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The effects of oxidative air pollutants on plant cuticles cuticular transpiration plant water balance and growth

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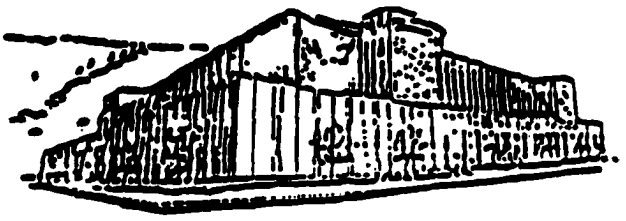

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The Effects of Oxidative Air Pollutants on Plant cuticles, cuticular transpiration, plant water balance, and growth

By

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M.S. Air Quality, 1993

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Abstract

Morphological, physiological and growth effects of acute chlorine gas (Cl_2) exposure were examined over three growing seasons in Western Montana, Rocky Mountains, USA, for two conifer species, *Pinus ponderosa* and *Pseudotsuga menziesii*. Acute damage symptoms after exposure consisted of chlorosis, necrosis, necrotic mottling, and defoliation. Cuticles of exposed *P.menziesii* needles and needles that flushed after Cl_2 exposure were more wettable. Moreover, foliage of both species had higher cuticular transpiration rates and lower total water content compared to control foliage, up to one year after exposure. Lower photosynthetic efficiencies, measured as F_v/F_m ratios, were observed for exposed foliage five months after exposure. Foliage on trees that flushed two months after Cl_2 exposure had higher foliar injury and lower needle longevity compared to controls, suggesting higher susceptibility to secondary stress factors. Exposed trees also had lower annual stem increment growth and cone production. Higher tree mortality was observed for *P.menziesii* but not for *P.ponderosa*.

Saplings of two poplar species, *Populus Euramericana Robusta* and *Populus nigra Brandaris*, and *P.menziesii*, were exposed to ozone (O_3) concentrations characteristic for growing seasons in urban areas and high elevations. Ozone exposure increased leaf wettability of poplars only temporarily, but increased cuticular transpiration of *P.Euramericana*. Both poplar species had lower foliar biomass in the ozone treatments compared to controls because of lower leaf growth and higher leaf abscission. Ozone effects on *P.menziesii* were limited to increased leaf wettability, which can affect ozone deposition and gas exchange. Leaf surface wetness, in the form of simulated dew, rain and mist, increased ozone deposition to poplar leaves 1.5-5 times. This increase was the result of lower ozone deposition due to stomatal closure, and ozone deposition to water present on leaf surfaces. Leaf surface wetness also decreased photosynthesis and CO_2 emissions in dark conditions.

This research has shown that acute Cl_2 exposure and chronic ozone exposure caused similar morphological, physiological and growth effects on trees, although ozone effects were less severe. Both pollutants may have long-term impacts on drought tolerance and growth of coniferous and deciduous trees. However, responses were highly dependent on species, both for conifers and deciduous trees.

Acknowledgements

This work was made possible by the assistance of many people. I am particularly grateful for the consistent support, both intellectual and personal, from my major professor, Dr. Carol Brewer, who spent many hours discussing and editing the contents of this dissertation. I am also grateful for the time and feedback from past and present committee members, who helped me to grow intellectually and stay on track. So, thanks to Dr. Anna Sala, Dr. Donna Boggs, Dr. Colin Henderson, Dr. Garon Smith and Dr. Paul Alaback. I would like to thank other faculty members, Dr. Vicky Watson, Dr. Tom Deluca and Dr. Diana Six, for their valuable input in the chlorine research, and Mark Heaphy and Dr. Colin Henderson for their assistance with the statistical analyses.

In the Netherlands I thank Dr. Bert van Hove at the Wageningen University and Research Center, for his generosity in time, advise and support, both in equipment and personal, that enabled me to carry out the ozone studies. At the Department of Plant Physiology, I thank Taede Stoker and Leen Peterse for their professional care of the plants and growth facilities, and Ria van den Noort, who instructed in me in the analyses of stomata, and was always a helping hand when needed. At the Department of Air Quality I thank Paul Heeres, who helped me to keep things going when equipment broke down (usually on Friday night at 6 pm...).

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CHAPTER 1
Introduction: Effects of strong oxidative air pollutants on plants

Humans have a long history of causing air pollution. Especially since the industrial revolution at the end of 1800's, not only has the production volume of goods dramatically increased, but so have emissions of air pollutants. At the modern day levels of emission, negative impacts of air pollutants have been observed and documented. The scale at which adverse effects of air pollution on vegetation are observed varies from the local to regional and global scales. For example, at a very local scale, emissions from a copper smelter in Anaconda, Montana, USA, led to severe damage to the surrounding coniferous forests in the form of foliar injury, reduced growth rates, increased levels of heavy metals in the forest ecosystem, and high tree mortality (Carlson, 1978; Bissell, 1982).

Air pollutants that affect vegetation on a regional scale include, for example, acid rain and photochemical smog. The main sources of acidifying air pollutants such as SO_2 (sulfur dioxide), NO_x (nitrogen oxides) and NH_3 (ammonia) are industry, vehicular combustion of fossil fuels, and agriculture, respectively (Schreuder, 1995; Stanners and Bourdeau, 1995). The main component of photochemical air pollution is ozone (Cape, 1997). Air pollutants, such as acid deposition and photochemical smog have been reported to cause crop damage (e.g., MacKenzie and El-Ashry, 1989), and they may play a role in the forest decline phenomenon that has been observed in the United States (Chappelka and Samuelson, 1998), and Western and Central Europe (McLaughlin, 1985; Smith and Lefohn, 1991; Stanners and Bourdeau, 1995). Although the severity and extent of forest decline has been lower than was initially predicted (Kandler and Innes, 1995),

large scale inventories show a general worsening of forest conditions in many parts of Europe (Lorenz, 1995). It is believed that air pollution predisposes trees to environmental stress factors (McLaughlin, 1985; Tomlinson and Tomlinson, 1990) such as drought stress (Schmieden and Wild, 1995; Chappelka and Freer-Smith, 1995), frost damage (Chappelka and Freer-Smith, 1995), nutrient deficiencies (Schmieden and Wild, 1995), and insect damage (e.g., Cannon, 1993).

Current examples of air pollutants leading to changes at a global level include increased concentrations of carbon dioxide (CO_2) in the troposphere and in the stratosphere and CFC's which influence stratospheric ozone levels. Higher CO_2 concentrations in the troposphere, the atmospheric layer between the earth's surface and the stratosphere, have been correlated with a slow increase in the temperatures at the earth's surface, referred to as the greenhouse effect (ICCP, 1995). This may result in drastic climate changes and shifts in the growth rates and distribution of vegetation (Winnett, 1998). Stratospheric ozone concentrations have been reported to be decreasing, which may lead to higher levels of UV-B radiation at the earth's surface, and subsequent adverse effects on public health (Burnett et al., 1997; Kunzli et al., 1997) and vegetation (Barnes et al., 1996).

In an effort to increase our understanding of the role of air pollution in forest health, this dissertation will focus on the effects of two strong oxidative air pollutants, chlorine gas and ozone, on physiological processes and growth of trees. These two pollutants have their influences at different scales. Chlorine exposure is generally caused by accidental releases, and effects are acute and observed on a local scale. Ozone

exposure is chronic in many regions of the world, and the adverse effects on vegetation is considered to influence vegetation at a regional scale.

Chlorine gas

Chlorine gas (Cl_2), produced electrolytically from sediment salts and seawater (Compaan, 1992), is widely used in the synthesis of an array of organic products (e.g., Poly vinyl carbon plastics; Compaan, 1992; Westervelt and Roberts, 1995), as well as for bleaching pulp and paper, treatment and disinfection of (drinking) water, and in the pharmaceutical industry (Faust and Aly, 1983; Richardson et al., 1996; Yosie, 1996). Chlorine has a green-yellowish color, is about 2.5 times denser then air, and is moderately soluble in water. It usually is transported in refridgerated tanks as a its liquid.

Accidents involving chlorine gas releases are not uncommon. For example, an evaluation of the Hazardous Substances Emergency Events Surveillance System over the period of 1990-1992 reported 138 accidental releases involving chlorine gas in nine states in the USA (Hall et al., 1996). About 25 % of these accidents involved human injuries and about 30 % led to evacuations. Since the production volume of chlorine gas is expected to increase over the next decade (Westervelt and Roberts, 1995), the risk of accidents with chlorine gas will likely increase.

Chlorine gas is highly toxic to humans as well as vegetation. Human exposure to chlorine gas causes irritation to eyes, nose and airways, and severe damage to the respiratory system (Baxter, Davies and Murray, 1989; Griffiths and Megson, 1984; Whithers and Lees, 1985). Health effects on the respiratory and nervous system can

persist over many years (Baxter et al., 1989). The IDLH concentration (= immediately dangerous to life and health) of chlorine gas is 30 ppm (Singh, 1990).

In comparison to human health effects, relatively little is known about the effects of chlorine gas on vegetation. A few studies following accidental chlorine leaks and spills have been reported (Brennan, Leonne and Holmes, 1969), whereas only a few controlled studies have been carried out (Brennan, Leonne and Daines, 1956 and 1966; Griffith and Smith, 1990). Reported morphological effects of chlorine gas exposure on broadleaved and coniferous plant species consisted of chlorosis (bleached appearance of leaves), necrosis (death of tissues), and necrotic mottling (Brennan et al., 1965; Brennan et al., 1969; Heck, Daines and Hindawi, 1970; Vijayan and Bedi, 1989). These symptoms are similar to those caused by acid rain and mist (Forsline, Dee and Melious, 1983; Whitney and Ip, 1991). In conifers, necrosis occurs as tipburn, an orange-brown coloring extending from the tip to the base of the needle (Brennan et al., 1966). Foliar injury due to chlorine gas exposure has been reported at concentrations as low as 0.1-1.5 ppm Cl_2 after exposures of 4 to 24 hr (Brennan et al., 1965; Griffith and Smith, 1990). Leaf tissue pH values as low as 1.0 were observed after exposure of tomato plants to 63 - 1000 ppm Cl_2 over 16 hr, and indirect effects may occur due to acidification of the soil (Thornton and Setterstrom, 1940). Pine trees seem to be more resistant to chlorine gas exposure than most herbaceous species (Brennan et al., 1966). Older and middle-aged conifer foliage was more sensitive to chlorine gas exposure than young foliage (Brennan et al., 1965; Griffith and Smith, 1990); branches generally were not killed by chlorine gas exposure (Brennan et al., 1966).

In contact with water hydrochloric acid (HCl) and hypochlorous acid (HOCl or bleach; pK_a= 7.58 at 20^{o}C) are formed:

$$Cl_2 + H_2O \longrightarrow H^+ + Cl^- + HOCl$$

This is a disproportonation reaction, leaving one Cl atom in a oxidation state of –1, and the other, i.e. the atom in HOCl, of +1. Plant injury can occur by several mechanisms. Although chloride ions (Cl^-) are a natural component of plant cells, accumulation of chloride ions in cell tissues can be toxic to plants (e.g. Fürher and Erisman, 1980). Second, exposure to hydrochloric and hypochloric acid may lead to a highly acidic solution in the apoplast, that disrupts the pH regulation of cells. Moreover, at very low pH, H^+ ions may compete with the Mg^{2+} ions present in chlorophyll. This may explain the chlorosis symptoms that are often observed after exposure to chlorine gas. Finally, HOCL, or bleach, is a strong oxidant in its dissociated form, that can injury several biochemically important molecules in plant cells.

Long-term effects of acute chlorine exposure on physiological functions such as tree water balance, water loss through the stomata or cuticle, photosynthesis, and growth have not been reported (e.g., Figure 1.1). The leaf cuticle is a complex mixture of waxes that acts as a barrier against excessive water loss and pathogen infection (Martin and Juniper, 1970; Cutler, Alvin and Price, 1982; Kerstiens, 1996). When stomates are open, water loss via the cuticle is generally a very small fraction of total leaf conductance (van Hove, 1989; Kerstiens and Lendzian, 1989b). However, cuticular water loss may be a significant fraction of total leaf conductance during summer drought when the stomates tend to be closed (e.g., Mengel, Hogrebe and Esch, 1989), and in winter conditions (Barnes and Davison, 1988). Cuticular water loss during winter is important because

water evaporated from foliage during clear winter days cannot be replaced when soil and stem water are frozen (Sowell, Koutnik and Lansing, 1982). Cuticular water loss during winter has been found to be an important factor in high altitude forests, especially because trees at timberline often are exposed to high wind speeds and ice abrasion, which enhances cuticular erosion (Baig and Tranquillini, 1976 and 1980; Sowell et al., 1982; Hadley and Smith, 1989). Cuticular characteristics can be changed by environmental factors such as low water availability, abrasion by rain and wind (Bengtson, Larsson and Liljenberg, 1978; Svenningsson and Liljenberg, 1986; Günthardt-Görg, 1994), as well as air pollutants. Evans, Gmur and Kelsch (1977) reported damage to cuticles of bean leaves after exposure to simulated acid rain, although acid rain did not seem to affect cuticles of birch and bean plants (Paparozzi and Tukey, 1984). Foliar injury of bean leaves exposed to HCl decreased with increasing amounts of cuticular waxes (Swiecki, Endress and Taylor, 1982). Chlorine gas would be expected to have similar effects to HCl on cuticles, because of the strong acid solutions that Cl_2 forms in water.

Chlorine gas exposure also may affect photosynthesis and growth. Exposure to acid mist is well known to decrease rates of photosynthesis (Roberts, 1990; Velikova and Yordanov, 1996; Velikova et al., 1997; Momen, Anderson and Helms, 1999), as well as photosynthetic capacity (measured as F_v/F_m ratios; Führer et al., 1990; Velikova and Yordanov, 1996). For example, irreversible damage to photosynthetic capacity was reported when *Phaseolus vulgaris* was exposed to mist of pH <2.0 (Velikova et al., 1997). Chronic chlorine exposure led to lower photosynthetic biomass, fruit yield, chlorophyll content, protein content and carbohydrate content in exposed fruit trees in India. These symptoms were accompanied by higher foliar chloride concentrations

(Vijayan and Bedi, 1989). In conifers, necrotic foliage generally defoliated (e.g., Heck et al., 1970; Chapter 2, this dissertation), causing significant declines of photosynthetic biomass. This can result in decreases in height growth (Carlson, McCaughey and Theroux, 1988; Salemaa and Jukola-Sulonen, 1990; Krause and Raffa, 1996), annual stem increment growth (Vosko and Klubica, 1992; Christiansen and Fjone, 1993; Krause and Raffa, 1996), and total biomass (Krause and Raffa, 1996; Sanchez and Wagner, 1999).

Ozone

Ozone (O_3) is present naturally in the troposphere, the part of the atmosphere between the earth's surface and the stratosphere. Tropospheric ozone is generated predominantly by photochemical processes, i.e., under the influence of solar radiation (Krupa and Manning, 1988). Chemical precursors of tropospheric ozone are nitrogen oxides and volatile organic carbons. These trace gasses occur naturally in the atmosphere, but are generated to a much larger extent by human activities such as car traffic, power generation, and solvent use (Krupa and Manning, 1988; Stanners and Bourdeau, 1995). Tropospheric ozone concentrations have increased substantially over the last century and are expected to increase by ~0.3 to 1 % per year over the next 50 years (Thompson, 1992). Ambient ozone concentrations observed in many parts of Europe and the United States are high enough to cause damage to natural vegetation and crops (Cooley and Manning, 1987; Reich, 1987; Krupa and Manning, 1988; Smith, 1990; Lefohn and Lucier, 1991; Lefohn, 1992; Scheel et al., 1997a).

Toxic effects of ozone on plants were first reported in agricultural areas near Los Angeles, where significant crop injury was found beginning in the 1950's (Heck, 1968). Acute ozone exposure caused foliar injury symptoms such as bronzing, chlorosis, and necrosis of plant tissues (Rich, 1964; Hill, Heggestad and Linzon, 1970; Krupa and Manning, 1988). Chronic exposure to ozone also reduced growth, yield, and quality of crops (Heck, 1968; Laurence and Weinstein, 1981; MacKenzie and El-Ashry, 1989). Moreover, ozone is considered one of the primary air pollutants playing a role in forest decline in Europe and the United States (Schmieden and Wild, 1995; Reich, 1987; Chappelka and Samuelson, 1998).

Ozone enters plants mainly via stomatal pores (Figure 1.1; Wieser and Havranek, 1993; Neubert et al., 1993; Fredericksen et al., 1996). In the stomatal cavity, ozone reacts with the aqueous phase (Laisk ,Kull and Moldau, 1989; Alscher, Donahue and Cramer, 1997). The breakdown products of these reactions are strong oxidants, and tend to interfere with plant membranes (Pääkkonen et al., 1995; Anttonen, Sutinen and Heagle, 1996) and plant metabolism (Sandermann, 1996). Ozone injury starts with the collapse of mesophyll and guard cells (Günthardt-Görg et al., 1993), which can cause increased stomatal opening during the daytime and incomplete stomatal closure at night (Hassan, Ashmore and Bell, 1994). Thus, ozone exposure can increase plant susceptibility to drought (Maier-Märcker and Koch, 1992; Hassan et al., 1994; Maier-Märcker 1999;).

Ozone exposure has been reported to cause decreased rates of photosynthesis (Darrall, 1989; Grünhäge and Jäger, 1994; Van Hove and Bossen, 1994; Mikkelsen, 1995; Pääkkonen et al., 1998d; Kytoviita et al., 1999; Yun and Laurence, 1999), and increased rates of respiration in plants (Reich, 1983; Skarby, Troeng and Bostrom, 1987;

Pääkkonen et al., 1995; Matyssek et al., 1997; Maurer et al., 1997). Chronic exposure to ozone led to increased shoot to root ratios for some species of plants, and often to lower total plant biomass (Darrall, 1989; Pell, Schlagnhaufer and Arteca, 1994; Rennenberg, Herschbach and Polle, 1996). Carbon allocation to other sinks, such as flowers and seeds, also can be decreased by ozone (Cooley and Manning, 1987). For example, ozone exposure reduced height and stem growth, and photosynthetic and total biomass in poplars (Dickson et al., 1998; Mortensen, 1998; Yun and Laurence, 1999). Finally, ozone exposure frequently has been observed to accelerate senescence in plants (Baker and Allen, 1996; Mikkelsen and Heide-Jørgensen, 1996; Pääkkonen, Holopainen and Karenlampi, 1997; Beare, Archer and Bell, 1999; Yun and Laurence, 1999).

