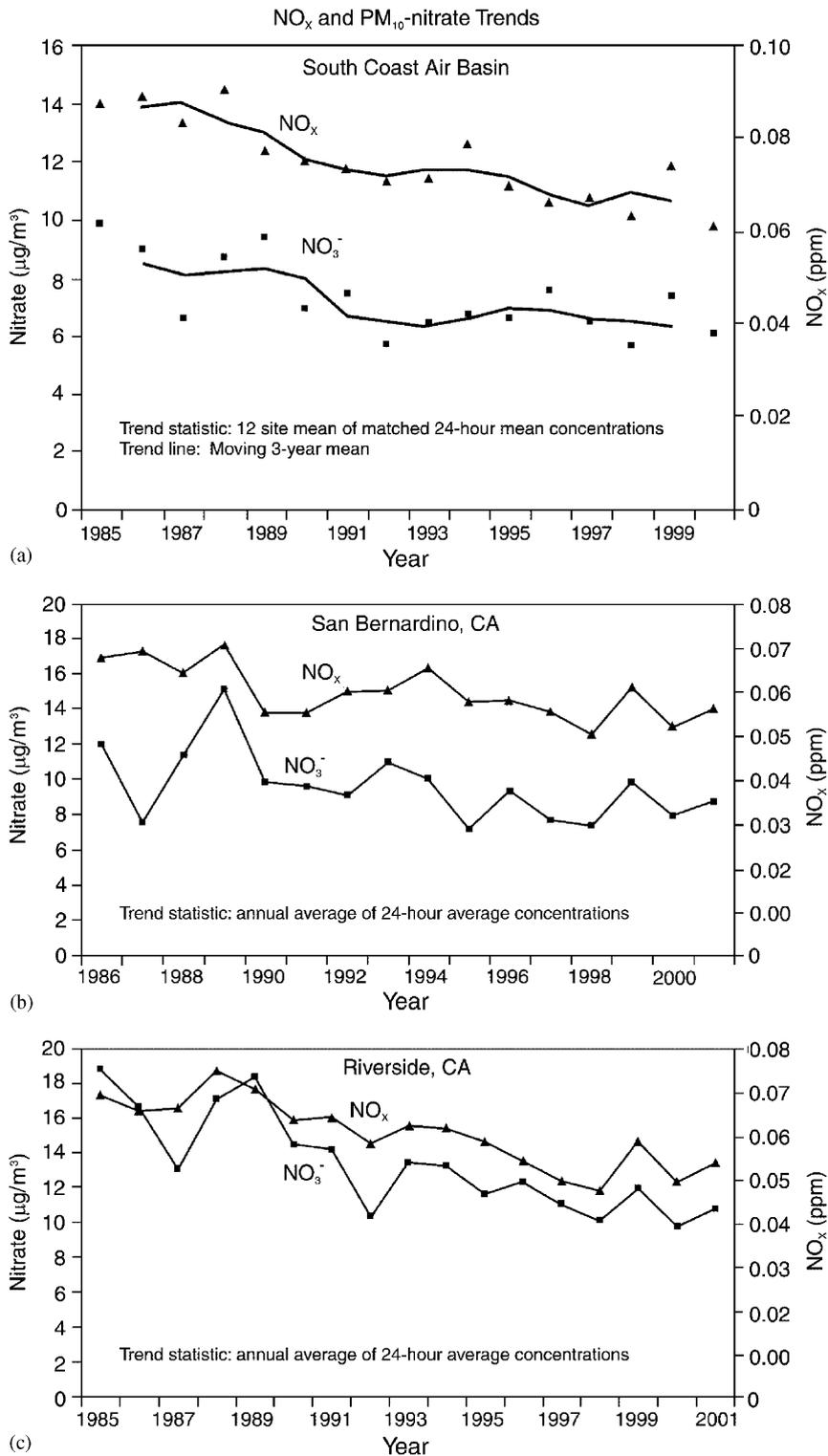


Fig. 3. Trend in annual NO<sub>x</sub> and PM10 nitrate for (a) California’s South Coast Air Basin, 1985–2000, (b) San Bernardino, CA, 1986–2001, and (c) Riverside, CA, 1985–2001. The plotted line in 3a is the moving 3-year mean value centered over the middle year. Source: N. Motallebi, California Air Resources Board, personal communication.

hydrologic index used might not represent an optimum metric to indicate whether a particular species was experiencing water stress. Recently, Edwards et al. (2004) investigated O<sub>3</sub> exposures and their implications for vegetation areas of the central Appalachian Mountains using an approach similar to that of Lefohn et al. (1997). Chappelka and Samuelson (1998) commented on what Lefohn et al. (1997) had discussed: the experimental database was limited and based on seedling responses.

### 2.3. Performance of exposure indices

Identification of a cumulative exposure index optimum for all plant species and all conditions does not appear to be possible due to (1) the limited information for assessing the relative performance of exposure indices for relating to vegetation effects and (2) the inherent differences in how plants respond to O<sub>3</sub> (Musselman et al., 1988; US EPA, 1996a). However, some indices have been shown to be less useful than others. US EPA (1996a) concluded that indices based on long-term averages were inadequate to differentiate among the different types of exposure regimes. Once such index is the SUM00, or the seasonal sum of all hourly average concentrations, also called the total exposure index. The SUM00 divided by the period of accumulation is defined as the long-term average concentration. This index weights all concentrations equally, thus focusing on the more numerous lower concentrations that have been found to be of less biological importance for assessing vegetation response. Given the importance of the higher hourly average O<sub>3</sub> concentrations, the SUM00 and average concentration metrics are inadequate for characterizing plant exposure to O<sub>3</sub>, except in those areas where numerous occurrences of high hourly average concentrations result in a high correlation between the peaks and the SUM00 index.

