TEMPORAL AND SPATIAL PATTERNS OF STOMATAL CONDUCTANCE,
OZONE CONCENTRATION, AND OZONE UPTAKE IN A
SUGAR MAPLE CANOPY

A Thesis Presented

by

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to

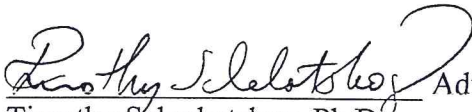The Faculty of the Graduate College

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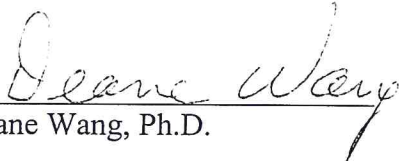Specializing in Forestry

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Accepted by the Faculty of the Graduate College, The University of Vermont, in partial fulfillment of the requirements for the degree of Master of Science, specializing in Forestry.

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Abstract

Tropospheric ozone is considered to be a contributory factor in widespread forest decline due to its phototoxicity and oxidizing capacity. Data from monitoring sites in the northeastern United States indicate that high ozone episodes are frequent in rural forested areas distant from ozone precursor sources. To develop a better description of potential ozone interactions in forest canopies, temporal and spatial patterns of stomatal conductance (g_s), ozone concentration (O_3), and other environmental variables were studied at five heights on a tower in an *Acer saccharum* Marsh canopy in Underhill, Vermont for 11 days. Both g_s and O_3 decrease with increasing depth in the canopy, with an average difference of 25% and 22% between the upper and lower canopy, respectively. Significant differences across time were found for both g_s and O_3. Both show a similar diurnal pattern reaching maxima in the early afternoon and decreasing in the evening. Regression analyses suggest that quantum flux density is the principal driving force for temporal and spatial patterns of g_s. Temperature was also found to influence both O_3 and g_s. Vertical differences in O_3 uptake per unit leaf area were a function of differences in g_s, while vertical variation in cumulative O_3 uptake was found to be a result of differences in leaf area density between heights. Uptake per unit leaf area ranged from 0.2 μmol m^{-2} h^{-1} to 34 μmol m^{-2} h^{-1}, and cumulative uptake ranged from 0.05 mmol ha^{-1} h^{-1} to 1000 mmol ha^{-1} h^{-1}. in the upper canopy. A large proportion (85%) of the total canopy O_3 uptake was observed in the upper crown (>10m) where the bulk (86%) of the total carbon gain in a sugar maple canopy occurs. Thus, the combined effect of higher O_3 and g_s in the upper canopy may result in decreases in carbon gain as O_3 and its precursors (NO$_x$ and VOC's) increase in the United States, as they are predicted to do. From this detailed evaluation of canopy processes it can be shown that scaling up from values of g_s and O_3 at one height and time underestimated total canopy ozone uptake by 50%.

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The Behavior of Ozone

Sources of Ozone

Ozone is a pervasive gas that exists both in the stratosphere and troposphere. Ozone in the stratosphere absorbs harmful UV rays, while ozone in the troposphere is a powerful oxidant which threatens human and plant health.

Some of the ozone in the troposphere is injected from the stratosphere. This process seems to involve large-scale eddies in the jet stream that occur when the boundary between the stratosphere and troposphere is deformed causing a downward fold which can be cut off from above by turbulent mixing (Lefohn 1992). This type of transport is extremely important to consider in the global budget of ozone. However, local and short term ozone budgets seem to be largely governed by photochemical pathways.

Ozone photochemistry requires the presence of hydroxyl (OH) radicals which are generated by either the photolysis of ozone (O_3):

$O_3 + hv \rightarrow O(^3P) + O_2$ and $O(^3P) + H2O \rightarrow 2OH$ (eq. 1) (hv=a quantum of radiation)
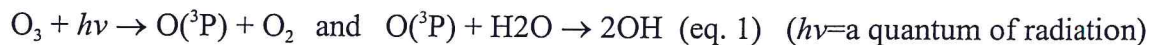
or, in the presence of peroxy radicals (HO_2), by the oxidation of nitrogen oxide (NO):

$HO_2 + NO \rightarrow OH + NO_2$ (eq. 2)

Hydroxyl radicals oxidize carbon monoxide (CO), methane (CH_4), or hydrocarbons (in a series of steps) to form peroxy radicals which in all cases react with NO to form NO_2 (see above). Nitrogen dioxide (NO_2) is the most important precursor to ozone production,

starting with its photolysis:

$$NO_2 + h\nu \rightarrow NO + O(^3P) \quad (eq.\ 3)$$
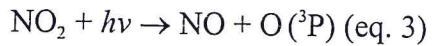
followed by the production of ozone:

$$O_2 + O(^3P) + M \rightarrow O_3 + M \quad (M \text{ is usually } O_2 \text{ or } N_2) \quad (eq.\ 4)$$

In the absence of NO the peroxy radicals can actually destroy ozone:

$$HO_2 + O_3 \rightarrow OH + 2O_2 \quad (eq.\ 5)$$

and ozone can also be destroyed by NO in the following reaction:

$$NO + O_3 \rightarrow NO_2 + O_2 \quad (eq.\ 6) \qquad \text{(Enders } et\ al.\ 1989, \text{Lefohn } 1992)$$

The production and destruction of ozone occurs at a stationary equilibrium (Leighton relationship, Lefohn 1992) resulting in only low ozone concentrations. This is shifted towards increased production of ozone in the presence of radicals like HO_2, which enhance the abundance of the precursors, while simultaneously producing OH radicals. Hydroxyl radicals can react with hydrocarbons which also results in ozone precursor formation. The rate of ozone production also increases in more polluted areas which are characterized by higher NO_x and volatile hydrocarbon concentrations, although both ozone and its precursors can be transported long distances. In the absence of high biogenic NO_x emissions the destruction of ozone is favored (Lefohn 1992). Both biogenic and anthropogenic emissions of hydrocarbons are comparable, while NO_x emissions are largely anthropogenic (Baumbach and Baumann 1989).