Effects of ozone exposure may be mediated by environmental factors such as temperature, solar radiation, and humidity (Heck, 1968; Neubert et al., 1993; Fredericksen et al., 1996). For example, ozone exposure led to decreased water use efficiency (van Hove and Bossen, 1994; Shan et al., 1996) and increased susceptibility to drought and low temperature stress in conifers (Maier-Märcker and Koch, 1992, 1995; Penuelas et al., 1994; Chappelka and Freer-Smith, 1995). Trees in forests exhibiting forest decline symptoms had lower foliar water content and higher transpiration rates (Rosenkranz et al., 1989; Badot and Garrec, 1990; Bussotti and Ferretti, 1998). Higher transpiration rates in a declining stand of spruce trees in the Northern Alps were attributed to decreased stomatal control (Maier-Märcker and Koch, 1995; Maier-Märcker 1999).

Air pollutants have been found to change cuticular characteristics (e.g., Günthardt-Görg, 1994). Ozone exposure accelerated wax erosion (Barnes, Davison and

Booth, 1988; Bytnerowicz and Turunen, 1994; Mankovska, Percy and Karnosky, 1999), decreased cuticular thickness (McQuattie and Rebbeck, 1994), and changed the chemical composition of cuticular waxes (Kerfourn and Garrec, 1992). However, ozone exposure generally did not affect the amount of cuticular waxes (Barnes et al., 1990b; Thornton et al., 1993; Cape, Sheppard and Binnie, 1995). Ozone-induced changes of plant cuticles, especially changes of the structure (Turunen and Huttunen, 1990), increased cuticular transpiration (Delucia and Berlyn, 1984; Kerstiens and Lendzian, 1989b; Hadley and Smith, 1990). Tree water loss may increase due to higher water loss from foliage via the cuticle (Figure 1.2). However, altered cuticular properties may not always lead to higher cuticular transpiration (Svenningson, 1988; Cape et al., 1995). Studies that reported negative effects of ozone exposure on cuticles and cuticular water loss generally experimented by exposing intact cuticles. Conversely, studies on isolated cuticles did not show effects of ozone exposure (Kerstiens and Lendzian, 1989a, 1989b; Schmid and Ziegler, 1992). It is not clear if using isolated cuticles is the best method to assess effects of ozone on cuticles, because indirect effects and feedback mechanisms from living cells cannot be easily accounted for. Increased water losses via the cuticle also can potentially interfere with the stomatal regulation because stomatal opening and closure are mediated by the water status of epidermis cells (Sheriff, 1984; Zou and Kahnt, 1988; Kerstiens, Federholzner and Lendzian, 1992; Kerstiens, 1995).

Although many effects of ozone on plant cuticles have been reported, little is known about ozone effects on cuticular transpiration and the extent to which these effects contribute to decreased tolerance to low water availability during drought periods (e.g., Figure 1.2). Moreover, few studies have related the effects of changed cuticular

properties to cuticular water loss and leaf wettability. Water layers can be present on needle surfaces at atmospheric humidities as low as 50 % (Burkhardt and Eiden, 1994), and have several types of negative influences on leaves. Water layers can increase the leaching of K^+, Mg^{2+} and Ca^{2+} ions from needle tissues, which has been described one of the symptoms of forest decline (McLaughlin, 1985; Tomlinson and Tomlinson, 1990). The presence of water films on leaf surfaces also can lead to decreased photosynthetic gas exchange (Brewer and Smith, 1994 and 1995). Ozone and acidic fog have been shown to increase the wettability of spruce needles (Barnes and Brown, 1990; Percy, Jensen and McQuattie, 1992), enhancing formation of water films on needles, and potentially higher ozone deposition rates to the leaf surface. Although ozone is mainly taken up via stomatal pores, ozone also is taken up via the cuticles. Higher rates of ozone deposition have been documented on wet leaf surfaces for individual leaves (Fuentes and Gillespie, 1992), as well as natural canopies after rain or dewfall (Fuentes et al., 1992, 1994). Grantz et al. (1995) reported a significant increase of non-stomatal ozone uptake by plant canopies in wet conditions, which was ascribed to chemical reactions of ozone with moisture present on the leaf surface. Increased air pollutant deposition rates to wet leaf surfaces compared to dry surfaces also have been observed for NH_3 and SO_2 (van Hove and Adema, 1996) and NO_2 (Weber and Rennenberg, 1996). However, leaf surface wetness does not always lead to increased deposition. Grantz et al., (1997) reported decreased ozone deposition to an amphistomatous cotton canopy.

Research questions

The work reported in this dissertation focuses on the effects of strong oxidants from air pollution on leaf cuticles, water balance, and growth of trees. Interestingly, chlorine gas and ozone, both oxidative air pollutants, had similar effects on vegetation. Only a few studies have addressed acute effects of chlorine gas exposure on vegetation. The work reported here is the first on long-term morphological, physiological and growth effects on plants, and will be important for our understanding of the effects of accidental chlorine gas releases on natural ecosystems. The second part of this dissertation focuses on possible mechanisms that may be responsible for the observed increase in susceptibility to drought stress that has been attributed to ozone exposure. The last chapter explores the effects of leaf surface wetness on ozone uptake by plants. Our current understanding of these effects is limited, although leaf surface wetness can have large impacts on ozone uptake by plants, as well as photosynthetic gas exchange.

The studies described in chapters 2 and 3 were initiated after a train derailment released ~ 55 metric tons chlorine gas into a montane, coniferous forest ecosystem, Rocky Mountains, U.S.A. Chapter 2 focuses on the acute effects of chlorine gas exposure, addressing the following questions: (1) What are the morphological symptoms of acute chlorine gas exposure on herbaceous plants, grasses, and conifers along a downwind gradient from the site of gas release; (2) What is the effect of chlorine gas on cuticles, cuticular transpiration and water content of conifer needles; and (3) Does acute chlorine gas exposure affect photosynthetic capacity of conifer needles?

Chapter 3 addresses the long-term physiological and growth effects of acute chlorine gas exposure, and focuses on the following questions: (1) What are the long-

term effects of chlorine gas exposure on cuticular transpiration and foliar water content; (2) What are the long-term effects of chlorine gas exposure on conifer growth; (3) Does chlorine gas exposure affect the susceptibility of trees to drought stress and insect damage; and (4) Does chlorine gas exposure affect tree mortality?

Chapter 4 addresses the following research questions: 1) Does ozone exposure affect leaf wettability of intact leaves, and, if so, do these effects occur via a direct or an indirect mechanism; 2) Do ozone-induced changes of cuticles affect rates of cuticular transpiration; 3) What are the effects of ozone exposure on plant growth; and 4) Do the responses of deciduous and coniferous tree species to ozone differ?

Chapter 5 addresses the effects of leaf wetness on ozone deposition and photosynthetic gas exchange, and focus on the following research question: 1) What are the effects of leaf surface wetness on ozone deposition, both in light and dark conditions; 2) What are the effects of leaf surface wetness on net photosynthesis and dark respiration; 3) What processes are responsible for the effects of leaf surface wetness on ozone deposition and gas exchange; and 4) Are the effects of leaf surface wetness on ozone deposition and gas exchange affected by pH and chemistry of the aqueous phase?

Chapter 6 is a description and summary of this dissertation written for a general audience, and is written like a popular article rather than a technical report.

The results indicate that acute, short-term chlorine gas exposure has long-term adverse effects on water relations, foliar biomass and growth of conifers. Thus, effects of accidental chlorine gas exposure need to be monitored over several growing seasons to address longer-term impacts on water loss, growth and mortality. This dissertation sheds more light on the effects of ozone on plant water balance and growth, as well as on the

mechanisms responsible for these effects. Moreover, it is shown that leaf surface wetness significantly increases ozone uptake by plants, and that these effects need to be taken into account in ozone deposition models.

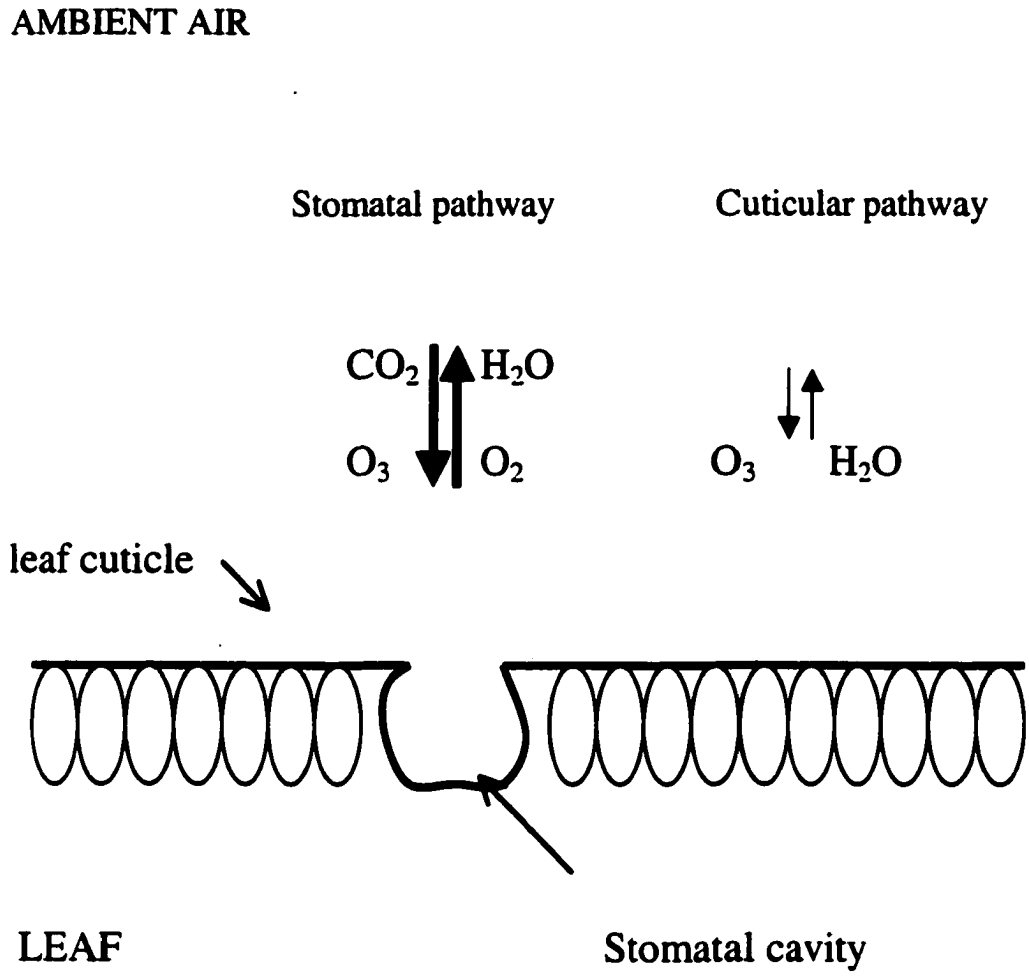
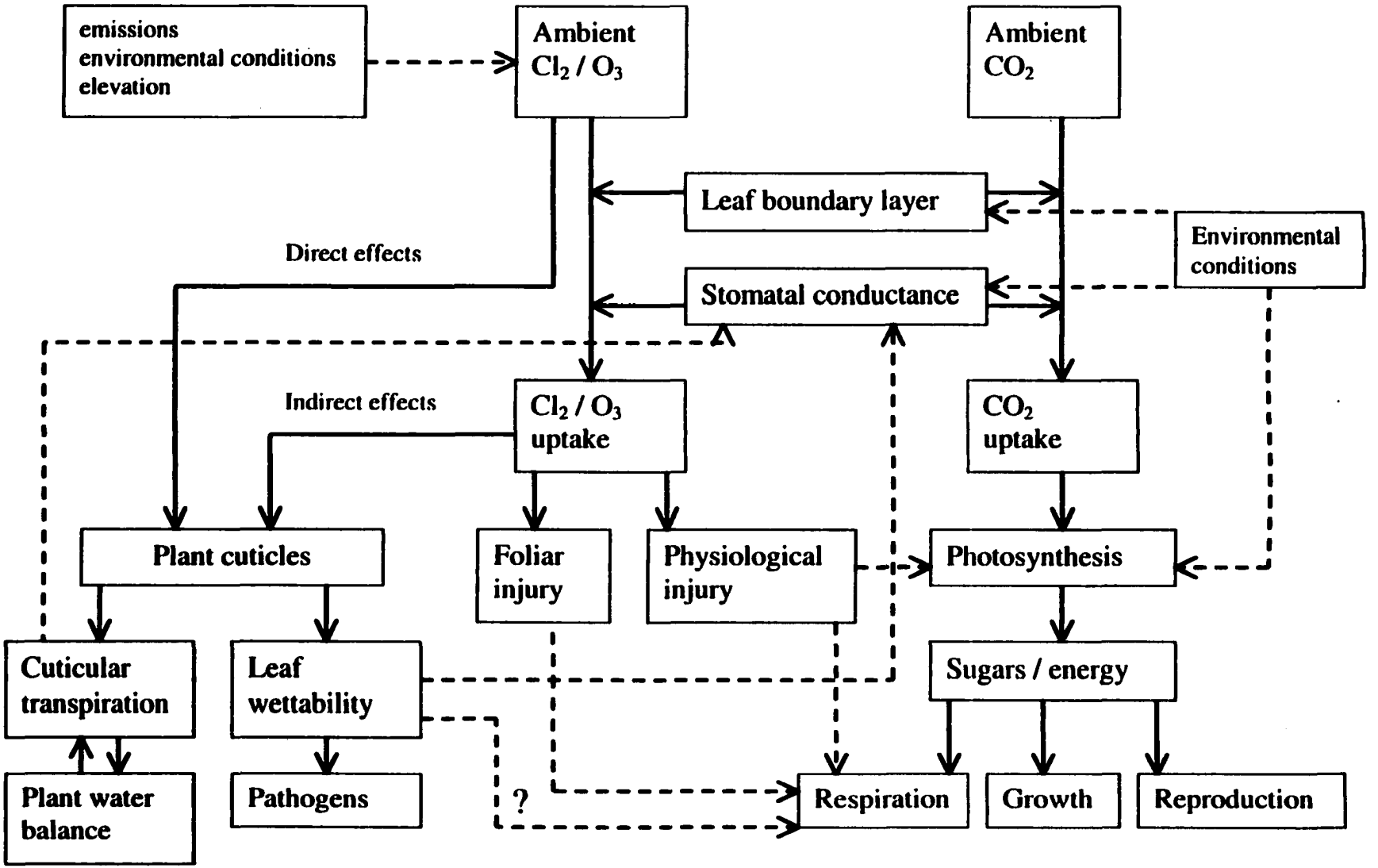


Figure 1.1: Schematic representation of gas exchange over a leaf surface. In light conditions the main pathway for gas exchange is via the stomata. Ozone deposition to the cuticle occurs by adsorption as well as uptake. Water vapor also is lost via the cuticle. The size of the arrows reflects the relative importance of the gas fluxes.

Figure 1.2: Chlorine (Cl_2) and ozone (O_3) effects on plant cuticles, photosynthesis and growth. Solid lines represent direct relationships and effects. Dashed line represent feedback mechanisms, both within plants as well as with the environment. Shaded boxes represent aspects that have been addressed in this dissertation.



CHAPTER 2

Morphological and physiological effects on forest vegetation after acute exposure to chlorine gas

ABSTRACT

I report on forest damage resulting from acute exposure to chlorine gas in a montane, coniferous forest in the Rocky Mountains, USA. On April 11, 1996, a train derailment released ~55 metric tons of chlorine gas into the atmosphere. Acute morphological and physiological effects of chlorine gas exposure were evaluated on two conifer species, *Pseudotsuga menziesii* (Douglas fir) and *Pinus ponderosa* (Ponderosa pine). Foliar injury, consisting of chlorosis, necrotic mottling, tip-burn, and necrosis was observed only on foliage that was directly exposed to chlorine gas. One-year old needles were directly exposed to chlorine gas whereas current-year needles flushed after the gas cloud had subsided. Necrotic needles on Douglas fir and Ponderosa pine defoliated during the months immediately following the gas release. Conifer buds were killed within 50 m of the gas release, which gave rise to secondary shoot growth in Douglas fir but not Ponderosa pine. Injury to plant cuticles was assessed, using droplet contact angles and droplet retention angles, on one-year old and current-year foliage. Chlorine gas exposure decreased droplet contact angles of both one-year old and current-year needles of Douglas fir ($P<0.0001$), even when no visible injury was apparent, but not for Ponderosa pine. Chlorine exposure led to increased cuticular water loss and decreased total water content of one-year old and current-year needles of Douglas fir, and of one-year old needles of Ponderosa pine ($P<0.0001$). Moreover, one-year old and current-year foliage on exposed trees had lower F_v/F_m ratios ($P<0.0001$), indicating decreased photosynthetic efficiency and changes in chloroplast membranes. These results indicate that exposure of conifer needles to chlorine gas can increase drought susceptibility and may potentially decrease photosynthesis of conifer needles, through both direct and indirect influences. This may have long-term effects on tree growth. This study also showed that plant responses to chlorine gas are species specific and affected by variation between study sites, and the stochastic character of movement of chlorine gas clouds.

INTRODUCTION

This study reports on the effects of chlorine gas (Cl_2) on natural vegetation after a train derailment in northwest Montana, USA. Few long-term data on the effects of acute chlorine exposure on physiological functions such as tree water relations, photosynthesis, and growth have been reported in the literature. Chlorine gas, derived from sediment salts and by the electrolysis of seawater (Compaan, 1992), has many applications in the chemical (Compaan, 1992), pharmaceutical, and paper industries (Yosie, 1996), as well as for disinfecting water (Richardson et al., 1996). Chlorine, a green-yellowish gas, is about 2.5 times more dense than air, is moderately soluble in water (EPA, 1998), and usually is transported in its liquid state. Accidents involving chlorine gas releases are not uncommon. For example, an evaluation of the Hazardous Substances Emergency Events Surveillance System over the period of 1990-1992 reported 138 accidental releases involving chlorine gas in 9 states in the USA (Hall et al., 1996). About 25 % of these accidents involved human injuries and about 30 % led to evacuations of people (Hall et al., 1996). Since the production volume of chlorine gas is expected to increase over the next decade (Westervelt and Roberts, 1995), the risk of accidents with chlorine gas will likely increase.