However, it is important to note that others have not necessarily agreed with these findings. For example, Heagle and Stefanski (2000) reported that both peak-weighted and non-peak-weighted exposure indices performed similarly. Heagle and Stefanski (2000) “pooled” the data from San Bernardino (CA) and Riverside (CA) with data from Amherst (MA), Corvallis (OR), Kennedy Space Center (FL), Raleigh (NC), and Blacksburg (VA) and found that high O<sub>3</sub> exposures were much greater at the two California sites (indicated by high

W126, SUM06, W95, and AOT40 values) in comparison with the other locations. Because the data from all of the sites were pooled, the large number of high hourly average O<sub>3</sub> concentrations that occurred at the California sites may have resulted in the exposure indices being highly correlated with one another and made it difficult to separate out the most optimum sets of indices from the other indices used in the analysis. Similarly, Arbaugh et al. (1998) reported that the SUM00 exposure index performed better for describing injury than the SUM06, W126, number of hours greater than or equal to 0.08 ppm, and the number of days between measurement periods. For areas with numerous high hourly average O<sub>3</sub> concentrations, such as southern California, many different O<sub>3</sub> exposure parameters relate well to plant response (Musselman et al., 1988).

An important concern with using the various cumulative exposure indices in predicting yield loss for agricultural crops or trees is that the same value of an exposure index may relate to different vegetation responses (Lefohn and Foley, 1992). Results reported by Yun and Laurence (1999a) showed that the same SUM06 value resulted in very different foliar injury when exposure regimes with different numbers of high concentrations were applied. Similarly, Hogsett et al. (1985) showed that the same SUM07 value resulted in different yield when exposure regimes, some containing peaks and some without peaks, were used.

To eliminate the concern that the same exposure value of an exposure index might provide different vegetation responses, Lefohn and Foley (1992) recommended that an additional exposure parameter, the number of hourly averaged O<sub>3</sub> concentrations  $\geq 100$  ppb (N100), combined with either the W126 or the SUM06 exposure indices be used to modify NCLAN exposure–response equations to predict yield loss. The N100 modification would then be used as a surrogate for the frequent number of peak hourly average concentrations that were experienced in the chambers with the highest exposures in the NCLAN experiments. Independently, Nussbaum et al. (1995), using identical AOT40 exposure regimes with some that contained peaks and some without peaks, suggested that peak concentrations  $>0.11$  ppm were important for describing the effect of O<sub>3</sub> on total forage yield.

Fundamentally, by focusing too much attention on the absolute value of the cumulative exposure index, instead of on the combination of the value of

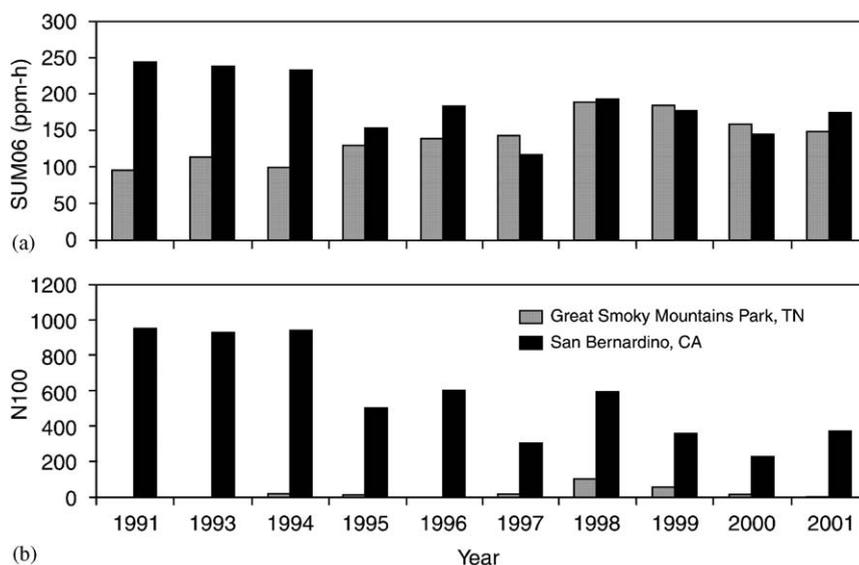


Fig. 4. A comparison of the (a) annual SUM06 exposure values and (b) number of hourly average concentrations greater than or equal to 100 ppb (N100) for the period 1991–2001 at a site in the Great Smoky Mountains National Park (gray bars) and at Crestline, San Bernardino, CA, a site in the San Bernardino National Forest (black bars). Source: US EPA AQS database.

the exposure index and the number of high hourly average concentrations included in that exposure index, inaccurate conclusions can be reached. We illustrate this point by comparing a site in the San Bernardino National Forest in southern California with a site in the Great Smoky Mountains National Park. Although the SUM06 cumulative exposure values are similar for both locations for most of the years between 1995 and 2001 (Fig. 4a), the San Bernardino site experienced as much as 956 times more hourly average concentrations  $\geq 0.10$  ppm than the Great Smoky Mountains National Park site (Fig. 4b). While foliar symptoms of  $O_3$  injury are observed in the Great Smoky Mountains National Park, the magnitude of the damage that is observed in San Bernardino Mountains, in terms of crown dieback, loss of older needle whorls of conifers, and death of trees is much greater. This example illustrates that two areas can have similar cumulative exposure index (SUM06) but different numbers of number of peak concentrations (hourly average concentrations  $\geq 0.10$  ppm, N100). Thus, assuming similarity of susceptibility of ecosystems in these two areas because of similar SUM06 values is inappropriate. Yun and Laurence (1999a) provide additional evidence that the same SUM06 value with different numbers of high concentrations can cause different foliar response. We expect  $O_3$  fluxes may be different between the two sites because of

differences in moisture conditions favoring uptake. However, flux data differences between the two sites are not available for comparison; any flux comparisons would be limited given the two sites have different species with differences in their susceptibilities and defense mechanisms.