Temporal and Spatial Patterns of Ozone

Many studies have shown temporal and spatial patterns of ozone in the troposphere. Ozone tends to exhibit a strong diurnal pattern, with minima in the early morning or late night, followed by a rapid increase in the morning, reaching maxima in the mid to late afternoon (Baumbach and Baumann 1989; Enders *et al.* 1989; Fuentes *et al.* 1992; Taylor and Hanson 1992; Coe *et al.*1995; Frederickson *et al.* 1995; Pilegaard *et al.* 1995; Skelly *et al.* 1996). Maxima ranged between 50 ppb and 200 ppb of ozone in all studies. Higher ozone concentrations during the day have been attributed to photochemical production (which occurs only in the presence of photoradiation) of ozone by the photolysis of NO_2 (eq.3) (Fontan *et al.* 1992). The accumulation of NO_2 during the night is a result of NO production by soil microorganisms, and the subsequent oxidation of NO to NO_2 (eq. 6) (Baumbach and Baumann 1989). Authors also speculate that NO is oxidized by ozone, thus driving the nighttime destruction of ozone, which would support findings of lower nocturnal ozone concentrations (Frederickson *et al.* 1995). Lower ozone concentrations during the night may also be due to the emission of long wave radiation which causes radiative cooling, and hence a temperature inversion, resulting in nocturnal stability, consequently preventing mixing from above (Enders *et al.* 1989; Fontan *et al.* 1992; Güsten *et al.* 1998). Some studies showed that ozone concentrations can remain enhanced during the night. This may be due the break up of temperature inversions caused by surface heating resulting in rapid downward mixing from the canopy boundary layer (Enders 1992). Seasonal variation in ozone concentration has also been observed.

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Ozone concentrations are highest during summer months due to lower wind speeds, higher light intensity, and greater NO emissions (Coe *et al.* 1995; Baumbach and Baumann 1989). Skelly *et al.* (1996) found that the vertical variation of ozone in a deciduous forest canopy increased in summer due to larger temperature gradients.

Ozone has also been found to exhibit spatial patterns, showing increased concentration with increasing height and altitude (Frederickson *et al.* 1995; Skelly *et al.* 1996; Güsten 1998). It is proposed that vertical ozone profiles are caused by the combination of the physical process of dry deposition and chemical reactions with advected air masses containing NO_x's and hydrocarbons. Lower ozone concentration at the forest floor are due to the NO emitted by microorganisms, thus driving the destruction of ozone at this level (Fontan *et al.* 1992; Frederickson *et al.* 1995). As NO is depleted, peroxy radicals formed from the oxidation of hydrocarbons can drive further destruction of ozone (eq.4), and the photolysis of NO_2 (formed from the oxidation of NO) is inhibited in the absence of solar radiation at the forest floor (Enders *et al.* 1989). Johansson and Janson (1993) found that under certain conditions reactions of ozone with terpenes emitted from coniferous forests could be an important nighttime sink for ozone and that the depletion of terpene concentrations above a spruce canopy corresponded with maximum ozone concentration..

Seasonal and diurnal patterns of ozone have not been reported for high elevation sites, but rather, these sites have been characterized by a flat pattern of permanently higher ozone concentrations (Chappelka *et al.* 1997; Baumbach and Baumann 1989). The flat pattern, which is observed at elevations as low as 600 m (Whiteface Mt., NY), may be

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driven by the dynamics of an isolated atmospheric reservoir in the troposphere, which is somewhat uncoupled from the photochemical and scavenging pathways (Taylor *et al.* 1992). Perhaps at higher altitudes greater irradiance drives faster photolysis of NO_2, driving the production of ozone, although there is no evidence to support this idea. Baumbach and Baumann (1989) describe an inversion present at approximately 1500 meters which prevents air from mixing from below. Skelly *et al.* (1996) found higher ozone concentrations and the greatest vertical ozone gradient at night in a deciduous forest, supporting the idea that nocturnal stability inhibits mixing between layers thus resulting in a vertical gradient and nighttime minima. It is also possible that the absence of vegetation, which acts as a filter, causes higher concentrations.

Ozone concentrations in open areas do not vary as much with height due to the absence of filtering from vegetation (Taylor 1992; Frederickson *et al.* 1995). This suggests that forests act as a filter for ozone.

Ozone Flux above Canopies and Sinks

Most research on the behavior of ozone in forests have used the eddy correlation technique to calculate ozone flux in the boundary layer above forest canopies. Eddy correlation methods are used to measure the flux of ozone in relatively uniform pockets of air which are constantly changing in size and shape due to interaction with adjacent air pockets (eddies). Fluctuations of gases within these pockets are correlated with vertical wind speed. (Lefohn 1991; Nobel 1991). This technique effectively integrates fluxes of trace gases over large areas. However, it requires instruments with fast response times

and fast logging systems (usually less than 1 second), and is does not accurately estimate temporal and spatial variation in canopies which may affect ozone concentrations (Hargreaves *et al.* 1992).

The eddy correlation flux is calculated by $F_c = -K_m (w'c')$ (eq. 7), where K_m is the diffusion coefficient for irregular eddying of gases in the canopy boundary layer, w is the time averaged vertical wind velocity, and c is the concentration of ozone at height z. The prime indicates the instantaneous deviation from the mean (Coe *et al.* 1995; Pilegaard *et al.* 1995).