In the gaseous form, chlorine is a highly toxic air pollutant that can cause severe, acute effects on human health. Human health effects of chlorine gas exposure include irritation to eyes, nose and airways, as well as more severe damage to the respiratory system (Baxter, Davies and Murray, 1989; Griffiths and Megson, 1984; Whithers and Lees, 1985). The IDLH (= immediately dangerous to life and health) of chlorine gas is 30 ppm (Singh, 1990). Health effects on the respiratory and nervous system can persist over

many years (Baxter et al., 1989).

In comparison to effects on human health, only a few studies have reported on the effects of chlorine gas exposure on vegetation. These studies either followed accidental chlorine releases into the environment (Brennan, Leone and Holmes, 1969), or were carried out under controlled conditions (Thornton and Setterstrom, 1940; Brennan, Leone and Daines, 1965 and 1966; Griffiths and Smith, 1990; Schulze and Stix, 1990). The most common foliar injury symptoms after exposure to chlorine gas include chlorosis, (bleaching and yellowing of leaf tissues) and necrosis (death of cells and cell tissue). These effects can be observed on foliage of both deciduous plants and coniferous plants (Heck, Daines and Hindawi, 1970). Necrosis may be confined to leaf margins, extending from the edge to the center and base of a leaf, and be interveinal (Brennan, et al. 1965; Heck et al. 1970). In monocots, such as corn, onion, and grass species, necrosis occurs in a streaky pattern following the course of veins. Damage appears first at the tip and extends downward to the base of the leaf (Brennan et al., 1965 and 1969). On conifer needles, chlorine gas exposure causes tipburn, an orange-brown coloration extending from the tip to the base of a needle, which eventually kills the whole needle (Brennan et al., 1966). Necrotic mottling (small necrotic spots scattered over the leaf surface) also has been observed after chlorine gas exposure (Brennan et al., 1965 and 1969; Heck et al., 1970). Completely necrotic foliage generally is dropped from the plant, and can lead to significant reductions of photosynthetic leaf area (Heck et al., 1970).

Threshold chlorine concentrations that cause visible injury depend on the plant species and duration of exposure (Brennan et al., 1965; Griffiths and Smith, 1990). Chlorosis and necrosis have been found at chlorine concentrations as low as 0.5 – 3.0

ppm (Thornton and Setterstrom, 1940). Brennan et al. (1965) reported that several crop species developed chlorosis within 4 hrs, and necrosis within 1 day after exposed to concentrations as low as 1.0 -1.5 ppm Cl_2. These toxicity thresholds concentrations depend on several factors, such as plant species, duration of exposure, and stomatal conductance (Brennan et al., 1965; Griffiths and Smith, 1990).

Foliar injury symptoms caused by chlorine gas exposure are similar to those of acid rain and acid mist (Hindawi, Rea and Griffiths, 1980; Forsline, Dee and Melious, 1983; Vijayan and Bedi, 1989; Whitney and Ip, 1991). This is expected because chlorine gas can form highly acidic solutions in the aqueous phase, forming hydrochloric acid (HCl) and hypochlorous acid (or bleach; HOCl; pKa= 7.58 at 20^{o}C). Exposure to acid mist can affect plant cuticles (Schmitt, Ruetze and Liese, 1987; Percy, Jensen and McQuattie, 1992; Bytnerowicz and Turunen, 1994), which may increase cuticular water loss and thus susceptibility to drought stress (Mengel, Hogrebe and Esch, 1989; Hadley and Smith, 1989). Changes in cuticular characteristics can be assessed using droplet contact and retention angles. Droplet contact angles indicate how much water droplets spread out over the leaf surface, which has important influences on photosynthetic gas exchange and susceptibility of plants to diseases (e.g., Brewer et al., 1994). Moreover, exposure to acid mist can decrease photosynthetic rates (Roberts, 1990; Velikova et al., 1997; Momen, Anderson and Helms, 1999), as well as photosynthetic efficiency (Führer et al., 1990; Bong and Hee, 1995; Velikova and Yordanov, 1996).

Foliar injury can be caused by cell plasmolysis due to the accumulation of acid in the apoplast (Heath, 1980), as well as by chloride accumulation in plant tissues (Ashworth, Gaona and Surber, 1985; McCune, 1991). Leaf tissue pH values as low as 1.0

were observed after exposure of tomato plants to 63 - 1000 ppm Cl_2 gas over 16 hrs (Thornton and Setterstrom, 1940). Chloride accumulation disturbs the ionic balance in plant tissues (Führer and Erisman, 1980). Consequently, accumulation of chloride in roadside trees due to deicing salts leads to similar foliar injury symptoms as exposure to chlorine gas (Shortle and Rich, 1970; Lumis, Hofstra and Hall, 1973; Führer and Erisman, 1980). Although accumulation of chloride in plant tissues has been observed after exposure to chlorine gas (Liegel and Oelschlager, 1962; Vijyan and Bedi, 1989), this is not necessarily the case (Brennan et al., 1965).

This study was initiated after a train derailment released chlorine gas into a montane, coniferous forest ecosystem in April, 1996. In light of this extensive chlorine spill, I addressed the following questions: (1) what are the morphological symptoms of acute chlorine gas exposure on herbaceous plants, grasses, and conifers over a down-wind gradient from the site of gas release; (2) what is the effect of chlorine gas on cuticles, cuticular transpiration and water content of conifer needles; and (3) does acute chlorine gas exposure affect photosynthetic capacity of conifer needles?

MATERIALS AND METHODS

Study site

The site of this study was located in a narrow valley in the Rocky Mountains, ~2 km west of Alberton, Montana, USA (47°00'N, 114°30'W). On April 11, 1996, at 0400 hr, a 72-car train derailment at the site released ~55 metric tons of chlorine gas into the atmosphere and the surrounding forest. Over the following week chlorine concentrations at the site of the gas release varied from 12-20 ppm to ~50 ppm (1-hr average), with peak

concentrations reaching ~1400 ppm (Olympus Environmental, 1996). Atmospheric dispersion models reported similar concentrations, with peak chlorine gas concentrations ranging from ~165 ppm at about 1.2 km, to ~5 ppm at 9 km downwind from the point of release (ATSDR, 1997). Forests up to ~14 km downwind from the derailment site were exposed to chlorine gas (Olympus Environmental, 1996). In addition to chlorine gas, several chlorophenol compounds were formed due to a ruptured railroad car containing potassium cresylate. Data on atmospheric concentrations of these organic pollutants were not made available, but concentrations in the soil were well below levels reported to adversely affect public health (Olympus Environmental, 1996). Residues of toxic chlorinated organic compounds were removed from the site by excavation of the contaminated soil layers.

For this study, four sites that had been exposed to chlorine gas were established, i.e., within 50 m of the site of the gas release (foliage completely necrotic except for current-year needles), 0.2 (foliage mainly chlorotic), 0.8 (not visibly injured two months after gas exposure) and 1.5 km downwind (not visibly injured two months after gas exposure) from the release (Table 2.1). Control sites were established at ~65 km downwind from the site of the gas release (CD; $46^{o}70'$N, $114^{o}00'$W), and ~4 km upwind (CU; Table 2.1). There were no indications that chlorine gas reached this upwind control site. All field sites were comprised of mixed coniferous forests, dominated by *Pseudotsuga menziesii* (Douglas fir) and *Pinus ponderosa* (Ponderosa pine)(Hitchcock and Cronquist, 1994), and similar understory vegetation and soils (Table 2.1). A schematic map of the study sites and their location in relation to the site of gas release is shown in Figure 2.1.

Visible morphological injury

Morphological injury to vegetation was assessed immediately after it was safe to access study sites on May 15 (1 month post spill) and again 2 months after the spill on June 10, 1996. Visible injury to foliage, characterized as chlorosis, necrosis and necrotic mottling, was assessed in the field for Douglas fir and Ponderosa pine. Visual assessment of tree conditions (whole tree), based on presence of green foliage, chlorosis and necrosis, within each site were uniform. At each study site 10 trees representative for the site were randomly chosen for each species (2 branches per tree). Light microscopy techniques were used to examine foliar damage for different species. Foliage was then scored using the following visual injury scores: non-visibly injured foliage (0), necrotic mottling (1), chlorosis (2), tipburn (3) and complete necrosis (4). Data for the two control sites were based on foliar injury observed in March 1997 and 1998 (1-3 year old foliage).

Secondary shoot growth

Survival rates of exposed buds (as indicated by flush or no flush) of Ponderosa pine and Douglas fir were recorded at the end of May 1996. Data on secondary shoot growth were collected in the summer of 1999 to assess longer-term damage. Again at each site, 5 randomly chosen trees of each species were evaluated, with two replicate branches per tree, and the presence or absence as well as the number of secondary shoots was recorded. All branches sampled for this and the following analyses were taken from the lower tree canopy (1.5 to 2 m above the ground surface).

Cuticular injury

Cuticular injury was evaluated for Ponderosa pine and Douglas fir. Two branches were collected from 5 randomly selected trees at sites ~0.8 km downwind from the spill site, 0.2 km downwind, 50 m downwind, and at an unexposed down-wind control site (CD, ~65km downwind). Exposed one-year old needles (flushed in 1995) and non-exposed current-year needles (flushed in 1996) were evaluated.

Effects of chlorine gas exposure on the cuticle were assessed using droplet contact angles (CA) and droplet retention angles (RT). For this analysis 4 to 10 needles were selected for analysis for each species (Brewer, 1996; Brewer and Smith, 1997; Staszewski et al., 1998).

Cuticular transpiration was determined by measuring the change in weight of the samples periodically over the course of 3 d (Hadley and Smith, 1990). Randomly chosen stems, two per species and per site for each needle age class were weighed, water saturated overnight, and weighed again. Stems were then sealed with paraffin wax to avoid water loss via the edges of the stem, and subsequently dried at ~30 ^{o}C in a drying oven. Minimal conductance to water vapor of the needles (Kerstiens, 1996) was derived from these measurements using data on temperature and relative humidity in the drying oven, and specific needle area (SLA). SLA was determined using the glass bead method (Thompson and Leyton, 1971). This method estimates SLA from the difference in weight of conifer needles before and after the needle is covered with a single layer of small glass beads (125 μm diameter). Total water content (TWC, expressed as gr H_2O per gr dry weight) and relative water content (RWC, expressed as the ratio of fresh-dry weight to saturated –dry weight) of foliage were derived from data on the fresh, saturated, and dry

weights of needle samples.

Photosynthetic efficiency

The two most common conifers in the study area, Douglas fir and Ponderosa pine, were chosen to study physiological effects. Chlorophyll fluorescence measurements were made with an Opti-Sciences Modulated Fluorometer (Model OS-100, PP Systems, MA). Measurements were taken between 10.00 and 14.00 hrs, with a saturated light pulse of 0.8 s, F_0 100-125. Needle samples of Douglas fir (n=6 trees, with 2 replicates per tree) and Ponderosa pine (n=6 trees, with 2 replicates per tree) were dark-adapted for 15 min prior to measurements. F_o (dark fluorescence yield) and F_m (maximum fluorescence) were measured to calculate the variable fluorescence ($F_v = F_m - F_0$), and the efficiency of excitation (F_v/F_m-ratio).

Statistical analysis

Data on cuticular changes, foliar water content and chlorophyll fluorescence were analyzed using the SPSS statistical package (SPSS, 1995). Data that met the requirements for normal distribution were analyzed using one-way analysis of variance (reported as F, P-value). Pair wise comparisons were made using a Bonferroni post-hoc test. The experimental design was a nested analysis of variance, with two replicates (F_v/F_m) to five replicates (CA and RT) per tree. Subsamples were tested for significant differences, and were pooled if within subsample differences were not significant ($P<0.05$; Sokal and Rohlf, 1997).

Data that did not meet normality assumptions were analyzed using a Kruskal-

Wallis (reported as H, P-value) test or the Kolmogorov-Smirnov (reported as ksz, P-value). This was the case for data on droplet retention (RT). Cuticular transpiration experiments were done for randomly selected subsamples from 5 randomly selected trees at each site, resulting in sample sizes varying from n=2 to n=4. Repeated measures (RM) techniques were used to analyze foliar water loss data, with two repeated factors, time of drying and sampling date.

RESULTS

Morphological injury symptoms

Visual injury to existing needles on coniferous trees was apparent. Needles on both Douglas fir and Ponderosa pine showed extensive necrosis and tipburn (Figure 2.2A and 2.2B). Needles on most Douglas fir trees at the spill site and up to ~500 m from the spill were almost completely necrotic, with a few chlorotic needles and no green needles present 2 mo after exposure. Necrotic needles dropped over the course of the summer. Some Douglas fir trees 1 and 1.5 km downwind from the spill had chlorotic, necrotic and green needles. Most exposed Douglas fir trees developed new green foliage. Ponderosa pine foliage was less visibly affected than Douglas fir. Ponderosa pine trees at the derailment site and ~200 above the derailment site had mainly necrotic and chlorotic needles, as well as newly flushed green needles present. However, needles on Ponderosa pine trees ~0.2 to 1.5 km downwind were mostly green, with only minimal visual injury observed.

The degree of foliar damage generally decreased with increasing distance from the site of the gas release for both Douglas fir and Ponderosa pine (T_2=2.016, P<0.05;

Figure 2.3). However, foliar injury downwind from the release site was higher than at the two control sites, especially for Douglas fir. Variation in foliar injury, between patches of trees as well as within individual trees, was high. For example, a Ponderosa pine tree ~0.8 m downwind from the derailment was completely necrotic on the bottom half of the tree, while the upper half showed no visually injury. Healthy green foliage, as well as chlorotic and necrotic foliage, necrotic mottling, and tipburn often occurred within the same tree.

Secondary shoot growth

By the end of May 1996, 100 % of the buds on Douglas fir trees had burst on trees at control sites and sites further than 0.1 km downwind from the release site. Yet, a significant number of the buds on Douglas fir trees within 50 m of the release had been killed compared to control sites (~30%; Fisher's exact test, P=0.005). The lack of healthy buds gave rise to the growth of secondary shoots. Secondary shoots did not appear over a whole branch, although there was no specific pattern in the distribution of the secondary shoots over the shoot increments of different ages. At the spill site, the youngest age class with secondary shoots was 5 years old (± 0.4 SE; n=20) and the oldest was 13 years (± 0.4 SE; n=20). An average of 9 secondary shoots (± 2 SE; n=20) were formed on each major branch examined. Further than ~200 m downwind and uphill from the gas release, Douglas fir buds survived. These buds gave rise to new foliage several weeks after the gas release. Similarly, 100 % of buds on Ponderosa pine opened at all study sites, except within 50 m of the gas release, where only 25 % of the buds opened (Fisher's exact test, P=0.031). Although bud mortality within 50 m of the gas release for Ponderosa pine was

much higher than for Douglas fir, no secondary shoots were produced on Ponderosa pine in 1996.

Wettability of cuticles

Exposure to chlorine gas changed the interaction of needle cuticles with water droplets. The droplet contact angle (CA) of exposed one-year old Douglas fir needles decreased in comparison to control needles (Table 2.2), thus increasing leaf wettability and the tendency to form of water layers over the waxy cuticle. However, on Ponderosa pine needles, lower CA's were only apparent for one-year old needles of trees growing 0.8 km downwind from the release site (Table 2.2). Current-year Ponderosa pine foliage that had not been exposed to chlorine gas did not show changes in leaf wettability (Table 2.2). However, current-year Douglas fir needles from 1996 showed significantly lower CA at the two sites closest to the release site compared to control needles (Table 2.2). Significant changes of droplet retention (RT) were found only for Douglas fir foliage that was directly exposed to chlorine gas (Table 2.2).

Cuticular water loss

Observed changes in cuticular water loss as shown on water loss curves (Figure 2.4) confirm that chlorine gas exposure affected the cuticles of exposed needles. Cuticular water loss of exposed, one-year old Ponderosa pine foliage was significantly higher compared to control needles ($F_{3,96}$=66.14, P<0.001; Figure 2.4A and 2.5A). Similarly, exposed Ponderosa pine needles at two of the exposed sites had increased minimal conductance to water vapor over the cuticles and closed stomata, $G_{min,H2Ov}$

($F_{3,96}$=33.72, P<0.001; Figure 2.4B and 2.5B). Interestingly, this was not the case for Ponderosa pine needles closest to the release site. Ponderosa pine needles that flushed in 1996 on trees growing 0.8 km downwind had increased levels of cuticular water loss compared to control needles, expressed as both relative water loss ($F_{3,96}$=44.19, P<0.001; Figure 2.5A) and as $G_{min,H2Ov}$ ($F_{3,96}$=6.73, P=0.006; Figure 2.5B). However, since current-year needles on chlorotic and necrotic Ponderosa pine trees did not have increased cuticular water loss or $G_{min,H2Ov}$, there was no consistent trend that could be attributed to chlorine gas exposure.

Effects on cuticular transpiration were more pronounced for Douglas fir foliage than Ponderosa pine. One-year old foliage and current-year foliage of Douglas fir (Figure 2.6A, $F_{3,96}$=6.60, P=0.007 and $F_{3,96}$=4.25, P=0.029 respectively) had increased relative water loss compared to control needles. However, relative water loss of trees from control and necrotic foliage near the release site did not differ for one-year old foliage (Figure 2.6A). Moreover, necrotic one-year old foliage of Douglas fir (50 m from release site) had significantly lower $G_{min,H2Ov}$ ($F_{3,84}$=12.75, P<0.001; Figure 2.6B), which can be attributed to the already low water content of the necrotic foliage. Interestingly, current-year foliage on exposed Douglas fir trees had higher $G_{min,H2Ov}$ when compared to control needles ($F_{3,84}$=7.03, P=0.006; Figure 2.6B). The increased water loss and minimal conductance observed for both Ponderosa pine and Douglas fir suggests that chlorine exposure may lead to increased susceptibility to drought stress, especially for directly exposed foliage. However, responses were species dependent, and did not necessarily correspond with the severity of foliar injury, or distance to the release.

Water content

TWC of exposed one-year old needles decreased with increasing severity of visual injury (Table 2.3). Foliage of both conifer species within 0.2 km of the release site had lower TWC than control needles or needles that were further downwind. However, current-year needles on trees within 50 m of the release site had significantly higher TWC than at the other sites (Table 2.3). The differences in RWC were less pronounced than for TWC (Table 2.3). Only one-year old Douglas fir needles within 50 m of the release site had significantly lower RWC compared to control needles (Table 2.3).