### 3. Ozone stomatal flux and effective flux

#### 3.1. Stomatal conductance

Any flux-based index must be based upon gaseous pollutant movements. In order to determine actual  $O_3$  flux, it is necessary to understand the movement of  $O_3$  from ambient air to plant surfaces,  $O_3$  uptake through stomata, and interactions of  $O_3$  within plant tissue. Any reduction in the ability for  $O_3$  to move into the leaf would lead to smaller effects because of less  $O_3$  available for interaction with leaf tissue. Stomata conductance is the principal control point of entrance of  $O_3$  into the sensitive leaf tissue and so has been thought for years to be the most important element to define flux-based indices (Heath, 1980).

The movement of  $O_3$  from the free air (often called bulk air) environment into the leaf proper is controlled by three main processes: movement from the free air into the canopy (canopy conductance), in which the placement of plants and leaves play a

role; boundary layer conductance in which the flow of air close to leaves with the air just at the stomata is governed; and stomatal conductance which describes how the air at the stomata moves into the leaf. Ozone concentration in a plant canopy is influenced by environmental factors such as light, temperature, and wind speed, and atmospheric concentrations of  $\text{NO}_x$  and VOC products as well as the structure of the canopy including the soil underneath it (Coe et al., 1995; Wang et al., 1995; Lendzian and Kerstiens, 1991).

Stomatal flux of  $\text{O}_3$  is controlled by ambient  $\text{O}_3$  concentration and by canopy and stomatal conductances and so will depend the concurrence (also referred to as coherence or symmetry or synchrony) of the diurnal concentration and conductance phases (Runeckles, 1992; Lee and Hogsett, 1999). Stomatal conductances are often highest in mid-morning due to high water content and low VDP while high ambient  $\text{O}_3$  concentrations generally occur in mid to late afternoon. Kurpius et al. (2002) describe the decoupling of the time period when the highest  $\text{O}_3$  concentrations and uptake occur in natural systems.

Since  $\text{O}_3$  flux is important in plant response and is dependent upon both conductance and ambient  $\text{O}_3$  concentration, the combinations of high conductance/low concentration in the morning and low conductance/high concentration in the afternoon would seem to suggest morning and afternoon to be somewhat equivalent for total flux. However, this generalization may be inappropriate. The low conductance in the afternoon may be more limiting to flux than the low concentration in the morning. In addition, generalization on lack of coherence of conductance and concentration must be made with caution. Grulke et al. (2002) reported high conductance when  $\text{O}_3$  concentrations were high under some conditions.

### 3.2. Nocturnal conductance

Stomata often remain partially open at night for most plant species (Musselman and Minnick, 2000), although conductance at night is at a much reduced level compared to daytime conductance slowing possible  $\text{O}_3$  movement. However, the level of turbulence at night is often low with resulting stable boundary layers. Nevertheless, nocturnal turbulence does intermittently occur and can result in non-negligible  $\text{O}_3$  flux into the plants. This nocturnal uptake can be an important contributor to plant

response (Matyssek et al., 1995; Musselman and Minnick, 2000; Grulke et al., 2002, 2004; Massman, 2004; Uddling et al., 2004). Grulke et al. (2004) report that for ponderosa pine in the San Bernardino (CA) National Forest, the stomatal conductance at night ranged from one tenth to one fourth that of maximum daytime gas exchange. In June, at a high-elevation site, 11% of the total daily  $\text{O}_3$  uptake of pole-sized trees occurred at night; while in late summer,  $\text{O}_3$  uptake at night was negligible. Massman (2004) suggested that nocturnal stomatal  $\text{O}_3$  uptake accounted for about 15% of the cumulative daily effective  $\text{O}_3$  dose that was related to predicted injury.

In addition, plants may be more sensitive to  $\text{O}_3$  at night because there is less production of metabolites and energy-containing compounds for detoxification processes in the absence of photosynthesis (Musselman and Minnick, 2000; Grulke et al., 2004). Research has shown that plants exposed to  $\text{O}_3$  at night can show greater reductions in growth than those exposed to  $\text{O}_3$  in daylight (Matyssek et al., 1995). In another experiment, *Brassica rapa* plants were exposed to  $\text{O}_3$  during either the day or night, with little significant difference in amounts of injury or reduced growth response to treatment even though the conductance was 70–80% lower at night (Winner et al., 1989). Lee and Hogsett (1999) reported that tissue biomass of ponderosa pine seedlings was significantly reduced when seedlings were exposed to either daytime or nighttime episodic profiles; although biomass reductions were greater with daytime peak concentrations than with nighttime peak concentrations.