Using the calculated ozone flux the deposition velocity is calculated by $V_d = F_c/c$ (eq. 8), where F_c is the flux and c is the concentration of ozone. The reciprocal of the deposition velocity, V_d, is the total resistance to transfer, r_t. The total resistance is broken down into many components, but the most common are the aerodynamic resistance (r_a), the boundary layer resistance (r_b), and the canopy resistance (r_c). The canopy resistance can be comprised of many resistances both in series and in parallel, depending on the parameter of interest (i.e.: mesophyll, epidermis, cuticle, stomata). Stomatal resistance is inferred by comparing canopy resistance to the canopy resistance to water vapor (Fuentes *et al.* 1992; Coe *et al.* 1995). One of the unique properties of ozone is its surprisingly high deposition velocity onto vegetation (Taylor and Hanson 1992). Because ozone has a low solubility in water compared to other trace gases such as sulfur dioxide and nitric acid, the deposition velocity for ozone would be expected to be lower than other trace gases (Taylor and Hanson 1992). Wesely and Eastman (1982) found ozone to have a higher deposition

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velocity than nitrogen dioxide over a soybean field, with values ranging from 0.3 cm s^{-1} during the night to 0.8 cm s^{-1} during the day, which were thirty percent higher than deposition velocities of NO_2 on average.

Pilegaard et al. (1995) also found a maximum deposition velocity of 0.8 cm s^{-1} over a spruce forest, which occurred in the mid-morning. Fuentes et al. (1992) observed maximum deposition velocities at midday and the minimum at night over a Populus grandidentata and Acer rubrum forest. Both studies found a diurnal pattern of deposition velocity which followed the pattern of stomatal conductance. This suggests that stomata play an important role in the removal of ozone within the canopy. Coe et al. (1995) and Wesely and Eastman (1982) also found the maximum deposition in the late morning, and very low ozone deposition during the night. They suggest that stomata are the primary deposition pathway for ozone. Research at the Harvard Forest showed that stomatal uptake of ozone was the dominant factor influencing deposition (Munger et al. 1996).

Pilegaard et al. also observed high ozone deposition velocities during the night and the winter when stomata are closed, indicating that some other sink for ozone must exist. They speculate that reactions with terpenes and NO (already described) play a role in ozone destruction, thus accounting for some of the destruction.

Using a resistance model Coe et al. (1995) compared the total canopy resistance to stomatal resistance and found that the overall uptake of ozone was greater than that of stomatal uptake. They conclude that there is another mechanism which is responsible for ozone uptake. Because the calculated deposition velocity at night is very low they

conclude that the unknown sink is closely linked with daytime conditions including sunlight and temperature. The authors suggest that the cuticle acts as an efficient sink for ozone under strong light conditions.

Rondon et al. (1993) conducted experiments to study the deposition of ozone over Pinus sylvestris and Picea abies forests. They also found that the deposition values for ozone exceeded stomatal uptake and conclude that the cuticle is a significant sink for ozone. Uptake by the cuticle is probably driven by photochemical reactions on the cuticular surface during high light conditions, although they were unable to separate light and temperature effects in this study.

In contrast to these findings Kerstians and Lendzian (1989) found that deposition to isolated permeable leaf cuticle was four orders of magnitude less than that for whole leaves. Thus ozone uptake at the canopy level should focus on the pathway through stomata since it is the most significant pathway of ozone uptake.

Taylor et al. (1988) also explained the unexpectedly high deposition velocity of ozone by the existence of an unrecognized sink within the canopy; they believe that cuticular resistance is negligible, and therefore the additional sink is likely to be the soil.

Fuentes et al. (1992) found that in dry conditions stomatal conductance of ozone governed removal from within a Populus grandidentata and Acer rubrum canopy. However, in wet conditions, when stomata are presumably closed, the authors identified an additional sink for ozone. When sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) dissolve in water they form bisulfite (HSO_3^-) and nitrite (NO_2^-), respectively. In this study ozone was found to oxidize nitrite (NO_2^-) into nitrate (NO_3^-) and bisulfite (HSO_3^-)

into bisulfate (HSO_4^-) in surface water on the vegetation. The authors concluded that water on foliage acts as a sink for ozone.

Although many sinks for ozone have been identified within the canopy, most studies concluded that stomata are significant in ozone removal. Experiments to measure the surface deposition of ozone onto vegetation have shown that the resistance to ozone uptake increases by several orders of magnitude when stomata are closed. This suggests that stomata are the major pathway for ozone destruction in forests (Wesely and Eastman 1982; Fontan et al. 1992; Munger et al. 1996; Ollinger et al. 1997).

Understanding the temporal and spatial patterns of ozone in canopies is extremely important when trying to quantify canopy level uptake of the pollutant. Forest decline, partially attributed to ozone exposure, may be further explained with the integration of canopy level experiments and plant fumigation experiments to examine the biological and physiological response to ozone. Plant fumigation experiments clarify the potential effects of ozone on vegetation, and canopy level experiments offer insight into the mechanisms responsible for the uptake of this harmful pollutant.

It is necessary to investigate temporal and spatial patterns of stomatal conductance in canopies since it is primarily through this pathway that ozone enters the plant an variation is plant responses is largely related to differences in conductance (Ollinger et al.1997). It is also necessary to provide an overview of plant responses to elevated ozone exposure in order to validate the urgency of continued research regarding ozone behavior in natural ecosystems. Almost all of the research involves exposing plants to above ambient concentrations of ozone. These data can be used to speculate about potential

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damage as the troposheric ozone increases with burgeoning urbanization and fossil fuel emissions from motor vehicles. This will be the topic of the final section in the literature review.