Chlorophyll fluorescence

Exposed one-year old needles of both Ponderosa pine and Douglas fir had significantly lower F_v/F_m ratios (Table 2.4). Moreover, current-year needles on exposed trees also had decreased F_v/F_m ratios (Table 2.4). The reduction in F_v/F_m ratio for one-year old foliage was more pronounced in Ponderosa pine than in Douglas fir needles. These results suggest that both one-year old and current-year foliage on exposed trees had decreased photosynthetic efficiency.

DISCUSSION

This is the first study ever to report physiological responses of natural vegetation after accidental chlorine gas exposure. Acute exposure to chlorine gas has immediate effects on native vegetation in a spill area and these negative effects also can be found some distance downwind from the immediate spill area. Acute foliar injury symptoms, consisting of chlorosis, necrotic mottling , tipburn and necrosis, were reported for

conifers, shrubs, herbaceous species and grasses. Necrotic needles defoliated over the course of the growing season following exposure. Foliage that was still in bud at the time of the gas release generally was not visibly affected. The results of this study indicate that chlorine exposure not only leads to visual foliar injury, but may also affect long-term tree growth through defoliation, increased susceptibility to drought stress and decreased photosynthetic efficiency. However, these effects vary by species and on a landscape scale, probably due to the patchy movement of chlorine gas clouds.

Morphological injury symptoms

The most prevalent foliar injury symptoms observed on Douglas fir and Ponderosa pine were chlorosis, necrosis and tipburn. These symptoms are similar to foliar injury of chlorine gas reported for other conifer species (Brennan et al., 1966; Heck et al., 1970). In the vicinity of the gas release, all necrotic foliage and some chlorotic foliage defoliated during the months immediately following gas exposure. Beyond 200 m from the site of gas release, exposed buds produced new foliage, even when entire shoots were necrotic and defoliated. However, within a radius of ~50 m of the gas release, 30% of Douglas fir buds and 75% of the Ponderosa pine buds were killed. Brennan et al. (1969) reported that exposure to 10 ppm Cl_2 killed conifer foliage but not the shoot or the buds. The fact that buds were killed in this current incident may be explained by the considerably higher chlorine gas concentrations present at the site of the gas release, i.e., up to 1040 ppm Cl_2 (peak concentration, Olympus Environmental, 1996) and ~165 ppm Cl_2 (1-hour average, ATSDR, 1997). Douglas fir responded to defoliation by producing secondary shoots. Formation of secondary shoots has been reported for species other

coniferous species as well. For example, the number of secondary shoots increased with increasing defoliation for *Picea abies* (Salemaa and Jukola-Sulonen, 1990). Ponderosa pine did not produce secondary shoots, even though more buds were killed than on Douglas fir. Thus, new Ponderosa pine foliage only arose from buds that survived. It has been reported that older and middle-aged foliage is generally more sensitive to chlorine gas than young foliage (e.g., Brennan et al., 1965; Griffiths and Smith, 1990). However, I did not address the relationship between the severity of foliar injury and leaf age directly.

Deciduous tree species were not visually injured by chlorine exposure, possibly because broadleaved species had not yet experienced bud break at the time of gas exposure, and foliage was not directly exposed to the chlorine gas cloud. Acute foliar injury symptoms found on shrubs and herbaceous species also consisted of chlorosis, necrotic mottling and necrosis (Appendix 2.1), similar to injury symptoms reported for chlorine exposure injury to shrubs (Brennan et al., 1969; Heck et al., 1970), flowers (Brennan et al.,1969), crop species (Brennan et al., 1965; Schulze and Stix, 1990), and weeds (Brennan et al., 1969; Griffiths and Smith, 1990). Injured foliage of shrubs, herbaceous species, and grasses was replaced over the following growing season.

Foliar injury symptoms were most severe within a radius of about 100 m from the site of gas release. Foliar injury can be caused by two mechanisms, chloride accumulation in plant tissue (Führer and Erisman, 1980; Ashworth et al., 1985; McCune, 1991), and cell plasmolysis due to the accumulation of acid in the apoplast (Heath, 1980). Foliage within a radius of 70 m from the gas release had a chloride content of 25,000-30,000 ppm Cl^- (Olympus Environmental, 1996). These chloride concentrations are up to

27 times higher than the mean natural background reported for native plants in boreal forests in Canada (~1100 ppm Cl^- range 15-5000 ppm; Sheppard, Evenden and MacDonald, 1999). Compared to the range reported naturally for chloride concentrations in foliage of crop species (~100 ppm Cl^- minimum and ~1000 maximum ppm Cl^- Liegel and Ölschlager, 1962; Führer and Erisman, 1980) the foliar chloride concentrations observed in this study were 30-300 times greater. Thus, chloride accumulation may be implicated, in part, as a possible cause of the foliar damage observed after chlorine exposure in western Montana. These plants also suffered a second tissue insult. Foliage pH values at the release site were 1.0 to 2.5 (Olympus Environmental, 1996), which is considerably lower than the typical pH range of plant tissues (7.0 to 8.5, Smith and Raven, 1979). Using chemical modeling the pH of the chlorine gas cloud in the vicinity of the spill site could have been as low as pH 1 (Schreuder, unpublished data). Under such strong acidic conditions in plant cells, competition of H^+ ions with Mg^{2+} in chlorophyll may occur. This is a potential causal mechanism for chlorosis, observed after chlorine gas exposure. Although either damage mechanism described above is plausible, the rapid appearance of foliar injury points to the acidity of the gas cloud as the most likely immediate cause of the foliar injury. However, some of the long-term effects could be related to chloride accumulation in exposed foliage. Foliar chloride concentrations and pH levels at distances greater than ~500 m from the site of the gas release were within the expected range of naturally occurring concentrations even though foliar injury was present up to at least 2 km downwind from the release site (Olympus Environmental, 1996).

The large variation in the extent of foliar injury within trees, as well as between

study sites, can be explained in part by rapid changes of chlorine gas concentrations in the chlorine gas cloud (e.g., Koopman et al., 1982, Blackmore, Herman and Woodward, 1982). Moreover, heavy gasses tend to remain close to the ground (Griffiths and Fryer, 1988), especially at wind speeds as low as reported at the time of the gas release (e.g., Koopman et al., 1982). For example, a Ponderosa pine ~1 km downwind had completely necrotic needles on the lower 2 m of the tree and did not recover over the period of this study. Yet, the upper part of the tree remained visually unaffected. This same pattern of injury partitioning was observed on a *Juniperus scopulerum* ~1.5 km downwind, suggesting highly variable behavior of the chlorine gas cloud.

Influences on cuticles and leaf moisture content

Droplet contact angles and droplet retention for Ponderosa pine foliage generally were not affected by chlorine gas exposure (Table 2.2). In comparison, the changes in leaf wettability and droplet retention were more pronounced for Douglas fir. Chlorine gas exposure led to decreased droplet contact angles on Douglas fir needles of both exposed one-year old needles and unexposed current-year needles (Table 2.2), although the decrease in droplet contact angles was not related to the severity of observed foliar injury or distance to the release site. This suggests that needle cuticles were affected both through direct exposure to chlorine gas, as well as through interference with de novo wax synthesis. Decreased droplet contact angles lead to increased leaf wettability, which in turn can lead to reduced photosynthetic gas exchange (Brewer and Smith, 1994; Ishibashi and Terashima, 1995). Droplet retention of directly exposed foliage was only lower for Douglas fir needles. Decreased contact angles after exposure to acid mist have been

reported for *Picea rubens* (Barnes and Brown, 1990; Percy et al., 1992). Paoletti, Raddi and La Scala (1998) reported that leaves of beech trees from declining stands had lower droplet contact angles compared to trees from healthy forest stands. One direct effect of acid rain on plant cuticles is the erosion of cuticular waxes (Paparozzi and Tukey, 1984). Indirect effects of acid rain on cuticles mediated through wax biosynthesis include decreased cuticular thickness amount of waxes (Garrec and Kerfourn, 1989; Percy, Krause and Jensen, 1990; Percy et al., 1992; Bytnerowicz and Turunen, 1994,) changes of the ultrastructure (Schmitt et al., 1987; Percy et al., 1990; Bytnerowicz and Turunen, 1994) and changes of the chemical composition of the cuticle (Percy et al., 1992; Günthardt-Görg, 1994). The amount of waxes present on the cuticle has been negatively correlated with the incidence of glazing and necrosis after exposure of bean leaves to HCl (Swiecki, Endress and Taylor, 1982), suggesting that a thicker cuticle offers some protection against injury.

One-year old needles of Ponderosa pine had higher water loss from exposed foliage, measured as relative water loss (Figure 2.5A), and $G_{min,H2Ov}$ (Figure 2.5B). One-year old Douglas fir foliage showed increased relative water loss after chlorine gas exposure, but increased $G_{min,H2Ov}$ was not observed (Figure 2.6A and 2.6B). $G_{min,H2Ov}$ of one-year old necrotic needles within in 50 m of the release was not different from control needles for Ponderosa pine (Figure 2.5B). However, one-year old necrotic needles of Douglas fir within in 50 m had a very low $G_{min,H2Ov}$ but high relative water loss (Figure 2.6B). This can be attributed to the low TWC of the necrotic needles (Table 2.3), as cuticular conductance of conifer foliage decreases with decreasing water content (Hadley and Smith,1990). Moreover, since relative water is loss is based on TWC, low TWC can

also lead to high relative water losses, even if these needles lost less water than control needles. Interestingly, current-year Douglas fir needles on exposed trees had increased cuticular water loss and $G_{min,H2Ov}$ (Figure 2.6A and 2.6B). This indicates that chlorine exposure may affect these cuticles indirectly via wax production. In Ponderosa pine however, an effect on cuticular transpiration of current-year needles was observed only at 0.8 km downwind of the release site, and not the other exposed study sites (Figure 2.5A and 2.4B). This suggests that chlorine gas did not affect cuticular water loss of Ponderosa pine, and observed differences may be due to natural variation between study sites. $G_{min,H2Ov}$ of both exposed and control needles reported in the present study agree well with the range of $G_{min,H2Ov}$ values reported in the literature (e.g., Kerstiens, 1996; Heinsoo and Koppel, 1999).

Previous studies have shown that exposure of *Picea abies* to acid fog (pH 3.0) led to increased cuticular water loss of excised twigs, as well as decreased water holding capacity of needles (Mengel et al., 1989; Esch and Mengel, 1998). Increased cuticular transpiration has been correlated with decreased cuticular thickness and increased wax erosion along elevational and climatic gradients (DeLucia and Berlyn, 1984; Hadley and Smith, 1989; Schreiber, 1994) as well as with exposure to anthropogenic pollution (Sase et al., 1998). The sharp decrease in $G_{min,H2Ov}$ during the drying treatments in this study (Figure 2.4B) indicates that stomata were closed after the first two hours. However, a stomatal component to $G_{min,H2Ov}$ cannot be excluded due to possible incomplete stomatal closure (Kerstiens, 1996). Incomplete stomatal closure may result from exposure to SO_2 and NO (Mansfield et al., 1988), ozone (Maier-Maercker and Koch, 1995; Maier-Maercker, 1999) and acid rain (Barnes et al., 1990; Eamus and Murray, 1993). However,

acid rain does not necessarily affect stomatal conductance (Reich and Amundson, 1985; Flagler, Lock and Elsik, 1994; Anderson et al., 1997) since these effects are influenced by factors such as ambient temperature (Momen et al., 1999), chemical composition of the simulated acid rain (Eamus and Murray, 1993), and tree age (Momen et al., 1997). Stomatal responses to vapor pressure deficit (VPD) and light may also be changed by increased cuticular transpiration via whole leaf feedback mechanisms (Sheriff, 1984; Zou and Kahnt, 1988; Kerstiens, 1997).

Increased minimal conductance to water vapor and water loss from exposed needles may lead to increased susceptibility to drought (Mengel et al., 1989) and winter desiccation (Sowell, Koutnik and Lansing, 1982; Hadley and Smith, 1989, 1990). Field observations over the course of the summer confirmed this prediction. Results from this study also indicate that chlorine gas exposure decreased TWC of directly exposed foliage for both species, and RWC was only affected for one-year old necrotic needles of Douglas fir (Table 2.3). Chlorotic Ponderosa pine needles were abscised from the trees during conditions of drought stress, whereas control needles were not visibly affected by the summer drought. Although chlorine gas exposure generally did not affect foliar water content of needles that flushed after gas exposure, these needles did show substantial drought damage over the summer, especially for Ponderosa pine. New foliage on necrotic Ponderosa pine and Douglas fir had increased TWC (Table 2.3), which can be attributed to increased water availability, since these new needles formed the only living foliage on these trees.

In my study leaf samples were taken in the lower canopy (1.5-2 m from the ground). It is possible that chlorine gas effects resulting in foliar injury were most

prevalent in the lower half of the canopy, since chlorine gas is denser than air. Therefore, even if effects on cuticular water loss and TWC in the lower canopy of a mature tree were found, it is not clear if foliage higher in the tree canopy was affected to some extent. Thus, these data on chlorine gas effects on foliar water balance should be used with caution to extrapolate influences to the whole tree level.

Influences on photosynthetic efficiency

Exposure to chlorine gas led to decreased F_v/F_m ratios for one-year old needles of both Douglas fir and Ponderosa pine (Table 2.4), suggesting decreased photochemical efficiency of exposed foliage compared to foliage on control trees. Moreover, foliage that developed after the chlorine release on exposed trees also had decreased F_v/F_m ratios (Table 2.4), suggesting that photosynthetic tissues of these needles were indirectly affected by the gas exposure. Given that these measurements were made in the lower canopy, the extent to which F_v/F_m ratios of foliage in the upper tree canopy changed is not known.

My observations agree with reported injury in other studies. Exposure to acid rain adversely affected chlorophyll fluorescence in *Picea abies* (Šiffel et al., 1996) and *Pinus* spp. (Bong and Hee, 1995; Shan, 1998). Foliage exposed to acid rain had lower F_v/F_m ratios in bean plants (Velikova and Yordanov, 1996), and these effects were irreversible at pH values below 2.0 (Velikova et al., 1997). Other studies have reported that foliage exposed to acid rain had reduced chlorophyll levels (Shan et al., 1997), and lower rates of photosynthesis (Forsline et al., 1983; Neufeld, Jernstedt and Haines, 1985; Roberts, 1990; Ashenden, Bell and Rafarel, 1995; Shan et al., 1996 and 1997; Velikova et al., 1997;

Momen et al., 1999). Führer et al. (1990) reported that the effects of acid-mist on the photochemical efficiency were dependent on age-class and genetic factors. The combination of defoliation, increased drought susceptibility of foliage on exposed trees and decreased photochemical efficiency may lead to tree growth reductions as well as increased tree mortality (Salemaa and Jukola-Sulonen, 1990; Christiansen and Fjone, 1993; Krause and Raffa, 1996) and tree mortality (Webb, 1981).

CONCLUSIONS

This study has shown that acute chlorine gas exposure did not only cause visible injury on conifers, but also affected exposed plant cuticles and increased water loss through cuticles. Moreover, chlorine gas exposure decreased photosynthetic efficiency of directly exposed Douglas fir foliage, but not of Ponderosa pine. Both species lost photosynthetic biomass due to defoliation. These effects may lead to higher susceptibility to drought stress and lower growth for exposed conifers. Thus, effects of acute chlorine gas exposure need to be studied over a time period of least several years to address longer-term implications of exposure. However, observed physiological effects were species specific. Moreover, there was considerable variation in effects of chlorine gas exposure between study sites. This may have been due to natural variation between study sites, as well as the unpredictable movement of the chlorine gas cloud and the fast changes in chlorine gas concentrations in the cloud, both of which are characteristic for heavy gas clouds. Finally, more research is needed to address the effects for deciduous trees, when tissues in buds as well as mature leaves are exposed to chlorine gas.

Table 2.1: Physical description of study sites. Data shown are elevation, soil type, and habitat type (Pfister et al., 1977). *Pinus ponderosa* was the second most abundant tree species at all study sites. All sites were level benches in close vicinity to the Clark Fork River, western Montana.

Site and downwind distance	Elevation (m asl)	Soil type	Habitat type
Upwind Control (~4 km)	876	Coarse loam	*Pseudotsuga menziesii/ Arctostaphylos uva-ursi*
50 m downwind	897	Fine loam	*Pseudotsuga menziesii/ Physocarpus malvaceus*
0.2 km downwind	898	Fine loam	*Pseudotsuga menziesii/ Physocarpus malvaceus*
0.8 km downwind	900	Fine loam	*Pseudotsuga menziesii/ Physocarpus malvaceus*
1.5 km downwind	902	Fine loam	*Pseudotsuga menziesii/ Symphorica albus*
Downwind control (~65 km)	976	Fine loam	*Pseudotsuga menziesii/ Physocarpus malvaceus*

Table 2.2: Droplet contact angle and droplet retention angle of one-year old (1995; n=10), and current-year (1996; n=10) needles, measured in July 1996. Letters indicate statistically significant differences within each species and factor (within columns; Nested one-way ANOVA for CA and Kolmogorov-Smirnov for RT; P<0.0001; 5 replicates per tree for the 1995 needle age class and 2 replicates per tree for the 1996 needle age class). Values are means (±SE).

Species + category	Contact angle (CA) degrees		Droplet retention (RT) degrees	
	1995	1996	1995	1996
Ponderosa pine				
Control, downwind	59 (3) a	82 (5)	86 (3) a	89 (1)
0.8 km downwind	37 (3) b	85 (3)	59 (5) b	85 (5)
0.2 km downwind	57 (3) a	91 (1)	89 (1) a	90 (0)
50 m downwind	59 (3) a	89 (3)	85 (3) a	89 (1)
Douglas fir				
Control, downwind	73 (3) a	93 (8) a	89 (1) a	90 (0)
0.8 km downwind	31 (3) c	96 (3) a	78 (5) b	90 (0)
0.2 km downwind	40 (3) b	50 (5) b	44 (5) c	90 (0)
50 m downwind	47 (5) b	74 (5) c	78 (3) b	90 (0)

Table 2.3: Total water content (n=4) and relative water content (n=2) of one-year old needles (1995) and current-year needles (1996), measured in July 1996. Letters indicate statistically significant differences within each column, for each species separately (One-way ANOVA). Values are means (±SE).