### 3.3. Ozone interaction within plant tissue

Plant stomatal conductance is central to all models of  $\text{O}_3$  deposition. Grünhage and Hanel (1997) and Emberson et al. (2000), using the Jarvis (1976) model, have described a multiplicative algorithm of stomatal conductance of  $\text{O}_3$ . Such models include functions for the effects of phenology, light, temperature, VPD, and soil water potential on the stomatal conductance of several species. Models of this type usually describe  $\text{O}_3$  deposition in terms of resistances associated with different uptake surfaces. In this paradigm, stomata are one of several possible deposition routes. To be useful for setting standards, however, these models, which are basically one-dimensional (vertical) models that are applied at a single point, must be scaled

to include horizontal spatial scales. Grünhage and Jäger (2003) extend a one-dimensional point model to horizontal scales by employing a Lagrangian model to infer information on the horizontal distribution of deposition. Nussbaum et al. (2003) and Bassin et al. (2004) describe a one-dimensional model that uses routinely measured gridded meteorological and  $O_3$  data and species distribution information to estimate  $O_3$  deposition at agricultural areas in Switzerland. Others have used adaptations of the Ball–Berry (Ball et al., 1987) conductance/photosynthesis model approach to estimate  $O_3$  uptake (Zeller and Nikolov, 2000; Nikolov and Zeller, 2003). These models might be expected to overestimate  $O_3$  effects on vegetation, since they do not account for the detoxification potential of vegetation that provides some amount of natural plant protection from  $O_3$ . This protection is not without cost since it consumes energy.

Classical plant physiology defines stomatal flux of any gas to be its conductance times the difference between the inside and outside concentrations. For uptake of  $O_3$  this is  $g_{O_3}[C_{O_3}^b - C_{O_3}^i]$  with  $O_3$  stomatal conductance ( $g_{O_3}$ ) being the major regulatory control of uptake of  $O_3$  within the leaf boundary layer ( $C_{O_3}^b$ ). The concentration of  $O_3$  within the substomatal cavity or inside the leaf is  $C_{O_3}^i$ . While the total flow of gaseous pollutants into leaves should include gaseous diffusion through the leaf boundary layer and from the substomatal cavity into the interior of the leaf cell, those paths are difficult to calculate and are generally ignored as being sites of high conduction. Ozone will attain a concentration in the cell, dependent upon its entry speed and its reactivity with the wall and plasma membrane constituents. In the past, the internal concentration ( $C_{O_3}^i$ ) has been set to be zero (Laisk et al., 1989) due to early studies, which found that no  $O_3$  could pass through a leaf. Moldau and Bichele (2002) have presented data at very high concentrations of  $O_3$  indicating that the concentration of  $O_3$  inside the leaf is low, but not zero. The authors (Moldau and Bichele, 2002) show a slow rise in internal  $O_3$  level within the first few minutes of exposure, suggested to be due to the depletion of an antioxidant. While  $C_{O_3}^i$  is assumed to be constant, due to the complexity of the internal structure of the leaf, the internal concentration of the pollutant cannot be uniform within the leaf.

In the leaf,  $O_3$  is consumed in chemical reactions, or changed into other species, and therefore  $O_3$  within the stomatal cavity drops in concentration.

Each reaction reduces the  $O_3$  concentration as it penetrates deeper to the site where actual injury is caused. Low levels of  $O_3$  are thought to be counteracted because the amount of detoxifying agents such as antioxidants is not limited; at high  $O_3$  levels the detoxification potentials by antioxidants can be overwhelmed and so the remaining  $O_3$  would cause more injury. As an antioxidant molecule is sacrificed reacting with ozone, it must be replaced, at an energy cost to the plant.

### 3.4. Plant defenses

Plant defenses include the ability of the plant to restrict entry of  $O_3$  into plant tissue (modifying the conductance) and to detoxify  $O_3$  once it enters the leaf (general antioxidant behavior). To adequately predict vegetation impacts associated with  $O_3$ , it is necessary to examine both  $O_3$  uptake and plant detoxification (Hogsett et al., 1988; Massman et al., 2000; Panek and Goldstein, 2001; Fuhrer and Booker, 2003; Massman, 2004; Matyssek et al., 2004; Grünhage et al., 2004).

The importance of detoxification processes has been experimentally demonstrated. For example, observed nonlinear relationships between  $O_3$  uptake and plant response indicate that additional mechanisms beyond uptake are responsible for vegetation effects (Amiro et al., 1984; Amiro and Gillespie, 1985; Lefohn and Tingey, 1985; Fredericksen et al., 1995). Amiro et al. (1984) and Bennett (1979) found that plant response increased nonlinearly with the  $O_3$  exposure duration, suggesting that there is some degree of internal detoxification. Species having high amounts of detoxification potential show less of a relationship of  $O_3$  stomatal uptake to plant response (Musselman and Massman, 1999). Karlsson et al. (2004) suggested that lack of relation between  $O_3$  uptake and response of trees may be related to detoxification capacity, since separating sensitive species from less sensitive provided better fit of uptake and response.

While any  $O_3$  uptake has the potential for causing a plant response, there may be nonlinear responses and lag times, which will affect how  $O_3$  will trigger a response from a toxicological perspective. Uptake is one of many interacting factors, such as time between episodes, temporal variation, phenology, canopy structure, physiological and biochemical processes, environmental conditions, genetics, and soil and nutrient conditions involved in plant response to  $O_3$ . In essence, injury occurs to

vegetation when the amount of pollutant absorbed exceeds the ability of the plant to detoxify  $O_3$  species or to repair the initial  $O_3$  induced events (Ariens et al., 1976; Tingey and Taylor, 1982; Tingey and Andersen, 1991). The magnitude of the response is determined by the actual amount of the pollutant reaching the target site and the ability of the plant to restore homeostatic equilibrium.