Spatial and Temporal Patterns of Stomatal Conductance

Studies have shown that stomatal conductance (of water vapor) exhibits a diurnal pattern which closely follows patterns of irradiance, as well as water availability. Stomatal conductance decreases in the afternoon in response to increased vapor pressure deficit (Beadle *et al.* 1985), and stomatal conductance stops when light levels decrease in the evening (Iacobelli and McCaughey 1993).

Vertical differences of stomatal conductance in canopies have also been reported. Iacobelli and McCaughey (1993), Reich *et al.* (1990), Roberts *et al.* (1990); Beadle *et al.* (1985), Frederickson *et al.* (1996), and Leverenz *et al.* (1982) observed that stomatal conductance rates decline with increasing depth into the canopy. Higher rates of stomatal conductance in the upper canopy are mostly attributed to greater levels of irradiance. For example, in a *Picea sitchensis* plantation 80% of the net radiation was absorbed by the upper canopy where 40-50% of the carbon gain occurred (Leverenz *et al.* 1982).

Profiles of sunlight in a canopy cause structural variation as well. Ellsworth and Reich (1993) found that leaves in the upper canopy have higher leaf mass per area (LMA) because they develop in high light and as a result are thicker and have greater mesophyll density. These structural differences account for some of the vertical variation in stomatal conductance. Reich *et al.* (1990) found that LMA decreases with increased

depth in an *Acer saccharum*, *Quercus rubra*, and *Tilia americana* canopy. Harley *et al.* (1996) also found that variation in conductance was due to these structural differences. It has also been suggested that the highest total stomatal conductance at the canopy scale will correspond to layers with the highest leaf area index (LAI), which is the middle and upper canopy levels in most forests (Ellsworth and Reich 1993).

Conductance to water vapor is also affected by external factors that alter the vapor pressure gradient between the leaf interior and the surrounding air. The steepness of the gradient rises with increasing dryness (decline in RH) and increasing temperature (Taiz and Zeiger 1998). Temperature and relative humidity are known to exhibit temporal and spatial pattern which may also influence patterns of stomatal conductance.

The aforementioned studies have clearly demonstrated that ozone concentrations and stomatal conductance are greatest in the upper canopy. Samuelson and Kelly (1996) found that stomatal conductance of ozone was at least double for *Quercus rubra* canopy trees and six times greater for *Prunus serotina* canopy trees than for understory saplings and seedlings of each. Frederickson *et al.* (1995) also found greater uptake of ozone through stomata of *Prunus serotina* trees at the upper crown level. They found greater stomatal density in seedlings than canopy trees which would result in a higher instantaneous dose, but the ozone concentration at this level was lower than the upper canopy. They also noted that the leaves in the upper canopy expanded earlier in the season thus resulting in an even higher cumulative dose.

Reich *et al.* (1990) calculated that 86% of the carbon gain (CO_2 is conducted into the leaf through stomata) occurred in the upper and middle canopy layers in an *Acer* spp. and

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Quercus rubrum forest, and that 66% of the carbon gain in an *Quercus* spp. and *Machia* forest occurred in the top third of the canopy. This is undoubtedly due in large part to greater stomatal conductance at these heights. Many researchers have found that ozone exposure caused decreases in stomatal conductance (Rebbeck *et al.* 1993; Samuelson 1994; Pearson 1995; Samuelson *et al.* 1996; Pääkonen *et al.* 1996, Momen *et al.* 1997; Zuethen *et al.* 1997).

The combined effect of higher ozone concentration and stomatal conductance rates in the upper canopy may result in an overall decline in carbon gain of forests as ambient ozone concentrations continue to increase. Reich *et al.* (1990) used a model to show that daily canopy carbon gain can be reduced by 57% at a high ozone dose. Skelly *et al.* (1996) found that uptake of ozone and stomatal conductance rates were highest in the upper canopy. Allen *et al.* (1992) found that most crown dieback associated with ozone injury is observed in upper canopy positions. This is most likely due to the large portion of carbon gain occurring there.

Other studies have shown that ozone exposure may lead to a loss of stomatal ability to close (Clark *et al.* 1996; Tjoelker *et al.* 1995). If this is the case, trees may be taking up larger doses of ozone, thus making them increasingly susceptible to physiological and biological dysfunction. Moreover, differential rates of conductance, stomatal density, and leaf morphology between species and individuals may play a large role in ozone response to ozone, since this variation will lead to different ozone doses into the leaf interior (Reich 1987).

Both scenarios described above seem possible based on the current literature, and both have the potential to result in severe changes in our forested ecosystems as ozone pollution continues to increase.

Studies using eddy correlation and resistance model techniques are useful in quantifying canopy level ozone uptake, while studies using porometry are useful in characterizing leaf level uptake and vertical and temporal variation in uptake. Taylor and Hanson (1992) compared the stomatal uptake of ozone to the calculated ozone deposition of ozone into stomata. The stomatal uptake of ozone was calculated as the stomatal conductance of water vapor measured with a porometer divided by the diffusivity coefficient of ozone (which is 1.68), and the ozone deposition into stomata was calculated using eddy correlation and resistance models. The authors found strong agreement between methods ($r^2=0.71$).

Leverenz et al.(1982) reported that stratified sampling of stomatal conductance increases confidence when estimating canopy scale conductance if the average conductance for each stratum is divided by the leaf area at that height. They also indicated that sample size (n) can be markedly lower if sampling at 5 to 7 strata within the canopy. These authors also compared canopy conductance from porometry measurements with calculated canopy conductance using eddy correlation and resistance models. They found strong agreement when using the stratified sampling method and by taking into account the leaf area at each height.