Species + category	Total water content, gr H_2O/gr dry weight	
	1995	1996
Ponderosa pine		
Control, downwind	1.21 (0.04) a	1.92 (0.06) a
0.8 km downwind	1.30 (0.01) a	2.03 (0.01) a
0.2 km downwind	1.01 (0.03) b	2.03 (0.01) a
50 m downwind	0.36 (0.04) b	2.14 (0.04) b
ANOVA	$F_{3,13}$=279.30, P<0.0001	$F_{3,5}$=8.55, P=0.026
Douglas fir		
Control, downwind	1.26 (0.03) a	1.63 (0.09) a
0.8 km downwind	1.12 (0.02) b	1.75 (0.03) a
0.2 km downwind	0.87 (0.02) c	1.70 (0.07) a
50 m downwind	0.12 (0.01) d	2.57 (0.34) b
ANOVA	$F_{3,13}$=644.07, P<0.0001	$F_{3,5}$=7.86, P=0.004

Species + category	Relative water content, %	
	1995	1996
Ponderosa pine		
Control, downwind	92 (1)	94 (1) a
0.8 km downwind	94 (0)	100 (1) b
0.2 km downwind	95 (2)	99 (1) a,b
50 m downwind	88 (1)	98 (0) a,b
ANOVA	$F_{3,13}$=5.38, P=0.069	$F_{3,5}$=11.07, P=0.021
Douglas fir		
Control, downwind	95 (3) a	94 (2)
0.8 km downwind	93 (1) a	96 (1)
0.2 km downwind	89 (1) a	99 (1)
50 m downwind	67 (1) b	91 (4)
ANOVA	$F_{3,13}$=65.05, P=0.0008	$F_{3,5}$=2.36, P=0.213

Table 2.4: Chlorophyll fluorescence measured as F_v/F_m ratios (n=6) of one-year old needles (1995) and current-year needles (1996), measured in July 1996. Letters indicate statistically significant differences within each column, for each species separately (Nested one-way ANOVA; 2 replicates per tree). Values are means (±SE). Needle age classes that were no longer alive are indicated as "n.a.".

Category	Fv/Fm ratio	
	1995	1996
Ponderosa pine		
Control, downwind	0.819 (0.003) a	0.793 (0.010) a
0.8 km downwind	0.785 (0.008) b	0.753 (0.013) a/b
0.2 km downwind	0.731 (0.024) b	0.748 (0.020) a/b
50 m downwind	n.a.	0.741 (0.031) b
ANOVA	$F_{3,9}$=25.87, P<0.0001	$F_{3,9}$=3.94, P=0.015
Douglas fir		
Control, downwind	0.824 (0.006) a	0.810 (0.006) a
0.8 km downwind	0.779 (0.009) b	0.751 (0.010) b
0.2 km downwind	0.778 (0.011) b	0.737 (0.021) b
50 m downwind	n.a.	0.769 (0.0013) b
ANOVA	$F_{3,9}$=16.00, P<0.0001	$F_{3,9}$=14.29, P<0.001

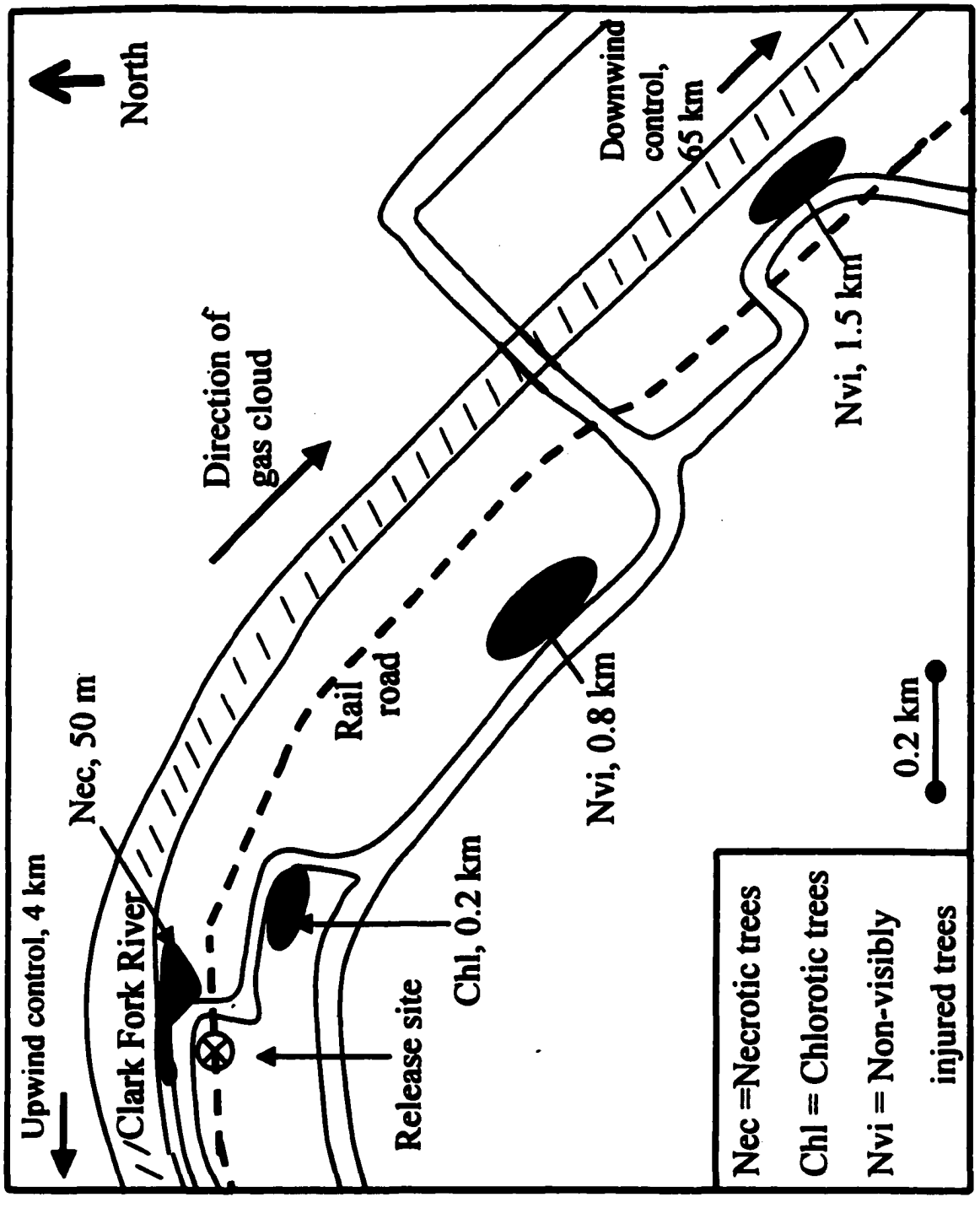


Figure 2.1: Schematic map of the release site and the main study sites downwind of the gas release.

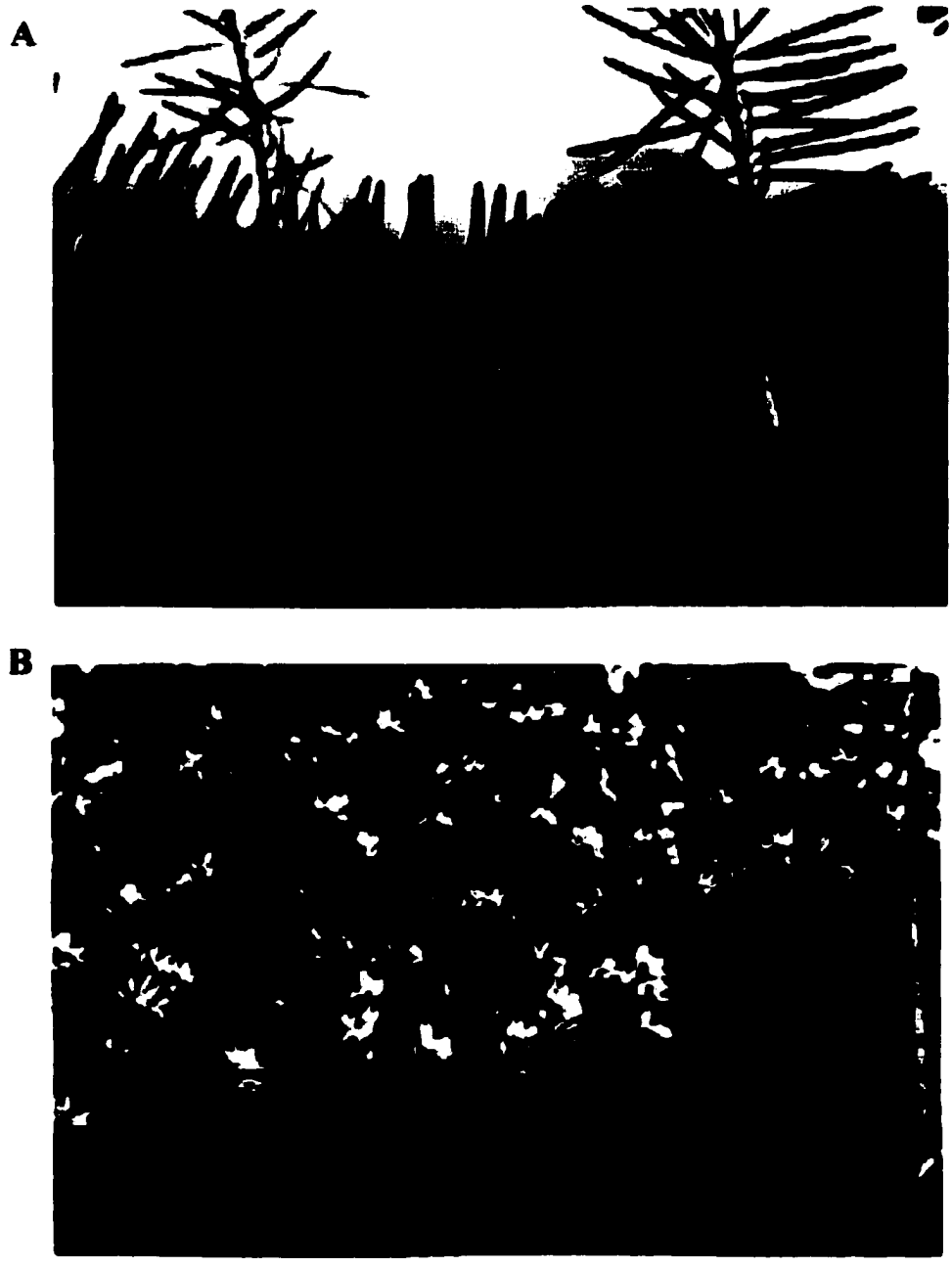


Figure 2.2: Acute visual injury after chlorine gas exposure. Shown are tipburn in *Pseudotsuga menziesii* (A) , and necrosis in *Pseudotsuga menziesii* with healthy *Amelanchier Alnifolia* (B).

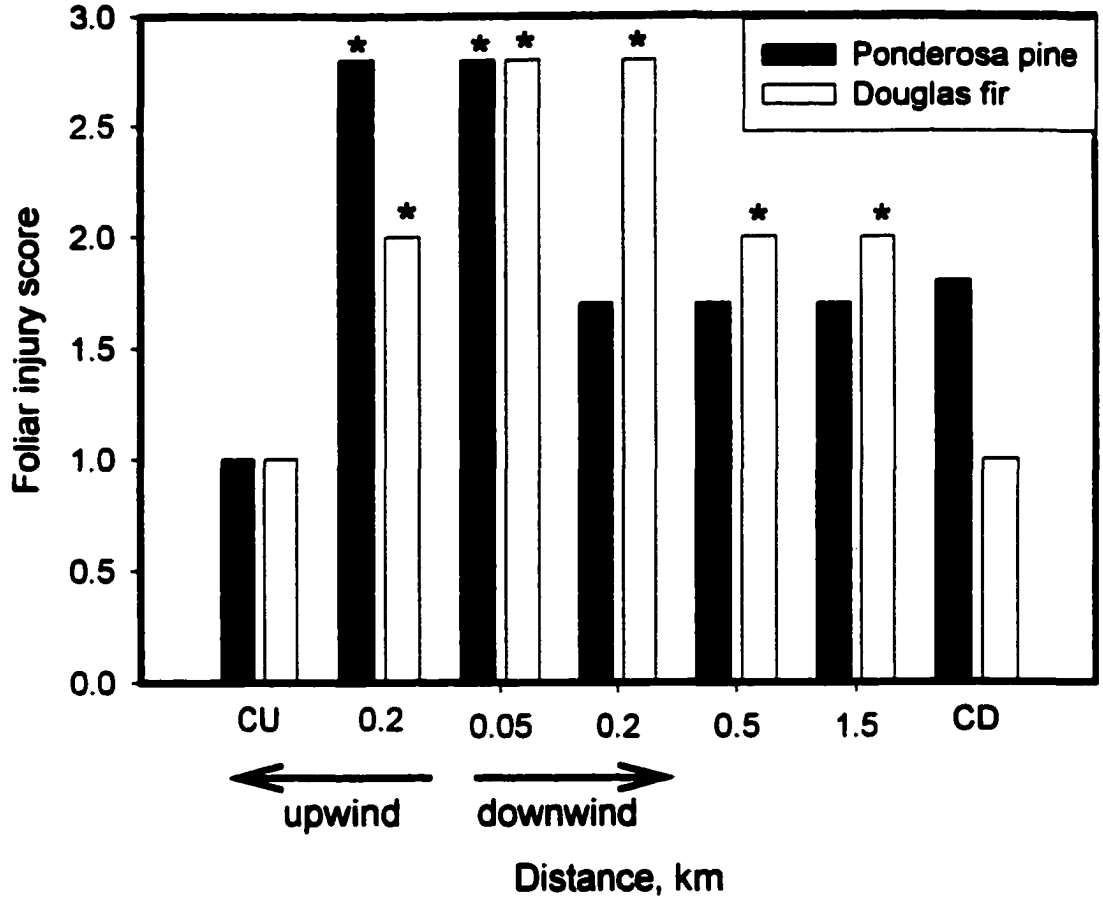


Figure 2.3: Foliar injury on Ponderosa pine (black bars) and Douglas fir foliage (open bars), for all needle age classes measured in June 1996, two months after exposure to chlorine gas. The scores are averages (n=10 trees, 2 replicate branches per tree) of the following visual injury categories: non-visibly injured foliage (0), necrotic mottling (1), chlorosis (2), tipburn (3) and complete necrosis (4). Data for the upwind and downwind control sides (respectively CU, 4 km upwind, and CD, 65 km downwind) are based on foliar injury observed in March 1997 and 1998 (1-3 year old foliage). Exposed sites are on level terrain, except one 0.2 km uphill of the gas release (0.2 up). "*" indicate sites that are statistically different from both control sites within each species (t-test, P<0.05).

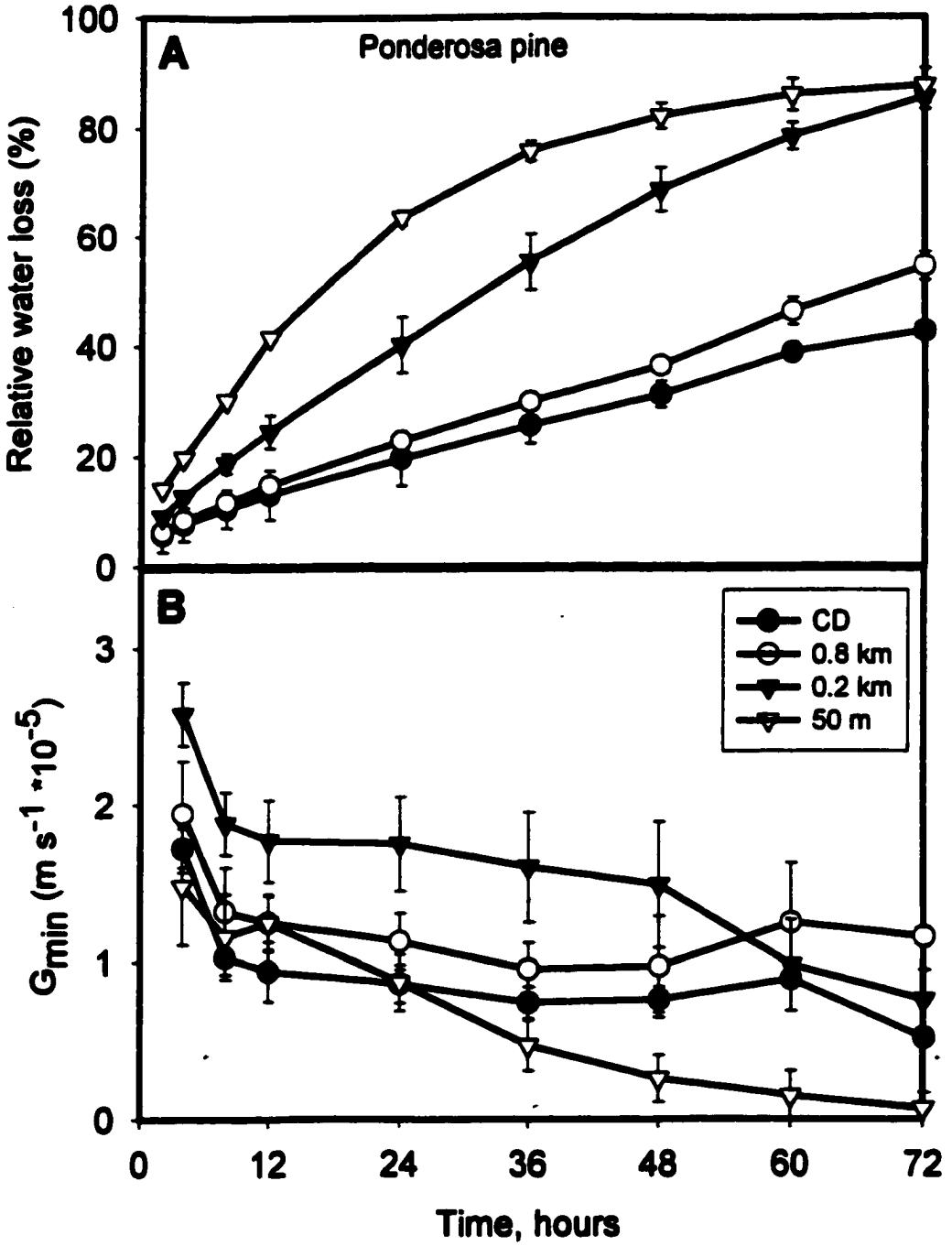


Figure 2.4: Relative water loss (panel A) and minimal needle conductance (panel B) of one-year old (1995) foliage of Ponderosa pine (± SE, n=4). Curves represent the downwind control site (solid circles), and sites 0.8 km downwind from the site of gas release (open circles), 0.2 km downwind (solid triangles), and 50 m downwind (open triangles).