Detoxification processes can be constitutive or inductive (noted as passive or active by Musselman and Massman, 1999). Constitutive detoxification processes are those biochemical pathways already present in the plant tissue, and immediately available for interaction with  $O_3$  (Heath, 1994). Typically this would involve a reduction of  $O_3$  or one of its early products. Plant species/varieties naturally differ in the amounts of constitutive antioxidants available for any type of detoxification; thus some plants are more able to neutralize an oxidative stress more than others. Further, the amount of constitutive antioxidants can vary with developmental age of the tissue and the environmental conditions. Ozone can react with many compounds within the substomatal cavity and on the surface of cells of the leaf to produce a variety of oxidizing and toxic chemicals. Reactions of  $O_3$  are very pH dependent and can easily form hydrogen peroxide in alkaline media. While reactions of the gases within the cell in the water phase at the cell's surface and the reaction of other species thus generated with the components within the wall region of the cell are poorly understood, some fundamental reactions have been described (Heath, 1987; Heath, 1988; Wellburn, 1990). Ozone reacts with organic molecules at double bonds to form carbonyl groups and, under certain circumstances, generates peroxides. Sulfhydryls are particularly easy targets, with the formation of disulfide bridges or sulfones (Mudd and Kozlowski, 1975). In water, the described reactions are hydrogen peroxide ( $H_2O_2$ ), hydroxyl radical (HO) (Heath and Castillo, 1987), and superoxide ( $O_2^-$ ). Effective detoxification reactions can occur via antioxidant metabolites and enzymes, such as ascorbate, glutathione, and superoxide dismutase, if they are present at high enough concentrations (Castillo et al., 1987; Scandalios, 1994).

Ascorbate within the wall declines when the tissue is exposed to  $O_3$  (Luwe et al., 1993; Moldau, 1998; Turcsányi et al., 2000; Zheng et al., 2000); a decline closely linked to the amount of  $O_3$  penetrating the leaf tissue. Certainly the variation in the types of biochemicals present in the apoplastic space can

give rise to a multiplicity of reactions with  $O_3$ , but the dominant antioxidant species is likely ascorbate. However, too rapid of  $O_3$  entry can overwhelm any antioxidant response by depleting it. These antioxidants produced do vary with stage of development and are influenced by other environmental stress pressures on the plant (Tingey and Taylor, 1982).

Inductive detoxification processes are those that must be formed de novo by the plant; a response to an  $O_3$  exposure would be by an active production of chemicals/antioxidants that interact with the  $O_3$  to neutralize its effect on plant tissue, often called a triggered response (Buchanan et al., 2000). The level of the induced response can be somewhat proportional to the level of the stress, but often, once triggered, exhibits a “saturated” biochemical process. Since the process must be induced, a delay or lag time occurs between the time of the application of the  $O_3$  stress and the production and activation of the detoxification process. This lag time will depend upon the intensity of the  $O_3$  loading, the quantity of constitutive detoxification chemicals already available, and the initial health and photosynthetic capacity of the plant and its ability to react to the stress. Inductive detoxification processes require energy for producing or regenerating the antioxidant or other detoxification biochemicals, but is difficult to show since it depends greatly upon the status of the plant.

#### 4. Using effective flux to establish biologically relevant plant response relationships

Plant defenses are difficult to quantify and to model, but some research is beginning to evaluate this component (Massman and Grantz, 1995; Chen et al., 1998; Plöchl et al., 2000; Barnes et al., 2002; Massman, 2004). Massman et al. (2000) developed a conceptual model of a dose-based index to establish plant injury response to  $O_3$  that is based upon the traditional exposure-based parameters. Most models to date do not explicitly include plant defense and, therefore, deal mostly with (simple) dose rather than effective dose. Furthermore, no existing model includes any feedback mechanism between  $O_3$  dose and the plant's ability to detoxify the subsequent  $O_3$  taken up through the stomata, by depletion of reserves or induction of new antioxidants.

Weighting of fluxes has been proposed (Grünhage and Jager, 1996; Massman et al., 2000; Pleijel et al., 2002; Danielsson et al., 2003). Massman et al. (2000) discussed the concept of time-varying-weighted fluxes

that can be used as a surrogate for plant defenses. Fig. 5 from Massman et al. (2000) illustrates the relationship at a grape vineyard site between stomatal conductance ( $g_{O_3}$ ), pollution uptake ( $F_{\text{stom}^*}$ ), and one possible time-varying-flux weighting factor ( $W$ ). The hypothetical time course of the weighting function takes into consideration the ability of a plant to neutralize the pollutant. Similar diurnal conductance and uptake models have been presented by others (Cape and Unsworth, 1988; Runeckles, 1992). Massman et al. (2000) included the defense mechanism. The model directly states that flux is not necessarily correlated with plant injury or damage because time-varying-defense mechanisms reduce the effectiveness of the fluxes. Clearly, the plant's biochemical response to  $O_3$  must be closely linked with stomatal flux and defense mechanisms in order to adequately predict vegetation effects.