Effects of Ozone on Plants

Biochemical Effects

Ozone is a unique molecule with some interesting qualities. It has a very short half-life on the order of minutes to hours as well as low solubility in water. As a result, ozone is highly reactive, generating free radicals of oxygen which react with and damage biomembranes. (Taylor and Hanson 1992)

Laisk (1989) reported that ozone concentrations in the intercellular space of a leaf rapidly decreases to zero. The ozone concentration was observed to decrease from 150 ppb to zero in the substomatal cavity, and this was explained by the high reactivity of ozone.

In contrast, other authors have suggested that ozone is likely to penetrate deep into the interior air spaces of a leaf due to its low solubility in water (McLaughlin and Taylor 1981; Taylor 1988).

Taylor and Hanson (1992) found that 23% of the ozone taken up by stomata reacts within the substomatal cavity, and that ozone uptake per cell decreases with increasing distance from stomata. The authors pointed out that cells in the substomatal cavity are more suberized than palisade mesophyll cells, suggesting that palisade cells are more sensitive to ozone.

This idea is supported by many experiments which found changes in chloroplasts, which are most abundant in the palisade cells. Changes in the chloroplast envelope and

thylakoid membrane in response to ozone have been reported (Schmieden and Wild 1995; Pääkkonen *et al.* 1996). Pääkkonen *et al.* (1996) observed curling and swelling of thylakoids, enhanced stacking of grana, and increased stroma density after two weeks of ozone exposure at 100 ppb.

Many studies also found decreased levels of chlorophyll a in ozone sensitive individuals (Rebbeck *et al.* 1993; Tjoelker *et al.* 1995; Pääkkonen *et al.* 1996) as well as decreases in rubisco (ribulose biphosphate carboxylase-oxygenase) enzyme levels (Samuelson 1994; Clark *et al.* 1996; Pääkkonen *et al.* 1996). Pell *et al.* (1992) showed that rubisco generation is sensitive to free radical ozone, which may oxidize macromolecules which, in turn, alter the levels of rubisco mRNA transcripts.

Some authors have suggested that the majority of ozone in a leaf reacts with the plasmalemma (Laisk 1989; Matyssek *et al.* 1995; Schmieden and Wild 1995). They found that detoxification and repair reactions in response to ozone increase in the apoplast. Ascorbate and glutathione have been identified as oxidant scavengers. These metabolites are generated by photosynthetic electron transport, and are exchanged between chloroplasts and cytosol, and then transported to the apoplast and plasmalemma (Laisk 1989). Chameides (1989) found higher concentrations of ascorbate in the apoplast of ozone tolerant species (up to 10^{-3} M), and that this pathway of oxidant depletion becomes saturated with increasing ozone dose. Matyssek *et al.* (1995) found that sun leaves had higher levels of antioxidants. This suggests that leaves growing in full sun may be more resistant to ozone damage.

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Photosynthesis and Stomatal Conductance

Most of the research on the deleterious effects of ozone on trees has focused on physiological responses such as photosynthesis, stomatal conductance, and carbon partitioning. Reich (1987) recommended using photosynthesis to determine plant responses to ozone since it reflects total biomass production, an important endpoint for healthy metabolism.

Many authors have observed decreases in net photosynthesis and stomatal conductance (of water vapor) in response to ozone. Samuelson (1994) and Pääkkonen *et al.* (1996) found decreases in photosynthesis and stomatal conductance in mature *Acer rubrum*, *Prunus serotina* , and *Betula pendula*. These authors attributed the observed decreases in photosynthesis to changes in biochemical processes (described earlier) as opposed to reductions in photosynthesis due to stomatal closure. They suggest that the stomata closed in response to high intercellular CO_2 concentrations as photosynthesis decreased. Reich (1987) also asserted that ozone inhibits photosynthesis directly, and that stomata closed in response to high intercellular CO_2.

Zeuthen *et al.* (1997) found photosynthesis and stomatal conductance to decrease in mature *Fagus sylvatica*. They concluded that the decrease in photosynthesis was largely due to biochemical dysfunction, although they believe that decreased stomatal conductance must play a role in the decrease as well. Eamus and Murray (1991) concluded that ozone directly caused stomata to close in mature *Fagus sylvatica*.

Pearson (1995) found that photosynthesis, stomatal conductance, and intercellular CO_2 decreased in *Eucalyptus globulus* seedlings, and concluded that ozone directly caused stomata to close, consequently causing reduced photosynthesis.

Tjoelker *et al.* (1995) found that photosynthesis and stomatal conductance decreased in mature *Acer saccharum*. They also found that ozone directly affected photosynthesis and that stomata closed initially in response to high intercellular CO_2. However, contrary to other studies, they found that eventually stomata opened despite continued decreases in photosynthesis. The authors suggest that ozone may impair stomata directly by delignifying guard cell walls, resulting in stomatal opening.

Clark *et al.* (1996) also found that both photosynthesis and stomatal conductance decrease initially in *Populus tremuloides* seedlings. They also attribute the decrease in photosynthesis to biochemical dysfunction. However, as Tjoelker *et al.* (1995) observed, they found that stomata eventually lose control and open in response to ozone. Impaired stomatal regulation, resulting in failure to close, normally can cause increased water loss and increased ozone uptake (Skärby *et al.* 1987).

Mansfield (1998) asserted that both interspecific variation in conductance and water status can explain different ozone effects on stomata.

From the literature it is clear that ozone does indeed affect photosynthesis and stomatal conductance. This is significant since both processes are necessary to maintain a positive carbon balance in a plant. However, there is some disagreement about which biochemical process is being directly affected by ozone. Decreases in photosynthesis may be caused by changes in the photochemical pathway or by stomatal closure in

response to ozone, or possibly both.

Ozone exposure also seems to eventually cause stomata to lose control. Perhaps some of the ambiguity can be blamed on the variation in ozone dose and duration between experiments. It is likely that ozone affects both the photochemical pathways and the stomata depending on the treatment (Lefohn 1991; Zuethen 1997). All of the previous experiments exposed the plants to ozone concentrations between 40 and 140 ppb.