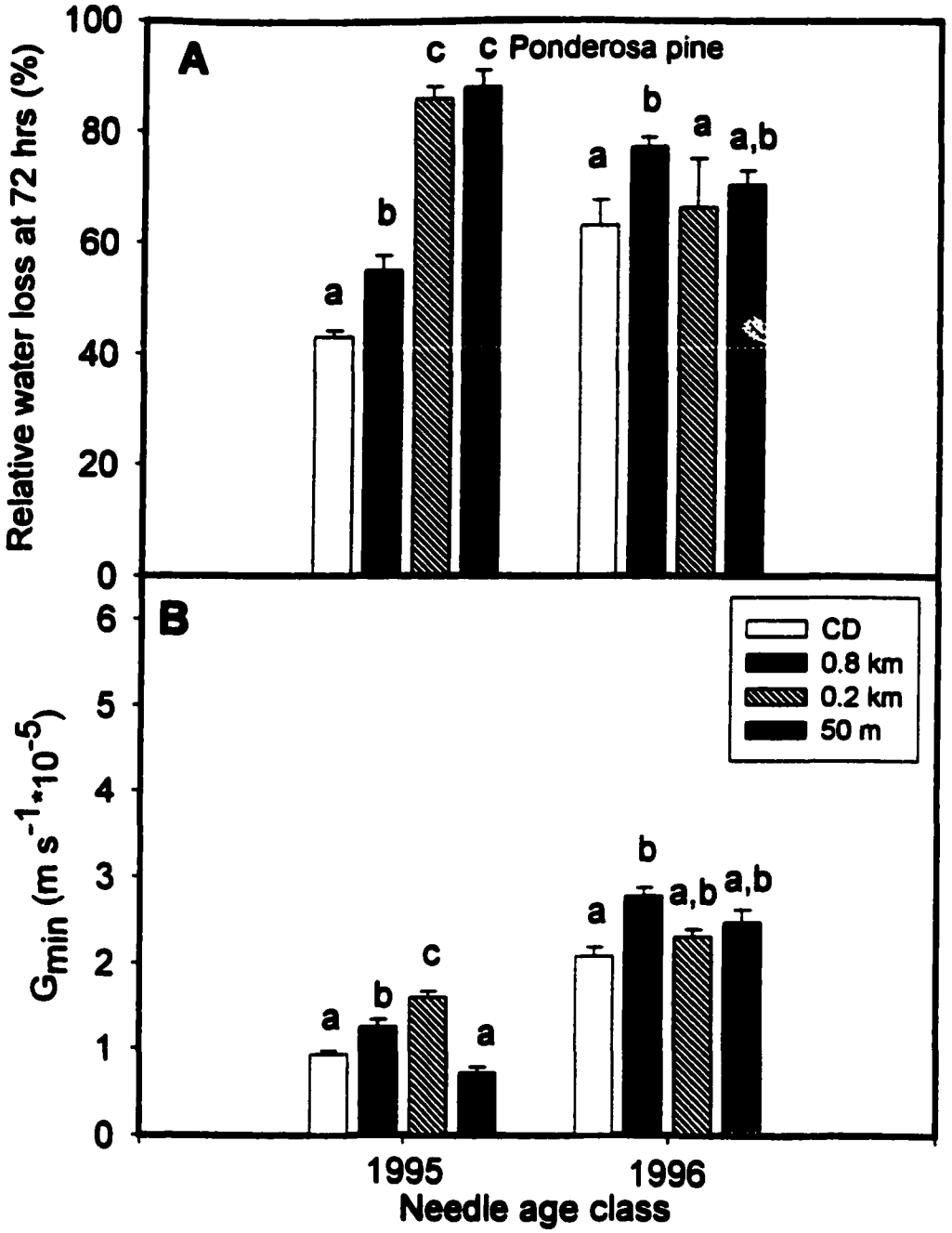


Figure 2.5: Relative water loss (panel A) and minimal conductance, $G_{min,H2Ov}$ (panel B) of one-year old (1995) and current-year (1996) foliage of Ponderosa pine. Relative water loss is shown after 72 hours (n=4), although statistical analyses are based on data from 0 to 72 hours (Nested one-way ANOVA, P<0.001; repeated factor: drying time, 10 measurements over time). $G_{min,H2Ov}$ is shown as the average from 2 to 72 hours, when stomates are assumed closed (n=4; nested one-way ANOVA, P<0.001; repeated factor: drying time, 9 measurements over time). Bars represent the downwind control site (open bars), and sites 0.8 km downwind (black bars), 0.2 km downwind (hatched bars) and 50 m downwind (double hatched bars). Error bars represent one SE.

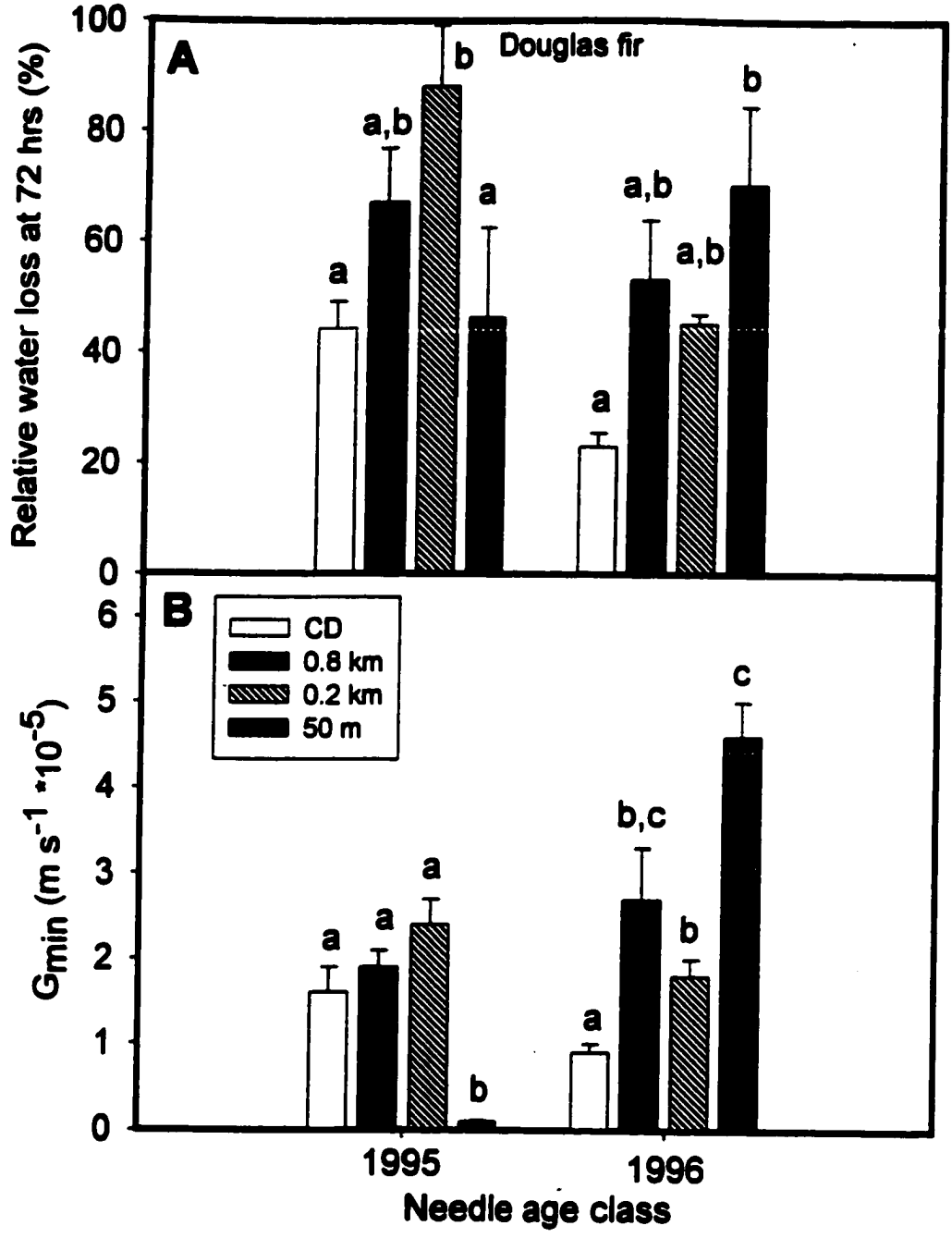


Figure 2.6: Relative water loss (panel A) and minimal conductance, $G_{min.H2Ov}$ (panel B) of one-year old (1995) and current-year (1996) foliage of Douglas fir. Relative water loss is shown after 72 hours (n=4, nested one-way ANOVA, P<0.001; repeated factor: drying time, 10 measurements over time). $G_{min.H2Ov}$ is shown as the average from 2 to 72 hours, when stomates are assumed closed (n=4; nested one-way ANOVA, P<0.001; repeated factor: drying time, 9 measurements over time). Bars represent the downwind control site (open bars), and sites 0.8 km downwind (black bars), 0.2 km downwind (hatched bars) and 50 m downwind (double hatched bars). Error bars represent one SE.

CHAPTER 3

Long-term effects of acute chlorine gas exposure on cuticular transpiration and growth of *Pinus ponderosa* and *Pseudotsuga menziesii*

ABSTRACT

The long-term effects of acute chlorine gas exposure on cuticles, cuticular transpiration, tree growth and mortality were studied in foliage of *Pinus ponderosa* (Ponderosa pine) and *Pseudotsuga menziesii* (Douglas fir), for three growing seasons. Chlorine gas exposure led to increased foliar injury of both exposed foliage and foliage that flushed after exposure ($P<0.05$). Chlorine gas exposure led to increased leaf wettability of directly exposed foliage ($P<0.001$) as well as increased cuticular transpiration for both types of foliage up to 1 yr for Ponderosa pine and directly exposed Douglas fir foliage up to ½ year after the spill ($P<0.001$), and decreased total water content (TWC) for directly exposed foliage of both species up to 1 yr after exposure ($P<0.001$). During the first year cuticular water loss, TWC, and relative water content (RWC) were significantly correlated with foliar injury ($P<0.05$). $F_{variable}/F_{maximum}$ (F_v/F_m) ratios of needles that flushed two months after exposure showed no effect after 1 yr. Exposure to chlorine gas did not affect needle length, specific leaf area, annual shoot increment growth, or the number of buds produced. However, longevity of foliage that flushed two months after exposure was reduced significantly ($P<0.001$). Annual stem increment growth decreased over at least three growing seasons following chlorine gas exposure ($P<0.001$), up to 0.2 km for downwind from the release for Ponderosa pine and 0.8 km for Douglas fir. Cone production was lower for exposed Ponderosa pine trees compared to controls ($P<0.05$). Tree mortality for Douglas fir was higher within ~50 m of the release site while mortality of Ponderosa pine was not affected. The pattern of growth parameters that were affected or not affected by chlorine gas exposure reflects the priority of carbon allocation when conifers experience defoliation. Over the lifetime of these conifers the impact on growth is comparable to disturbances such as several years of drought or defoliation due to insect damage.

INTRODUCTION

This study reports on the long-term morphological, physiological and growth effects of chlorine gas on conifers after a train derailment in 1996 in northwest Montana, USA. Accidents involving chlorine gas releases are not uncommon (Hall et al., 1996), and can result in toxic effects to humans, causing irritation to eyes, nose and the respiratory system (Baxter, Davies and Murray, 1989; Griffiths and Megson, 1984; Whithers and Lees, 1985). Chlorine gas exposure also causes foliar injury to vegetation, consisting of chlorosis, necrotic mottling, and necrosis (Chapter 2; Brennan, Leone and Daines, 1965; Brennan, Leone and Holmes, 1969; Heck, Daines and Hindawi, 1970). These symptoms are similar to those caused by acid rain and mist (Forsline, Dee and Melious, 1983; Vijayan and Bedi, 1989; Whitney and Ip, 1991).

Long-term effects of acute chlorine gas exposure on physiological functions such as tree water relations, photosynthesis, and growth have not been reported. Because chlorine gas can form highly acidic solutions in contact with water (Morris, 1946; Chapter 2; Schreuder, unpublished data), exposure to chlorine gas can have effects similar to acid rain and acid mist. Chlorine gas exposure may adversely affect tree water balance via influences on the waxy cuticle and on stomatal regulation. Acid rain and acid mist have been reported to change cuticular wax composition (Percy, Jensen and McQuattie, 1992; Kerfourn and Garrec, 1992; Günthardt-Görg, 1994), wax structure (Schmitt, Rütze, and Liese, 1987; Percy, Krause and Jensen, 1990; Bytnerowicz and Turunen, 1994), and decrease total wax production (Garrec and Kerfourn, 1989; Percy et al., 1990, 1992; Bytnerowicz and Turunen, 1994). These changes may increase cuticular transpiration rates (Hadley and Smith, 1989), rendering plants more susceptible to

drought stress, both in summer (Mengel, Hogrebe and Esch, 1989) and winter conditions (Barnes and Davison, 1988; Hadley and Smith, 1989).

Increased stomatal conductance has been reported after exposure to acid mist (Eamus and Fowler, 1990; Flagler, Lock and Elsik, 1994), and in forests that show symptoms of decline (Maier-Märcker and Koch, 1992, 1995). Exposure to acid mist can lead to decreased rates of photosynthesis (Roberts, 1990; Velikova et al., 1997; Momen, Anderson and Helms, 1999) as well as photosynthetic efficiency (Führer et al., 1990; Velikova and Yordanov, 1996). A one-time application of acid mist decreased F_v/F_m ratios and photosynthesis of *Phaseolus vulgaris* (Velikova and Yordanov, 1996), and these effects were irreversible at pH values below 2.0 (Velikova et al., 1997).

In conifers, necrosis generally begins as tipburn, an orange-brown coloring extending from the tip to the base of the needle which eventually develops into complete necrosis (Brennan, Leone and Daines, 1966), followed by subsequent defoliation of photosynthetic tissues (Chapter 2; Heck et al. 1970). Tree defoliation can lead to decreased height growth (Carlson, McCaughey and Theroux, 1988; Salemaa and Jukola-Sulonen, 1990; Krause and Raffa, 1996), stem increment growth (Vosko and Klubica, 1992; Christiansen and Fjone, 1993; Krause and Raffa, 1996), as well as total tree biomass (Krause and Raffa, 1996; Sanchez and Wagner, 1999). The adverse effects of defoliation on tree growth tended to increase with increasing severity of defoliation (Carlson et al., 1988), younger tree-age (Sanchez and Wagner, 1999), and lower starch reserves at the time of defoliation (Webb, 1981). Furthermore, the combination of defoliation and increased water stress may increase susceptibility to insect injury in conifers (Ferrel 1978; Christiansen and Fjone, 1993).

In this study I report on the long-term physiological and growth effects after acute chlorine gas exposure in a coniferous forest in western Montana, USA. Earlier studies at this site have shown that chlorine gas exposure led to significant foliar injury, and subsequent defoliation of exposed Ponderosa pine and Douglas fir trees (Chapter 2). Moreover, exposed conifer foliage had increased cuticular water loss and decreased photosynthetic efficiency (Chapter 2). I predicted that the combination of these influences would lead to long-term effects on tree water balance, growth and mortality. This study addresses the following questions: (1) What are the long-term effects of chlorine gas exposure on cuticular transpiration and foliar water content; (2) What are the long-term effects of chlorine gas exposure on conifer growth; (3) Does chlorine gas exposure affect the susceptibility of trees to drought stress and insect damage; and (4) Does chlorine gas exposure affect tree mortality?

MATERIALS AND METHODS

Study site

The study sites were located in a narrow valley ~2 km west of Alberton, Montana, in the Rocky Mountains (47°00'N, 114°30'W). On April 11, 1996, around 0400 hr, a train derailment released ~55 metric tons of chlorine gas (Cl_2) into the atmosphere and the surrounding forest. During the following week, chlorine gas concentrations at the site of the gas release varied from 12-20 ppm Cl_2 to ~50 ppm Cl_2 (1-hr average), with peak concentrations reaching ~1400 ppm (Olympus Environmental, 1996). Atmospheric dispersion models predicted similar concentrations, with peak concentrations ranging from ~165 ppm Cl_2 at about 1.2 km to ~5 ppm Cl_2 at ~9 km downwind from the site of

gas release (ATSDR, 1997). In addition, chlorophenols were formed due to a ruptured railroad car containing potassium cresylate. Soil layers polluted with these organic compounds were removed from the site by excavation, and concentrations in the soil were well below critical levels to public health. Data on atmospheric concentrations of these organic pollutants were not made available (Olympus Environmental, 1996).

Two control sites were established, one ~65 km downwind (CD; 46°70'N, 114°00'W) and another ~4 km upwind (CU) from the site of gas release. Four study sites that had been exposed to chlorine gas were established based on foliar injury symptoms observed within two months after exposure (Chapter 2). The exposed sites were located 50 m downwind from the site of gas release (foliage completely necrotic except for current-year needles), 0.2 km downwind (foliage mainly chlorotic), ~0.8 km and ~1.5 km downwind (foliage not visibly injured)(Figure 2.1). All sites were similar in elevation, vicinity to the river, soil type and vegetation (Table 3.1). Tree status (based on visual judgment of foliage) was judged to be uniform within each study site. My investigation focused on the two most common conifer species in the area, *Pseudotsuga menziesii* (Douglas fir) and *Pinus ponderosa* (Ponderosa pine) (Hitchcock and Cronquist, 1994). To cover the total distance over which chlorine gas appeared to affect Pseudotsuga menziesii (Olympus Environmental, 1996), some of the measurements were extended up to ~10 km downwind. Physiological and growth measurements were carried out over three growing seasons following the gas release. Samples for all measurements were collected from the bottom part of the canopy (1.5 to 2 m above ground). In this study the growing seasons will be referred to by calendar year and years since the chlorine gas release, expressed as years to spill (yts), in the following manner: 1994: -2 yts (2 years before spill), 1995: -1

yts (1 year before spill), 1996: 0 yts (spill year), 1997: +1 yts (1 year after spill), and 1998: +2 yts (2 years after spill).