Massman (2004) reported that the daily maximum potential for plant injury, based on effective dose, tended to coincide with the daily peak in  $O_3$  concentration; this observation was attributed to the diurnal changes in his hypothetical plant defensive mechanism (Fig. 6). The primary difference between these two injury metrics is most obvious between the hours of 0800 and 1200 h. Relative to the simple dose metric, plant photosynthesis ( $A$ ) tends to reduce the contribution of the effective dose at this time of day (when the low- and mid-level concentrations occur) in comparison to the daily cumulative dose, since plant defenses likely are greater during the morning to noon hours

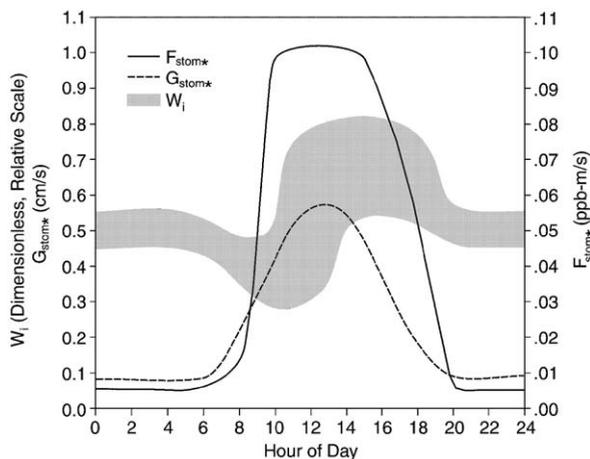


Fig. 5. Time-varying ozone stomatal flux ( $F_{\text{stom}^*}$ ) stomatal conductance ( $G_{\text{stom}^*}$ ) and an empirical weighting factor used as a surrogate for defense ( $W$ ). Source: Massman et al. (2000).

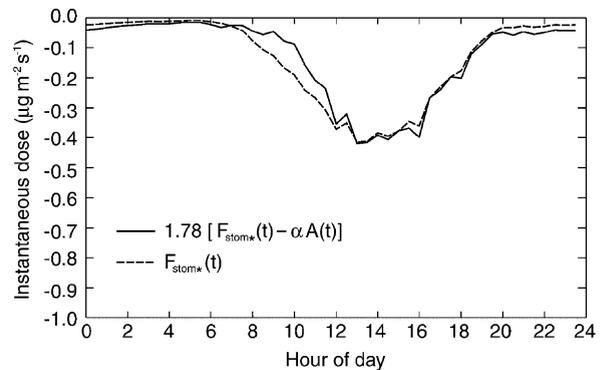


Fig. 6. Average daily cycle of the model estimates for the flux-based instantaneous ozone dose and the instantaneous effective ozone dose, at vineyard site during the CODE91. The instantaneous effective ozone dose has been multiplied by 1.78 for ease of comparison between the two flux-based metrics. Source: Massman (2004).

because photosynthesis is then maximal (Massman et al., 2000). Correspondingly, defenses are likely lower in mid-afternoon when  $O_3$  concentrations are higher. The results reported by Massman (2004) support the controlled and uncontrolled (i.e., San Bernardino National Forest) experimental results that the higher hourly average  $O_3$  concentrations appear to have a greater effect on vegetation than the mid and low levels.

Again, results reported by Yun and Laurence (1999b) showed that conductance alone was not adequate for predicting plant response; the authors reported similar stomatal conductance in two cultivars of aspen (*Populus tremuloides*) resulted in different levels of injury. These observations and the work by Massman (2004) indicate that the defense and repair components are very important in protecting the vegetation from the mid-level  $O_3$  concentrations. Defense mechanisms are less protective during the latter part of the daytime when the higher hourly average concentrations occur, possibly because they are overloaded by peaks or depleted by earlier exposures. As indicated earlier in this section, one must consider the effective dose, which consists of the combination of (1) integrated  $O_3$  flux and (2) detoxification mechanisms to make adequate predictions.

Pleijel et al. (2002) reported that an  $O_3$  uptake rate threshold of  $5 \text{ nmol m}^{-2} \text{ s}^{-1}$  performed best for both wheat and potato. Similarly, Danielsson et al. (2003) were able to show an improved relationship between uptake and yield of spring wheat using a threshold of  $5 \text{ nmoles m}^{-2} \text{ s}^{-1}$ . Pleijel et al. (2002)

report that an instantaneous flux threshold improved the  $r^2$  value from 0.30 to 0.45 for the relationship between  $O_3$  flux and potato yield. However, their data show that much of the variability was still not captured in the model.

A problem in using a statistically derived threshold is evident in the work by Pleijel et al. (2002). Most of the flux was associated with concentrations below 0.06 ppm because the conductance was highest when the concentrations were below 0.06 ppm. These results associated most of the measured effects with concentration at the lower end of the distribution, and do not agree with the results associated with controlled and uncontrolled experiments showing the importance of the higher  $O_3$  concentrations in plant response.

Models that ignore the combination of uptake and detoxification processes might not provide sufficient predictive power when applied under ambient ecosystem conditions. Detoxification processes are dynamic and cannot be represented in response modeling by a constant threshold value. Defense and repair mechanisms vary diurnally as well as seasonally and that may make it difficult to use simple flux thresholds in instantaneous flux measurements to compensate for detoxification processes. Flux-based models that use a fixed threshold do not allow for the temporal (i.e., daily and seasonal) variability of defense mechanisms and the predicted results associated with these models may not provide consistent results. Correspondingly, Grünhage et al. (2004) question the use of statistically derived constant flux thresholds for  $O_3$  uptake as suggested by Danielsson et al. (2003).

To examine the consequences of applying a (critical) flux threshold, we have employed the CODE91 vineyard data model (Massman et al., 1994; Pedersen et al., 1995; Massman, 2004) for partitioning the measured eddy covariance flux into stomatal ( $F_{stom}$ ) and non-stomatal components to yield half-hourly estimates of  $F_{stom}$  for the  $3\frac{1}{2}$  weeks of the experiment. A flux threshold ( $F_{thrsh}$ ) can now be applied to  $F_{stom}$  after which the average daily time course for the accumulated dose is determined by separating the data into  $\frac{1}{2}$ -h time slots and averaging the data within each of time slot. Only positive ( $F_{stom} - F_{thrsh}$ ) are included in the accumulated dose.