Carbon Partitioning and Growth

Plants exposed to ozone are believed to exhibit a stress response. This can be expressed as ozone injury, changes in carbon allocation and partitioning, and altered growth (Grulke and Miller 1994 and 1996; Schmieden and Wild 1995; Zeuthen *et al.* 1997).

Samuelson and Kelly (1996) found reduced carbohydrates in roots and leaves, and increased carbohydrates in branches in *Quercus rubrum* adults. Schmieden and Wild (1995) also referred to documented reductions in carbon partitioning to roots and foliar carbon retention. The authors suggest that ozone induces changes in carbon demand resulting in higher maintenance respiration and less carbon reserved in sinks, and that changes in carbon allocation and partitioning may indicate a compensatory response which may postpone reductions in above ground growth.

Friend and Tomlinson (1992) detected a shift in carbohydrate partitioning to starch and an increase in partitioning to repair compounds in *Pinus taeda* needles. Carbon transport to roots was also reduced in response to ozone exposure.

Samuelson (1994) found increased foliar carbon retention in *Acer rubrum* seedlings, but decreased foliar carbon retention in *Prunus serotina* seedlings coupled with increased carbohydrates in branches in response to ozone. Paynter *et al.* (1991) also observed increased carbon retention in *Pinus echinata* needles.

Chappelka *et al.* (1997) found decreases in herbivore defense compounds in ozone injured *Asclepias exalta* and *Prunus serotina*, which they attributed to increased carbon demand for stress repair mechanisms. These plants were growing in natural conditions.

Changes in carbon allocation and partitioning can both stimulate and suppress growth (Samuelson and Kelly 1996). Kress and Skelly (1982) exposed several eastern tree species to three concentrations of ozone (50, 100, and 150 ppb) for 28 days in fumigation chambers. At 50 ppb *Pinus taeda* exhibited a significant height growth suppression, while *Liriodendron tulipifera* exhibited a height growth stimulation. At 100 ppb *Pinus taeda*, *Liquidambar styraciflua*, *Pinus rigida*, and *Platanus occidentalis* exhibited a height growth suppression, while *Acer saccharum* exhibited a height growth stimulation. All species except *Pinus virginiana* exhibited a height growth suppression at 150 ppb. This study indicates that a species may be sensitive in terms of growth impact but tolerant in terms of foliar injury (*Pinus taeda*) or conversely, a species may be tolerant in terms of growth impact but sensitive in terms of foliar injury (*Liriodendron tulipifera*). It is clearly demonstrated by this study that changes in carbon allocation can cause very

different responses (i.e. growth vs. foliar injury). Laurence *et al.* (1996) support the findings of Kress and Skelly (1982). They found that *Acer saccharum* does not show signs of reduced biomass or growth in ozone

exposure of less than 140 ppb, at which point they exhibit growth suppression and lower foliar biomass. Many authors who observed decreases in photosynthesis found subsequent decreases

in growth rates (Pearson 1995; Pääkkonnen *et al.* 1996; Samuelson *et al.* 1996).

The reviewed literature clearly confirms that changes in carbon allocation and partitioning are caused in response to ozone exposure. However, each species seems to demonstrate a different combination of changes, which can both enhance or inhibit carbon partitioning and growth depending the plant part of interest, and ozone dosage.

Differential Responses to Ozone

Differential responses of plants to ozone have been characterized by Reich (1987) who observed that annual plants are the most sensitive to ozone exposure, followed by deciduous trees, and finally conifers, being the most resistant to the pollutant. He found a 73% decrease in photosynthesis in annual plants, a 36% decrease in deciduous trees, and a 7% decrease in conifers exposed to ozone.

Reich asserted that the variation in responses can largely be attributed to differences in stomatal conductance rates and carbon assimilation. For example, the foliage of conifers represents a greater relative energy investment because they are retained for longer periods of time; however; needles are less productive per unit time. Conifer needles have

comparatively lower rates of stomatal conductance, thus requiring more time to match the ozone uptake of a deciduous tree with higher conductance.

Variation in stomatal conductance rates exists among deciduous trees as well. Laurence (1996) found that *Acer saccharum* have lower stomatal conductance than *Populus tremuloides* trees, perhaps explaining the greater sensitivity to ozone of the latter.

Many authors have also found intraspecific differences in ozone sensitivity. Grulke and Miller (1996) examined the response of mature *Sequoiadendron giganteum* to ozone exposures. They found that some individuals were more sensitive to ozone. The most sensitive trees had the greatest stomatal conductance before the treatment.

Studies have shown that mature and juvenile trees of the same species also respond differently to ozone exposure. Samuelson *et al.* (1996) and Samuelson and Kelly (1996) found that mature individuals of *Quercus rubra*, *Prunus serotina*, and *Acer rubrum* all had greater stomatal conductance rates than seedlings and saplings of the same species before ozone treatment. They also found that the uptake of ozone was greatest for the mature individuals. The higher cumulative dose to mature trees was found to be a function of differences in stomatal conductance. Both studies reported that mature trees showed more physiological damage than juveniles.

Frederickson *et al.* (1995) also found that both stomatal conductance and ozone uptake were greater for *Prunus serotina* canopy trees than for seedlings and saplings. Similarly, they found that canopy trees had the greatest cumulative uptake of ozone as a result of

greater conductance. They also pointed out that canopy tree leaves expanded earlier, resulting in a longer exposure time.

Momen *et al.* (1997) also reported increased sensitivity in mature *Pinus ponderosa* compared to seedlings. Mature trees exhibited greater stomatal conductance prior to ozone exposure.