Visual and insect injury

Visible foliar injury due to drought and insects was assessed in March 1997 and 1998. Two branches with the needle age classes -1, 0, and +1 yts, were collected from 5 randomly selected trees at each site. Ten to fifteen randomly chosen needles from each branch, 50 to 75 in all, were scored in the lab according to the five following foliar injury categories: 1) 100 % green; 2) 5 to 25 % chlorotic; 3) >25% chlorotic; 4) 5 to 25 % necrotic; and 5) >25% necrotic. Beginning in March 1998, I monitored for evidence of insect injury in the field whenever the study sites were visited.

Cuticular injury and transpiration

Effects of chlorine gas exposure on needle cuticles were assessed using droplet contact angles (CA) and droplet retention angles (RT) (Brewer, 1996; Brewer and Smith, 1997). CA is a measure of leaf wettability, and can be used to study effects of air pollutants on cuticles (e.g., Staszewski et al., 1998). CA and RT were determined for foliage from 10 randomly selected trees at each site with two replicates per tree.

Cuticular water loss was determined for exposed 1995 foliage (-1 years to spill, yts), and non-exposed 1996, 1997, and 1998 (0, +1 and +2 yts respectively). One branch from was collected from 5 randomly selected trees at each site. From this sample, 2 to 4 samples were randomly selected for further analysis. Fresh weight, and saturated weight after soaking overnight were measured on foliage samples. Then stems of water-saturated

needle samples were sealed with paraffin wax, to avoid water loss via the stem, and dried at ~30 ^{o}C in a drying oven. Cuticular transpiration was determined by weighing the samples periodically over the course of 3 d (Hadley and Smith, 1989). Samples were dried for an additional 3 d at ~60 ^{o}C to determine dry weight. Minimal conductance to water vapor, $G_{min,H2Ov}$ (Kerstiens, 1996), was calculated from data on specific needle area (SLA), temperature and relative humidity in the drying oven, and the weight loss of the needles. SLA was estimated for foliage of all needle age classes for each sample date using the glass bead method (Thompson and Leyton, 1971). Total water content (TWC, expressed as gr H_2O per gr dry weight) and relative water content (RWC, expressed as the ratio of fresh weight-dry weight to saturated weight-dry weight) of foliage were derived from the fresh-, saturated-, and dry-weights of needle samples. Cuticular transpiration and foliar water content were measured periodically in until September 1998 (i.e., 9/96, 12/96, 3/97, 5/97, 10/97, 3/98, 6/98 and 9/98), a total of three growing seasons after exposure to chlorine gas.

Photosynthetic efficiency

Chlorophyll fluorescence measurements were made with an Opti-Sciences Modulated Fluorometer (Model OS-100, PP Systems, MA) in March and May 1997, one year after gas exposure. Needle samples of Douglas fir and Ponderosa pine (5 randomly selected trees for each species, 2 replicates per tree) were dark-adapted for fifteen minutes prior to measurements. F_o (dark fluorescence yield) and F_m (maximum fluorescence) were measured to calculate the variable fluorescence ($F_v = F_m - F_0$) and the efficiency of excitation (F_v/F_m ratio). Measurements were taken between 10.00 and 14.00

hrs, with a saturated light pulse of 0.8 s, F_0 100-125.

Growth measurements

Needle length was measured in March 1997 and 1998 for 15 randomly selected trees at each site, with 4 replicates per tree. Annual shoot increment growth was measured in the field in winter, spring, and fall of 1997, by measuring the distance between nodes on leading branches in the lower canopy. This was done for 5 to 15 randomly selected trees of each species with two replicate branches per tree, at each site. Bud counts were carried out in fall 1996, 1997, and 1998, and represent the three growing seasons after chlorine gas exposure. On each sampling date, the number of buds per leading branch was counted for 10 to 27 trees of each species, depending of tree availability at each site (two replicate branches per tree).

Retention of needle age classes was measured in the field in March 1998 at the upwind and downwind control sites and at nine sites exposed to chlorine gas (up to ~10 km downwind from the site of release), for 10 randomly selected trees per site with two replicates branches. In April 1999, this survey was repeated at the two control sites, an additional upwind control site, and eight exposed sites ($\leq$1.5 km downwind of the site of release).

Tree cores were collected in May 1997 at the upwind and downwind control sites and three exposed sites ($\leq$0.8 km downwind from the release site). Sampling procedures at each site were the same as those to estimate retention of living needle age classes. Cores were examined microscopically and annual core increment growth was calculated as the % change of the 18-year average (i.e., up to the year before the gas release). This

analysis was repeated in November 1998. In May 1998, large mature trees at all field sites were examined for the presence of cones. These data were then expressed as the % of trees that produced cones.

Tree Mortality

Tree mortality was assessed in May 1999, three years after the gas release, at two upwind control sites, as well as eight sites up to 1.5 km downwind of the site of gas release. At each site a 10 X 10 m plot was randomly established. All trees taller than 2 m were identified to species. I measured DBH (diameter breast height, 1.37 m), and whether trees were alive (living foliage present on branches) or dead (no living foliage present; personal communication, D. Six). This was a conservative estimate of tree mortality since a conifer can be dead while there is still living foliage present on the branches. Finally, tree mortality was simulated at the stand level, using the FOREST-BGC model (Running and Gower, 1991). Results were reported as relationships between foliar biomass and stem increment growth (Nichols, 1988). Input values for model calculations were derived from data collected in this study.

Statistical analysis

Data were analyzed using SigmaStat (SPSS, 1997). Data that met the requirements for normal distribution were analyzed using analysis of variance (reported as F, P-value). Pair wise comparisons were made using a Bonferroni post-hoc test. The experimental design was a nested analysis of variance. Samples were collected from 5 to 10 randomly selected trees with 2 to 15 replicates per tree. The actual number of samples

and replication depended on the measurement. If there were no significant differences based on subsamples, they were pooled to increase power of the statistical analysis ($P<0.05$; Sokal and Rohlf, 1997).

Data that did not meet the normality requirements were analyzed using a Kruskal-Wallis ANOVA on ranks (reported as H, P-value) or the Kolmogorov-Smirnov test (reported as ksz, P-value). Cuticular transpiration experiments were done for randomly selected subsamples from 5 randomly selected trees at each site, resulting in sample sizes varying from $n=2$ to $n=4$. Repeated measures (RM) techniques were used to analyze foliar water loss data, with two repeated factors, time of drying and sampling date. Frequency data, such as visual injury, % cone production and tree mortality were examined using a Chi-square analysis, an extended Kruskal-Wallis test, or Fisher's exact test.

RESULTS

Visual drought injury

Foliar injury was assessed in the two years following chlorine gas exposure. In March 1997 (+1 yts), foliar injury of –1 yts and 0 yts needles of Ponderosa pine was significantly higher within 0.2 km of the release site compared to control trees and trees 0.8 downwind (Table 3.2); foliage that was chlorotic in May 1996 had progressed to necrotic. Moreover, even though 0 yts foliage had a healthy appearance after it flushed, 35 to 75 % of needles had become necrotic by March 1997 on trees 0.2 km and 50 m downwind. There were no significant differences in foliar injury for Douglas fir trees, although there was a trend towards increased foliar injury in the –1 and –1 yts age classes

for the trees within 0.2 km of the site of release in March 1997 (+1 yts) and 1998 (+2 yts; Table 3.2). Because increased foliar injury was observed for both exposed foliage and foliage that flushed after exposure, chlorine gas exposure may have caused foliage from both –1 yts and 0 yts age classes to be more susceptible to secondary stress factors.

The extent of visual injury in March 1997 was strongly correlated with visual injury in March 1998 for both species (r^2=0.80, P=0.001). This correlation was stronger in 0 yts foliage (r^2=0.90, P=0.002) than in –1 yts foliage (r^2=0.58, P=0.23). Foliar injury of Ponderosa pine in March 1997 (+1 yts) was negatively correlated with TWC and RWC in September 1996 (r^2= -0.82, P<0.01) and in March 1997 (r^2= -0.67, P=0.05). Foliar injury of Douglas fir in March 1997 was positively correlated with relative water loss in December 1996 and May 1997, and with $G_{min,H2Ov}$ in December 1996 and October 1997 ($r^2 \geq 0.70$, P<0.05). Negative correlations for Douglas fir were present between visual injury in March 1997 and TWC in September 1996 and May 1997. RWC was negatively correlated with visual injury at all sampling dates through October 1997 ($r^2 \leq -0.70$, P<0.05). Thus, foliar injury one year after gas exposure was a good indicator of the effects chlorine gas exposure on cuticular water loss and needle water content. In March 1998, no significant relationships between visual injury and indices of water loss were found.

Cuticles and cuticular water loss

Two years after chlorine gas exposure, –1 yts foliage of Ponderosa pine trees 0.8 km downwind had lower CA's compared to controls, suggesting that chlorine gas exposure increased leaf wettability of conifer foliage, and that these effects persisted long

after the initial exposure (Table 3.3). However, statistically significant differences in CA for the other needle age classes could not be attributed to chlorine gas exposure, and were probably due to site to site variation. Thus, chlorine gas exposure only led to persistent effects on CA for directly exposed conifer foliage of Ponderosa pine. This also may have been the case for *Douglas fir* foliage as well, but at the time of measurement the directly exposed needle age class had already defoliated. CA decreased significantly with increasing needle age (Table 3.3) for both for Ponderosa pine and Douglas fir ($F_{123,66}$ and $F_{35,69}$ respectively; $P<0.0001$) at all study sites. Droplet retention angles (RT) did not differ significantly with different chlorine exposures for either species. However, there was a trend towards lower RT for Ponderosa pine with increasing needle age ($H_3=22.5$, $P<0.001$).

In September 1996, five months after chlorine gas exposure, 1-year old needles (-1 yts) of Ponderosa pine and Douglas fir had higher relative water loss relative to controls (Figure 3.1A and 3.2A). However, increased relative water loss was not reflected in significantly higher $G_{min,H2Ov}$ (Figure 3.1B and Figure 3.2B). Moreover, unexposed 0 yts needles on trees 50 m downwind also had higher water loss compared to the other sites, but again these differences were not reflected in significantly higher $G_{min,H2Ov}$ (Figures 3.1A and 3.2A). Relative water loss for –1 yts Ponderosa pine needles within 0.2 km of the release remained higher than control needles through October 1997 ($F_{2,56}=9.04$, $P<0.001$; Figure 3.3A and Appendix 3.1). Douglas fir foliage also continued to show increased relative water loss from –1 yts foliage within 0.2 km of the release through October 1997 ($F_{3,45}=5.50$, $P=0.007$; Figure 3.4A and Appendix 3.2). However, although there was a trend towards higher $G_{min,H2Ov}$ for –1 yts foliage within 0.2 km of the release,

these were not statistically significant (Appendix 3.1 and 3.2). There was a trend towards higher relative water loss from 0 yts foliage for both Ponderosa pine and Douglas fir trees within 50 to 200 m from the release (Figure 3.3B and Figure 3.4B; Appendix 3.1 and 3.2). This trend was not consistent over time for Ponderosa pine and foliage at exposed sites showed higher water loss compared to control sites at some dates but not at others. $G_{min,H2Ov}$ of 0 yts foliage for both Ponderosa pine and Douglas fir was higher within 0.2 km of the release compared to control sites ($F_{2,65}$=3.57, P=0.034 and $F_{2,73}$=4.24, P=0.019 respectively; Appendix 3.1 and 3.2). There were no effects of chlorine gas exposure on relative water loss and $G_{min,H2Ov}$ of Ponderosa pine needles that flushed in 1997 (+1 yts), the second growing seasons after chlorine gas exposure. However, the +1 yts needles of Douglas fir trees had increased relative water loss up to 0.8 km downwind and higher $G_{min,H2Ov}$ at 0.8 km downwind compared to control sites ($F_{2,33}$=11.66, P<0.001 and $F_{2,25}$=6.01, P=0.007 respectively; Appendix 3.1 and 3.2). These data suggest that chlorine gas exposure affected water balance of conifer foliage, via both direct and indirect mechanisms, for at least 2-3 growing seasons after exposure. However, responses were species dependent (higher for Douglas fir), and not always consistent over time. Although there was a good correlation between increased relative water loss and $G_{min,H2Ov}$ (mean r^2=0.69 ± 0.11 SE, n=19; 75 % of r^2 values >0.87), significant increases in relative water loss were not always reflected in significantly higher $G_{min,H2Ov}$ and vice versa.

Water content

TWC of foliage that flushed after chlorine gas exposure was lower at all exposed sites for Douglas fir in September 1996 (Table 3.4), but not after this date (Appendix 3.2). Directly exposed Ponderosa pine foliage within 50 m had lower TWC compared to the other sites (Table 3.4). TWC was lower in sites up to 0.2 km downwind compared to the control site and 0.8 km downwind for directly exposed (-1yts) Ponderosa pine foliage up to at least October 1997, 1 ½ years after chlorine exposure ($F_{2,62}$=6.81, P<0.001, Appendix 3.1). Similarly, directly exposed Douglas fir foliage (-1 yts) had lower TWC up to 0.2 km downwind compared to the control site and 0.8 km downwind up to at least October 1997, ½ years after chlorine exposure ($F_{2,53}$=16.75, P<0.001, Appendix 3.2). RWC was lower for directly exposed foliage within 50 m of the release in September 1996 (Table 3.4). This needle age class defoliated in Fall of 1996. Chlorine gas exposure did not affect RWC for either of the species nor any of the needle age classes after September 1996 (Appendix 3.1 and 3.2).

Chlorophyll fluorescence

There were no differences in F_v/F_m in late March 1997, and trees of both species were still in winter dormancy (F_v/F_m ratio 0.361 ± 0.015 SE). Similarly, in May 1997 there were no differences in F_v/F_m ratios for -1 yts Ponderosa pine and Douglas fir foliage 0.8 km downwind and 0 yts Douglas fir foliage (Table 3.5). Although there were significant differences in F_v/F_m ratios in the 0 yts needle age class (Table 3.5), these differences could not be attributed confidently to chlorine gas exposure, because of the inconsistent responses in relation to the distance to the release site.

Growth measurements

While needle length varied between sites, it did not appear to be affected by chlorine gas exposure. Ponderosa pine needles on trees at exposed sites had significantly longer needles than the downwind control site in the +1 and +2 needle age classes (Table 3.6). However, since these differences were present before the exposure to chlorine gas (Table 3.6), they cannot be attributed to gas exposure. Although there were some differences in needle length in Douglas fir in the -1 and 0 yts needle age classes, there was no consistent pattern that would link these differences to chlorine gas exposure (Table 3.6).

Specific leaf area (SLA) of Ponderosa pine and Douglas fir foliage was not affected by chlorine gas exposure (overall averages 84 $cm^2\ g^{-1} \pm 3$ SE (n=47) for Ponderosa pine; 93 $cm^2\ g^{-1} \pm 4$ SE (n=33) for Douglas fir). SLA decreased with increasing needle age in both Ponderosa pine and Douglas fir for both control and exposed trees. SLA of +1 yts Douglas fir needles was significantly higher than −1 yts needles ($F_{2,31}$=5.4, P=0.004), with SLA values of 111 $cm^2\ g^{-1} \pm 9$ and 84 $cm^2\ g^{-1} \pm 4$, respectively.

Although the natural variation in annual shoot growth (for branches 1.5-2 m off the ground) was considerable, there were significant differences between the study sites. Since there were no differences between the sampling dates and subsamples, all samples for the three sampling dates were combined for statistical analysis. Shoot growth was higher at the upwind control site compared to the other sites, for both Ponderosa pine and Douglas fir (Table 3.6). However, these differences cannot be attributed to chlorine gas exposure, since similar patterns were observed pre- and post-chlorine gas exposure (Table 3.6). Thus, it appears that the differences in annual shoot growth between study

sites were due to site-specific differences rather than to exposure to chlorine gas.

Ponderosa pine trees at sites 0.2 and 0.8 km downwind had more buds in 1996 (0 yts) and 1997 (+1 yts), the two growing seasons following chlorine gas exposure (Table 3.6). A similar pattern was observed for Douglas fir, with increased numbers of buds on trees 0.8 km downwind in 1996 (0 yts) and 0.2 km downwind in 1997 (+1 yts; Table 3.6). In 1998, there were no differences in bud counts for either species.

By spring 1998 (+2 yts), only 40-75 % of needle age classes were present on Ponderosa pine trees within 0.5 km of the site of gas release, compared to control trees and trees further downwind from the release site (Figure 3.5A). In 1999 (+3 yts), control trees still had significantly more needle age classes present than exposed trees (H_{10}=56.8, P<0.001). Moreover, in 1999, lower needle retention for Ponderosa pine was evident up to 1.5 km downwind (compared to 0.5 km in 1998), and exposed Ponderosa pine had even fewer needle age classes present than in 1998. Douglas fir trees up to 1.5 km downwind from the site of the gas release had fewer than 2 needle age classes present, compared to about 9 years at control sites (Figure 3.5B). In 1999, these differences were still present (H_{10}=$_{123.1}$, P<0.001). Patterns of lower needle retention by Douglas fir needles were observed up to 10 km downwind from the release site (Figure 3.5B).

There was no difference in annual core increment growth between study sites in 1994 and 1995, the two growing seasons before chlorine exposure (P>0.05). Moreover, there were no differences in annual core increment growth for Ponderosa pine from 1994 to 1998 at sites 0.2 and 0.8 km downwind (Figure 3.6A). The control sites showed an increasing trend from 1994 to 1998 ($F_{4,120}$=2.86, P<0.001; Figure 3.6A). In contrast, Ponderosa pine up to 0.2 km downwind from the release had significantly lower core

increment growth in the three years following chlorine gas exposure compared to before exposure ($F_{4,120}$=2.86, P<0.001; Figure 3.6A). Ponderosa pine at 1.5 km downwind had lower growth the first growing season after exposure, but this decrease was not statistically significant (Figure 3.6A). Similarly, Douglas fir at control sites had an increasing trend in core increment growth between 1994 and 1998 ($F_{4,120}$=5.61, P<0.001; Figure 3.6B). Douglas fir up to 0.8 km downwind from the release site had significantly lower core increment growth for at least three years after exposure to chlorine gas, compared to before exposure. In 1996, reductions of stem growth in Douglas fir corresponded to severity of foliar injury, with reductions of 26 % (± 6 %) and 56 % (± 7 %) for chlorotic and necrotic trees, respectively ($T_{3.3}$, P=0.009).