Fig. 7 shows the average daily time course of the cumulative dose associated with three different flux thresholds (3.8, 5.8, and  $7.5 \text{ nmol m}^{-2} \text{ s}^{-1}$ ) and the cumulative dose associated with  $F_{stom}$  only (i.e., no

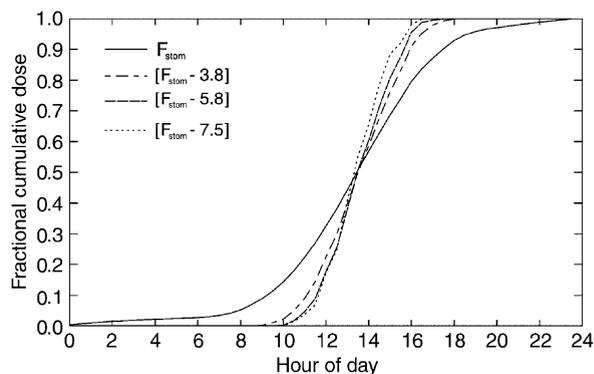


Fig. 7. Changes in fractional cumulative dose over time of day, using flux thresholds of 0, 3.8, 5.8, or  $7.5 \text{ nmol m}^{-2} \text{ s}^{-1}$ .

threshold). The  $5.8 \text{ nmol m}^{-2} \text{ s}^{-1}$  threshold is approximately the value of  $6 \text{ nmol m}^{-2} (\text{PLA}) \text{ s}^{-1}$  used by the participants of the United Nations Economic Commission for Europe International Cooperative Program (UNECE ICP) (Harmens et al., 2004). PLA is the Projected Leaf Area (one-sided) in the direction of the sun of leaves near the top of the canopy, which for this study was 3.4. For the present discussion  $\text{PLA} \approx 1$  is assumed for the vineyard. Comparison of the above with the other two values for  $F_{thrsh}$  ( $3.8$  and  $7.5 \text{ nmol m}^{-2} \text{ s}^{-1}$ ) demonstrates the sensitivity of the accumulation curve to the specific value of the  $F_{thrsh}$ .

Fig. 7 shows that employing a flux threshold preferentially weights the daylight hours between 10 a.m. and 3 p.m. The use of a flux threshold will reduce the cumulative dose during the morning hours, during which plant photosynthesis is high and detoxification chemicals are being produced. This is in agreement with the expectation that the morning  $O_3$  fluxes should contribute relatively less to the cumulative total. However, in the afternoon hours, when photosynthesis is reduced (from stress or carbohydrate loading) and defenses may become overwhelmed, additional accumulation of dose is occurring. The application of a flux threshold does not address the additional accumulation occurring during the late afternoon, nighttime and early morning hours. The application of a flux threshold underemphasizes or eliminates the fluxes occurring at these biologically important times.

## 5. European critical level

In the 1980s, several European countries were seeking an acceptable, scientific basis for designing

control strategies to reduce regional and trans-boundary air pollution. Under the auspices of the UN Economic Commission for Europe (UNECE) Convention on Long-Range Transboundary Air Pollution, it was agreed to adopt the critical levels approach for O<sub>3</sub>. A critical level is an exposure- or a flux-based parameter. Critical levels are set to prevent long-term injury and damage to the most sensitive elements of any ecosystem. They are used to map and identify areas in Europe in which the levels are exceeded and that information is then used to plan optimized and effect-based abatement strategies. As used by the UNECE, they are not air quality standards in the US sense, but they have been used as ecological targets for planning reductions in pollutant emissions.

While the nature of the significant harmful effects is not specified in the definition, which provides for different levels for different types of harmful effect (e.g., visible injury or loss of crop yield), there are different critical levels established for injury or for damage that apply to crops, forests, and semi-natural vegetation. These critical levels are revised periodically as new scientific information becomes available. The ‘Level I’ critical levels were used in the 1990s to map areas which exceed these levels, but research led to the conclusion that these simple, exposure-based levels lead to over-estimation of the effects in some regions and under-estimation in others (Kärenlampi and Skärby, 1996; Fuhrer et al., 1997). Different regions with similar AOT40 values can have considerably different O<sub>3</sub> flux values (Zeller, 2002) and research has shown the inadequacy of the AOT40 for estimating crop loss in Europe (De Santis, 1999; Ashmore et al., 2004; Uddling et al., 2004). Also, the AOT40 has additional limitations with mathematical robustness, which may further limit its usefulness (Sofiev and Tuovinen, 2000).

Therefore a decision was made to work towards a flux-based approach, with an objective goal of modeling O<sub>3</sub> flux-effect relationships for the three vegetation types (crops, forests, and semi-natural vegetation). Current critical levels for O<sub>3</sub> utilize three indices (i.e., exposure-based, modified exposure-based, and flux-based) depending on the crop and the scientific database available for the index. The critical levels terminology has now changed, such that Level I is called exposure-based and Level II is called flux-based (Harmens et al., 2004). Current information on Critical Levels is available online (Mills, 2004).

Flux-based indices are available only for wheat and potato, reflecting the limited database (Harmens et al., 2004). The AOT40, referred to as “concentration-based accumulated over a stated period of time,” is still used to predict effects on most crops and forest trees (Harmens et al., 2004). The “modified concentration-based” over a period of time (exposure-based) index uses VPD as the modifier (Harmens et al., 2004), since O<sub>3</sub> uptake and subsequent plant response is highly dependent on stomatal uptake as influenced by VPD. The modified concentration-based parameter is only used for crop injury, not damage.