In contrast, some authors found that juvenile trees are more sensitive to ozone. Grulke and Miller (1994) observed greater ozone uptake and stomatal conductance (prior to ozone treatment) in juvenile trees of *Sequoiadendron giganteum*. Seedlings and saplings showed marked increases in respiration, and eventual decreases in stomatal conductance after exposure to ozonecompared to mature trees.

Rebbeck *et al.* (1993) also observed the stomatal conductance of juvenile *Picea rubens* to be 50% higher than mature trees before ozone treatment. The juveniles exhibited more physiological and biochemical changes after ozone exposure, suggesting that their sensitivity was largely due to a greater cumulative dose.

Although some research indicated that juveniles had greater stomatal conductance, higher conductance in mature trees is more widely documented.

Conclusions

The current study focuses on the uptake of ozone by *Acer saccharum* trees. A reduction in sugar maple vigor has been reported throughout the Northeastern United States and Southeastern Canada in the past 20 years (Fortin *et al.*, 1997). This decline is generally associated with early leaf abscission, abnormal coloration, dieback, reduced productivity, and death. Frequently this weakens trees, predisposing them to attack by fungi or insects. Fortin *et al.* (1997) found that forest tent caterpillar larvae preferred sugar maple foliage treated with 3x-ambient ozone. This could signify that greater injury by the forest tent caterpillar will occur in sugar maple exposed to high ozone concentrations. Payette *et al.* (1996) concluded that sugar maple declines were more likely caused by discrete abiotic factors such as drought, which were further exacerbated by insect defoliation.

It is clear that the direct effects of any single pollutant are quickly obscured by the complex interaction between several other pollutants and environmental factors. It is very important to continue research and monitoring efforts so that the mechanisms of forest decline can be understood in the near future.

The behavior of ozone in canopies has yet to be fully explained, although with careful integration of research from atmospheric, ecological, and physiological disciplines the relevant processes will be highlighted and new questions will be generated. Ozone poses a threat to both human and plant health and should be closely monitored and studied by

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scientists all over the world to ensure a collective understanding of the effects that this harmful pollutant may have.

There is evidence that a commercially important tree species are affected by ozone potentially resulting in economic losses. For example, reductions in photosynthesis and stomatal conductance were observed in mature sugar maple trees exposed to 60 pbb throughout the day for 3 years (Gaucher *et al.*1997) and 75-115 ppb throughout the day for 6 months (Tjoelker 1996).

During the summer of 1993 a forest canopy at Proctor Maple Research Center in Underhill, Vermont was exposed to an excess of 300 hours of ozone concentrations above 60 ppb (probable threshold for sensitive plants), and at Mt. Equinox, in the southern section of the Green Mountain National Forest, Vermont ozone concentrations above 50 ppb have exceeded 300 hours during the summer in 5 out of the 6 years that ozone monitoring has occurred there (Manning 1994).

The goal of the current research was to examine the spatial and temporal patterns of ozone concentration, stomatal conductance, and hence, the uptake of ozone in a sugar maple canopy. Forest canopies are not homogenous. They have distinct structural properties, such as leaf mass and area, which influence patterns of radiation interception and moisture. To accurately estimate canopy scale uptake of ozone it was necessary to sample in multiple layers of the canopy at several time intervals to account for temporal and spatial variation in environmental and physiological factors. In this way, the

temporal and spatial patterns of conductance, ozone concentration, and ozone uptake were identified.

Temporal and Spatial Patterns of Stomatal Conductance, Ozone Concentration, and Ozone Uptake in a Sugar Maple Canopy

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Abstract

Tropospheric ozone is considered to be a contributory factor in widespread forest decline due to its phototoxicity and oxidizing capacity. Data from monitoring sites in the northeastern United States indicate that high ozone episodes are frequent in rural forested areas distant from ozone precursor sources. To develop a better description of potential ozone interactions in forest canopies, temporal and spatial patterns of stomatal conductance (g_s), ozone concentration (O_3), and other environmental variables were studied at five heights on a tower in an *Acer saccharum* Marsh canopy in Underhill, Vermont for 11 days. Both g_s and O_3 decrease with increasing depth in the canopy, with an average difference of 25% and 22% between the upper and lower canopy, respectively. Significant differences across time were found for both g_s and O_3. Both show a similar diurnal pattern reaching maxima in the early afternoon and decreasing in the evening. Regression analyses suggest that quantum flux density is the principal driving force for temporal and spatial patterns of g_s. Temperature was also found to influence both O_3 and g_s. Vertical differences in O_3 uptake per unit leaf area were a function of differences in g_s, while vertical variation in cumulative O_3 uptake was found to be a result of differences in leaf area density between heights. Uptake per unit leaf area ranged from 0.2 μmol m^{-2} h^{-1} to 34 μmol m^{-2} h^{-1}, and cumulative uptake ranged from 0.05 mmol ha^{-1} h^{-1} to 1000 mmol ha^{-1} h^{-1}. in the upper canopy. A large proportion (85%) of the total canopy O_3 uptake was observed in the upper crown (>10m) where the bulk (86%) of the total carbon gain in a sugar maple canopy occurs. Thus, the combined effect of higher O_3 and g_s in the upper canopy may result in decreases in carbon gain as O_3 and its precursors (NO_x and VOC's) increase in the United States, as they are predicted to do. From this detailed evaluation of canopy processes it can be shown that scaling up from values of g_s and O_3 at one height and time underestimated total canopy ozone uptake by 50%.

Introduction

Widespread forest decline in North America has been documented over the past two decades (Reich 1987; Gaucher 1997). Tropospheric ozone has been proposed as a contributory causal factor in this decline as a result of several documented deleterious effects on physiological and biochemical factors in plants (McLaughlin 1985)

In addition to reductions in agricultural and commercial forest yields due to reduced growth and survivability, exposure to ozone concentration has been linked to human respiratory ailments (Lefohn 1991).

Ozone levels have frequently exceeded national ambient air quality standards (NAAQS) in many parts of the USA and are expected to increase in the next 30 years by approximately 1% each year in the mid to high latitudes of the Northern Hemisphere (Hought and Derwent 1990; Ollinger et al.1997). Furthermore, rural forested areas frequently have high concentrations of ozone due to long distance transport and lower levels of scavenging compounds (Aneja and Zheng 1992).

It has been suggested that forests act as an efficient filter for many gases and aerosols because of their large surface area for gaseous exchange (Cavender and Allen 1990; Lefohn 1992). The behavior of ozone within the forest canopy is not well understood, however, many sinks for ozone within the canopy have been identified (Fontan et al.1992; Fuentes et al.1992; Taylor et al.1992; Rondon et al.1993; Kull and Moldau 1994; Coe et al.1995; Pilegaard et al.1995). Numerous experiments to measure the surface

deposition of ozone onto vegetation have shown the resistance to ozone uptake increases by several orders of magnitude when stomata are closed, suggesting that stomatal uptake of ozone is the dominant deposition pathway (Wesely and Eastman 1982; Fontan *et al.* 1992; Munger *et al.* 1996). It is well established that the daily course of stomatal conductance is significant in controlling the deposition of gaseous pollutants including ozone (Ollinger *et al.* 1997). From a forest health perspective it is necessary to focus on stomatal uptake of ozone, since it is primarily through this pathway that ozone enters the plant.

There is evidence that commercially important tree species are affected by ozone potentially resulting in economic losses. For example, reductions in photosynthesis and stomatal conductance were observed in mature sugar maple trees exposed to 60 pbb throughout the day for 3 years (Gaucher *et al.* 1997) and 75-115 ppb throughout the day for 6 months (Tjoelker 1996).

Ozone concentrations as low as 50 ppb are reported to cause physiological and biochemical changes in plants (Manning 1994; Pearson 1995, Laurence 1996). High doses of ozone have been shown to impair stomatal function, reduce photosynthetic and conductance rates, reduce growth, and cause changes in carbon partitioning and allocation (Rebbeck 1993; Samuelson 1994; Frederickson et al., 1995; Pearson 1995, Tjoelker *et al.* 1995; Laurence *et al.* 1996; Pääkkonen *et al.* 1996; Samuelson *et al.* 1996; Momen *et al.* 1997; Zuethen *et al.* 1997). Ozone injury occurs in the leaf interior where it oxidizes cell membranes, pigments, and photosynthetic enzymes (McLaughlin and Taylor

1981;Taylor 1988; Lefohn 1992). It is therefore imperative that factors affecting leaf gas exchange are identified.

During the summer of 1993 a forest canopy at Proctor Maple Research Center in Underhill, Vermont received in excess of 300 hours of ozone concentrations above 60 ppb (probable threshold for sensitive plants), and at Mt. Equinox, Vermont ozone concentrations above 50 ppb have exceeded 300 hours during the summer in 5 out of the 6 years that ozone monitoring has occurred there (Manning 1994).

Studies have shown that ozone concentrations decrease with increasing depth in the canopy (Waite and Scherbatskoy 1994; Frederickson 1995; Samuelson and Kelly 1996). Stomatal conductance has also been observed to decrease with increasing depth in the canopy (Leverenz *et al.* 1982; Beadle *et al.* 1985; Reich *et al.* 1990; Iacobelli and McCaughey 1993). The combined effect of higher ozone concentration and stomatal conductance in the upper canopy may result in enhanced uptake of ozone at this level (Reich 1990; Samuelson and Kelly 1996; Skelly *et al.* 1996).

The goal of the current research was to examine the spatial and temporal patterns of ozone concentration, stomatal conductance, and hence, the stomatal uptake of ozone in a sugar maple canopy. Forest canopies are not homogenous. They have distinct structural properties, such as leaf mass and area, which influence patterns of radiation interception and moisture. To most accurately estimate canopy scale stomatal uptake of ozone it was necessary to sample in multiple layers of the canopy at several time intervals to account for temporal and spatial variation in environmental and physiological factors. In this

way, the temporal and spatial patterns of conductance, ozone concentration, and ozone uptake were identified.

Methods

Site Description

Field studies were conducted at the Proctor Maple Research Center (PMRC) in Underhill Center, Vermont [Chittenden County] (44° 31' 42", 72° 51' 09", elevation 410 m). PMRC is located on the western slope of Mt. Mansfield with a northwesterly aspect. The regional climate is cool, moist, and submontane-continental with warm summers and cool winters. Average annual precipitation at PMRC is 1160 mm (1990-1997 data; NADP 1999) with approximately 40% of annual precipitation falling as snow. Average precipitation pH at PMRC is 4.37 for 1990-1997 (NADP 1999). The summer of 1998 was the rainiest on record since 1897, with 581 mm falling during the months of June, July, and August 1998.

The site is located on glacial till substrate derived from schists and gneisses of Cambrian origin, which tend to weather to base-cation poor acid soils. The soils are well developed Humo-Ferric Podzols of the Haplorthod great group (Canada Soil Survey Committee 1978) and are a coarse loam soil type (T.R. Wilmot, unpublished data).

The dominant forest trees at the study site include *Acer saccharum*, *Acer rubrum*, *Betula alleghaniensis*, and *Fagus grandifolia* with an occasional *Ostrya virginiana*. The understory consists primarily of *Cornus alternifolia*, *Viburnum alnifolium*, *Polygonatum pubescens*, *Arisaema triphyllum*, *Trillium* sp., *Smilacina racemosa*, *Prenathes alba* and