## 6. Additional research needs for replacing exposure indices with effective-dose indices

Both exposure and effective-dose modeling efforts have their weaknesses in predicting O<sub>3</sub> vegetation effects. Exposure indices do not take into consideration O<sub>3</sub> uptake and detoxification, which tend to be independent, when used in exposure-response predictive models. The sensitivity of vegetation as a function of time of day or period of growth, as well as edaphic conditions, may result in plants being exposed to high O<sub>3</sub> concentrations with little resultant injury or damage, while more moderate levels of O<sub>3</sub> exposures result in injury (Showman, 1991; US EPA, 1986, 1996a). Similarly, large amounts of O<sub>3</sub> uptake, occurring during periods when detoxification processes predominate, can result in overestimates of vegetation effects.

In Section 4, we pointed out that plant defenses are difficult to quantify, but some research is beginning to quantify this component (Massman and Grantz, 1995; Chen et al., 1998; Plöchl et al., 2000; Barnes et al., 2002; Massman, 2004). Clearly, additional research, especially in relation to diurnal variation of detoxification processes, is needed in this area before effective-dose models have the power that is required to adequately predict vegetation effects.

Monitoring technology is progressing so that measuring and analyzing O<sub>3</sub> deposition to vegetated canopies, although difficult and complex, is becoming more routine. Models of O<sub>3</sub> conductance into plant tissue within a canopy also exist (Wesely, 1989; Massman, 1993; Grünhage and Haenel, 1997) but should be improved. Grünhage et al. (1999) provide a model of the deposition of O<sub>3</sub> on plant and soil surfaces. Other models that partition O<sub>3</sub> uptake into stomatal and non-stomatal components

are available (Zeller and Nikolov, 2000; Nikolov and Zeller, 2003).

As new experiments are performed to add to the previous scientific knowledge base, future research efforts should focus on the patterns (i.e., combinations) of the hourly average concentrations that are used in the controlled experiments from which data are generated. If new experiments use realistic exposures in flux-based experiments, then the problems associated with using NCLAN-type data, which contained numerous peak concentrations, to determine exposure-effects relationships can be avoided. In addition, attention should be paid to the exposure regimes that are used in the control comparisons. Selecting hourly average concentrations in the control experiments that are too low could result in data that tend to overestimate effects (US EPA, 1996a; Tingey et al., 2002).

Other factors, including predisposition time (McCool et al., 1988; Hogsett et al., 1988) and crop development stage (Heagle et al., 1991; Tingey et al., 2002), contribute to variations in biological response, which suggests the need for weighting  $O_3$  concentrations to account for predisposition time and phenology. However, the role of biology influencing plant response varies considerably with species and environmental conditions, so that specification of a weighting function for general use in characterizing plant exposure is not yet possible.

Both exposure indices and flux-based models currently have their limitations in predicting vegetation effects. Routine monitoring in the United States and Canada for  $O_3$  is summarized as hourly average concentrations. Emission reduction strategies for implementing pollution control plans involve relating  $O_3$  concentrations and exposures with appropriate reductions in  $O_3$  precursors. Massman et al. (2000) and Massman (2004) stress that the product of the overlapping mathematical relationships of conductance, concentration, and defense mechanisms results in a much different picture of potential impact to vegetation than just the use of conductance and concentration in predicting vegetation effects. The controlled and uncontrolled experimental results provide the basis for focusing on the higher  $O_3$  concentrations, while including the mid and lower levels, when estimating the effects of emission reductions on vegetation. Results of those studies provide the biological framework from which relevant exposure–response, dose–response, and effective dose models can be

developed so as to provide a consistent relationship between effective dose and vegetation effects. As we gain further insight into detoxification processes and other defense mechanisms that will allow us to develop better relationships between effective dose and vegetation effects, it is anticipated that more appropriate links can emerge between exposure, effective flux, ambient air quality standards, and emission reduction strategies.

## 7. Conclusions and recommendations

Determining response of plants to  $O_3$  requires defining the proper  $O_3$  summary statistic to relate to the plant response. Early work related ambient hourly average  $O_3$  concentration to plant response. Subsequent work determined that cumulative exposure to  $O_3$  and peak  $O_3$  concentrations were important for plant response, so exposure-based  $O_3$  parameters were utilized. Since stomata are partially open at night, the 24-h  $O_3$  exposure period of time should be used for both exposure–response and effective–dose models.

The effects of  $O_3$  on individual plants and the factors that modify plant response to  $O_3$  are complex and vary with biological and physical factors such as plant species, environmental conditions, and soil moisture and nutrient conditions. Researchers recognize that current  $O_3$  exposure indices do not fully characterize the potential for plant uptake, detoxification, and resulting vegetation effects (US EPA, 1996a). The exposure indices, being measures of ambient condition, do not take into consideration the physical, biological, and meteorological processes controlling the transfer of  $O_3$  from the atmosphere through the leaf and into the leaf interior, and subsequent biochemical reactions within the leaf. Experimental results that identify and quantify defense mechanisms and their relationship to  $O_3$  uptake are an important refinement in the use of flux-based indices to predict plant response.

Because of a lack of a dose-based index that includes uptake and detoxification, researchers and policymakers continue to use concentration- or exposure-based indices instead of effective–dose based indices. Until effective–dose models are developed that integrate  $O_3$  uptake and detoxification with biological response, we anticipate that models based solely on  $O_3$  flux will overestimate vegetation effects. Without adequate effective–flux based models, exposure-based  $O_3$  metrics appear

to be the only practical measure for use in relating ambient air quality standards to vegetation response.

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