At the control sites, 75 % of Ponderosa pine produced cones in 1998 (+2 yts). However, at exposed sites up to 10 km downwind only 40 % (± 27 %) of trees produced cones (Fisher's Exact test, P=0.05 to 0.001; range 10 to 70 %). Cone production was lowest within 1.5 km downwind from the site of gas release, averaging only 29 % of the trees. One exception was a heavily managed Ponderosa pine stand ~2.5 km downwind, with 70 % of the trees bearing cones. Douglas fir had an overall average of 40 % (± 20 %) of trees bearing cones in 1998 (+2 yts; range 10 to 67 %). Trees at sites 50 m and 0.5 km downwind had significantly lower cone production than the control sites (only 10 % of trees with cones; Fisher's Exact test, P=0.02).

Mortality

Chlorine exposure increased susceptibility to insect damage on a very limited scale. By April 1998 (+3 yts), only two mature Douglas fir trees, weakened by defoliation

after chlorine gas exposure, had been killed by Douglas fir beetle (*Dendroctonus pseudotsugae* Hopkins). Several Douglas fir trees, which had been killed directly by chlorine gas, were infested by ambrosia beetles (*Trypodendron* spp.). There was no evidence of infestations by insects of Ponderosa pine.

In spring 1999, three years after gas exposure, there were no effects of chlorine gas exposure on mortality of Ponderosa pine, averaging 10 % (± 5 %) mortality over 9 sites within 1.8 km of the site of release (range 0 to 30 %). Douglas fir mortality was significantly higher at the two sites within 40 m of the site of gas release compared to control sites (Figure 5). Although Douglas fir mortality at distances beyond >50 m downwind of the release site was not significantly different from the upwind control sites, tree mortality at these exposed sites tended to be higher than at the control sites (Figure 5).

DISCUSSION

This is the first study ever to report on long-term physiological and growth effects on a natural forest ecosystem after acute chlorine gas exposure. Observed adverse effects consisted of influences on leaf water loss and growth. Chlorine gas exposure led to increased leaf wettability of directly exposed needles of Ponderosa pine. Moreover, relative water loss via the cuticle tended to be higher for exposed trees within 0.2 km of the release, which led to lower TWC for directly exposed foliage. Chlorine exposure caused severe defoliation, and led to decreased annual stem increment growth, cone production, and leaf longevity of photosynthetic biomass. These effects may have long-term adverse effects on tree health and survival, due to increased susceptibility to drought stress and lower rates of photosynthesis.

Cuticles and cuticular water loss

Two years after chlorine gas exposure, CA's on directly exposed Ponderosa pine foliage (-1 yts) 0.8 km downwind were lower than controls, suggesting that cuticles had not recovered to pre-spill conditions (Table 3.3, Chapter 2). Although exposed 0 yts Douglas fir needles had lower CA's 5 months after gas exposure (Chapter 2), no recovery had occurred two years after exposure (Table 3.3). However, CA's of this needle age class on exposed trees were not different from control trees, because CA's of control needles has decreased over time (Table 3.3). Lower CA's have been reported for other conifer species over time due to erosion of cuticular waxes (e.g., Staszewski et al., 1998). Other differences in CA could not be attributed to chlorine gas exposure, and were most likely due to site to site variation (Table 3.3).

Decreased CA's have been reported for *Picea rubens* after exposure to acid mist (Barnes and Brown, 1990; Percy et al., 1992) as well as for beech trees with forest decline symptoms (Paoletti, Radi and La Scala, 1998). CA and RT decreased with increasing needle age (Table 3.3), which can be attributed to erosion and degradation of cuticular waxes over time (Schreiber, 1994; Staszewski et al., 1998). The observed changes in CA of –1 yts Ponderosa pine foliage may have been caused, in part, by exposure to the chlorine gas cloud, since acid rain and mist can increase cuticular wax erosion (Paparozzi and Tukey, 1984), change wax composition and structure (e.g., Percy et al., 1990, 1992; Kerfourn and Garrec, 1992; Bytnerowicz and Turunen, 1994), and decrease wax production (Garrec and Kerfourn, 1989; Bytnerowicz and Turunen, 1994). These changes may have led to higher cuticular transpiration (DeLucia and Berlyn 1984;

Hadley and Smith, 1989; Schreiber, 1994), and lower TWC (Mengel et al., 1989; Esch and Mengel, 1998).

In September 1996, three months after gas exposure, –1 yts and 0 yts foliage of Ponderosa pine and Douglas fir had increased relative water loss (Figure 3.1A and 3.2A), further suggesting that cuticles had not recovered since the spill (Chapter 2). These effects persisted up to 1 year after exposure for Ponderosa pine foliage, and up to ½ year for Douglas fir foliage (Figures 3.3 and 3.4, Appendices 3.1 and 3.2), after which this needle age class was too sparsely represented on Douglas fir branches to be sampled for water loss. Interestingly, increased cuticular transpiration was significant for directly exposed –1 yts foliage measured as relative water loss, and for the 0 yts needle age class measured as $G_{min,H2Ov}$ (Figures 3.3 and 3.4, Appendices 3.1 and 3.2). Yet, the differences were only significant for one of the factors, either relative water loss or $G_{min,H2Ov}$. The factor that was not statistically significant showed the same general trend as the significant factor. Moreover, there was a reasonably strong correlation between relative water loss and $G_{min,H2Ov}$. Thus, it appears that chlorine gas exposure tended to increase cuticular water loss of conifer foliage within 0.2 km of the release. Increasing the sample size may have led to results that were more internally consistent. Cuticular damage to the –1 yts needle age class occurred through direct exposure to chlorine gas. Since cuticular transpiration of 0 yts of both species and +1 yts Douglas fir foliage also were elevated, these cuticles were most likely affected indirectly via effects on de novo wax synthesis. Disruption of wax synthesis also has been reported for *Picea rubens* in response to acid mist (Percy et al., 1992).

Adverse effects of chlorine gas exposure on cuticular transpiration lasted up to 1

year for Ponderosa pine and 0.5 year for Douglas fir. This led to lower TWC for –1 yts foliage within 0.2 km from the release (Appendices 3.1 and 3.2). However, chlorine gas effects on TWC were limited to directly exposed conifer foliage within 0.2 km of the release. These effects lasted up to May 1997, 1 year after exposure. RWC of conifer foliage was not affected by chlorine gas exposure (Appendices 3.1 and 3.2). RWC values were well above the lethal RWC threshold reported for Douglas fir in Montana (~55 %; Pharis and Ferrell, 1966). In September 1996, RWC values of necrotic and chlorotic foliage within 50 m of the site of gas release were close to this threshold, and may have led to subsequent defoliation. Moreover, lower RWC could have led to stomatal closure earlier during the day, and thus, lowered photosynthetic rates (Running, 1976; Pallardy, Pereira and Parker, 1991). Finally, all measurements for cuticular water loss and foliar water content were taken in the lower canopy. Since the chlorine gas cloud tended to stay close to the ground, the relationship of my findings to whole tree water balance are uncertain.

Chlorophyll fluorescence

In late March 1997, there were no differences in F_v/F_m ratios, and photosynthesis was still down regulated, as is common for conifers during winter (Havranek and Tranquillini, 1995). Similarly, in May 1997 there were no differences in F_v/F_m ratios of directly exposed –1 yts needles. There were some differences in F_v/F_m ratios of 0 yts foliage of both species. However, these could not be conclusively attributed to chlorine gas exposure and may have been caused by natural variation between study sites. These data suggest that photosynthetic efficiency had recovered since the previous summer,

when both –1 yts and 0 yts foliage on exposed trees had decreased F_v/F_m ratios (Chapter 2). Irreversible decreases of F_v/F_m ratios and photosynthesis have been reported for *Phaseolus vulgaris* after a one-time application of acid mist of pH<2.0 (Velikova et al., 1997; Velikova, Tsonev and Yordanov, 1999).

Growth

It has been reported that conifers allocate carbon to different tissues according to the following prioritization (Figure 3.8): 1) maintenance respiration, 2) growth respiration, 3) leaf growth and storage, 4) root growth and storage, 5) stem growth and storage, 6) protective chemical compounds, and 7) reproduction (e.g., Running and Gower, 1991; Weinstein, Beloin and Yanai, 1991; Dewar, Ludlow and Dougherty, 1994; Barnes et al., 1998). According to Mooney and Winner (1991) photosynthesis is a high priority in carbon allocation in evergreen trees. Others have suggested that carbon is allocated to the most limiting resource. For example, decreased light availability might lead to increased foliage production and shoot growth, whereas nutrient and drought stress to would precipitate allocation to increased root growth (Mooney and Winner, 1991; Luxmore et al., 1995). Based on this carbon allocation scheme (Figure 3.8), I predicted that chlorine gas exposure, and the subsequent defoliation, would have the greatest effect on tissues that tend to be lower priority for allocation.

Needle length, SLA, and annual shoot increment growth were not affected by chlorine gas exposure (Table 3.6). Although there were differences between study sites in these parameters, these differences appeared to be site specific and not related to chlorine gas exposure. There was a trend towards increased bud production at sites 0.2 and 0.8 km

downwind of the site of gas release (Table 3.6), suggesting higher investment in foliage over the two growing seasons following chlorine exposure. Foliar biomass of trees exposed to chlorine gas deceased considerably over the first growing season. Necrotic needles within ~50 m of the release, as well as some of the chlorotic foliages up to 0.2 km downwind site, defoliated during the summer following exposure. Reduced needle longevity also was observed for 0 yts foliage for both species (Figure 3.5). The downwind distance over which reduced needle longevity was observed for Ponderosa pine increased during the years following exposure, suggesting that both directly exposed and newly flushed needles on exposed trees were more susceptibility to environmental stress factors. This was particularly evident during an extended drought period in the summer of 1996, agreeing with data showing premature leaf senescence can be induced by decreased RWC (Pharis and Ferrell, 1966; Pallardy et al., 1991).

Both Ponderosa pine and Douglas fir had lower annual stem increment growth over at least three growing seasons following chlorine gas exposure (Figure 3.6). For Ponderosa pine these effects were significant up to 0.2 km downwind of the release, and for Douglas fir up to 0.8 km downwind. Moreover, other exposed sites up to 1.6 km downwind tended to have lower core increment growth the first growing season after chlorine exposure, and these effects subsided in the second growing season. Interestingly, stem increment growth of the deciduous western larch was not affected (Schreuder, unpublished data), suggesting that the growth decline observed in the evergreen conifers was related to direct exposure of foliage to chlorine gas and subsequent defoliation. Defoliation has been reported to decrease stem increment growth of *Pseudotsuga menziesii* and *Abies grandis* (Nichols, 1988), *Pinus ponderosa* (Miller and Wagner,

1989), *Pinus contorta* (Schoettle, 1990), *Picea abies* (Christiansen and Fjone, 1993), and *Pinus resinosa* (Clancy, Wagner and Reich, 1995). Possible contributing factors to the decrease of stem increment growth in this study include lower photosynthetic biomass, lower photosynthetic efficiency in the first growing season (Chapter 2), and increased susceptibility to drought stress.

Nichols (1988) described annual stem increment growth of *Pseudotsuga menziesii* and *Abies grandis* as a function of stem increment growth and percent foliage retained in the previous growing season. When this model was applied in the current study, predicted results agreed well with measured data for both Douglas fir (r^2=0.86, P<0.005), as well as Ponderosa pine (r^2=0.80, P<0.001). Based on this model, stem increment growth of Douglas fir was expected to return to normal 5 to 6 years after gas exposure and after 4 to 7 years for Ponderosa pine, depending on the severity of defoliation. These predictions were similar to predicted recovery times using the Forest BGC model (Running and Gower, 1991), i.e., 4 years for full recovery for Ponderosa pine and 7 years Douglas fir. Results also were consistent with observed recovery times for stem growth of defoliated conifer species, including Douglas fir (2 to 5 years; Alfaro and MacDonald, 1988; Maher and Shepherd, 1992).

Douglas fir and Ponderosa pine responded differently to chlorine gas exposure. Stem increment growth of Ponderosa pine and Douglas fir were affected up to 0.2 and 0.8 km downwind respectively (Figure 3.6). Moreover, needle defoliation was lower for Ponderosa pine than for Douglas fir, and the replacement rate of defoliated needle age classes was slower for Douglas fir. What factors may explain these differences? Ponderosa pine tends to be more drought-tolerant than Douglas fir, and has a deeper root

system. Moreover, Ponderosa pine replaces its foliar biomass at a faster rate (~4 years) compared to Douglas fir (~9 needle age classes on healthy trees). Current-year foliage of Ponderosa pine tends to contribute proportionally more (~70 %) to whole tree photosynthesis compared to Douglas fir (~36 %; Chabot and Hicks, 1982). Thus, one would expect faster recovery for Ponderosa pine compared to Douglas fir. This was observed for both stem growth as well as replacement of lost needle age classes. Furthermore, exposed Ponderosa pine tended to keep the youngest exposed needle age class (1-year old), where as Douglas fir dropped most exposed needles, including 1-year old needles. Also, defoliation was observed for Douglas fir on much larger area compared to Ponderosa pine (up to 10 km downwind from the release for Douglas fir and 0.5 km for Ponderosa pine, Figure 3.5). Thus, Douglas fir appeared more sensitive to acute chlorine gas exposure than Ponderosa pine. Although the 1-year old needle age classes of Douglas fir and Ponderosa pine contribute about equally to whole tree photosynthesis (~25 %; e.g., Chabot and Hicks, 1982; Rundel and Yoder, 1998), Ponderosa pine generally retained this age class, probably at a higher respiration cost, whereas Douglas fir dropped this age class of needles. This pattern may be explained by higher drought tolerance of Ponderosa pine, and higher relative photosynthetic gain from the current-year needle age class (Chabot and Hicks, 1982; Rundel and Yoder, 1998). Older Douglas fir needles contribute very little to whole tree photosynthesis (Chabot and Hicks, 1982), representing a cost rather than a benefit for a tree. This may be, especially true when needles are severely damaged, e.g., by chlorine gas exposure.

Repair of exposed tissues in Ponderosa pine may have led to higher respiration costs. These costs are added to those incurred because Ponderosa pine supports

proportionally more sapwood than Douglas fir (e.g., Margolis et al., 1988). Increased respiration costs for chlorine exposed trees may explain observations of lower cone production for Douglas fir and Ponderosa pine at exposed sites up to 0.5 and 1.5 km downwind of the gas release compared to control sites. Cone production is a considerable carbon sink of coniferous trees (Dewar et al., 1994; Gower, Isebrands and Sheriff, 1995). Allocation to cones has been reported to lead to decreased stem increment growth (Luxmoore et al., 1995). Sidhu and Stanforth (1986) reported decreased numbers of fertile trees, cones, and seeds per cone for *Abies balsamea*, *Picea mariana* and *Larix larcina* after exposure to fluorides. Thus, the range of possible causal factors for lower cone production in exposed trees includes toxicity of chloride, drought as well as defoliation. Because reproduction is a carbon sink with a lower allocation priority in these long-lived trees (Figure 3.8), allocation to foliar biomass may be a higher priority than reproduction.

Chlorine gas exposure, and the subsequent defoliation, did not affect needle length and shoot growth or bud growth. However, exposure did correlate with lower annual stem increment growth and reproduction. Thus, higher priority carbon sinks, i.e., needles, buds and branches, were not affected by chlorine gas exposure, whereas allocation to lower priority sinks, i.e., stem increment growth and reproduction, did seem to be negatively impacted (Figure 3.8). These observations also agree well with increased carbon allocation to shoots (Temple, 1988; Reich et al., 1993; Clancy et al., 1995), and decreased stem increment growth (Christiansen and Fjone, 1993) in defoliated conifers.

Tree mortality

Tree mortality of Ponderosa pine was not affected during the three years following chlorine gas exposure. However, mortality of Douglas fir was significantly higher at the two sites nearest to the release, and generally higher at the other exposed sites compared to control sites (Figure 3.7). Tree mortality within 30-50 m of the release most likely occurred within a few months after chlorine gas exposure. Douglas fir mortality was highest in the younger tree age classes, with ~90 % of the mortality occurring in trees with a DBH <6 cm. This is in agreement with reports that tree mortality after defoliation is highest among small, suppressed Douglas fir trees (Alfaro et al., 1982), which have lower carbon reserves than larger trees (Webb, 1981). Although smaller trees may come back more vigorously after partial defoliation (Clancy et al., 1995), defoliation exceeding ~80 % increased mortality of *Picea balsamea* (Margolis et al.; 1988). Model results of the Forest BGC model (Running and Gower, 1991) indicated that conifer defoliation exceeding ~85 % would lead to increased tree mortality due to a negative carbon balance. A conservative defoliation estimate, based on visual injury data two months after exposure within 50 m of the gas release, was ~ 75 % for Ponderosa pine and ~89 % for Douglas fir; thus some mortality at this site was expected, especially for Douglas fir.

CONCLUSION

These data suggest that acute exposure to chlorine gas not only leads to acute visible injury, but also to longer-term physiological and growth effects of conifers. Chlorine gas exposure may have led to increased susceptibility to drought stress, decreased photosynthetic biomass, and decreased stem growth and reproduction.

Moreover, increased tree mortality was observed, especially close to the site of gas release. Although effects on drought susceptibility lasted up to 0.5 to 1 year, effects on foliar biomass and growth can be expected to persist 4 to 7 years after chlorine gas exposure. Effects of exposure to chlorine gas were highly species specific. *P. menziesii* was more susceptible to defoliation than *P. ponderosa*, and defoliation patterns of *P. menziesii* were a useful indicator to of past presence of chlorine gas. Since chlorine gas tended to increase drought susceptibility of directly exposed foliage, tree responses may differ between dry and moist sites, as well as between dry and moist climates and growing seasons. Growth responses to chlorine gas exposure resembled those of defoliation due to severe drought or insect damage. Therefore, when extrapolating results from this study to deciduous tree species, known responses of trees to defoliation may help to assess what type of long-term responses to expect after chlorine gas exposure. Factors such drought tolerance, site characteristics, climate, and timing of exposure need to be taken into account for such an assessment.

Table 3.1: Physical description of study sites. Data shown are elevation, soil type, and habitat type (Pfister et al., 1977). *Pinus ponderosa* was the second most abundant tree species at all study sites. All sites were level benches in close vicinity to the Clark Fork River, western Montana.

Site and downwind distance	Elevation (m asl)	Soil type	Habitat type
Upwind Control (~4 km)	876	Coarse loam	*Pseudotsuga menziesii/ Arctostaphylos uva-ursi*
50 m downwind	897	Fine loam	*Pseudotsuga menziesii/ Physocarpus malvaceus*
0.2 km downwind	898	Fine loam	*Pseudotsuga menziesii/ Physocarpus malvaceus*
0.8 km downwind	900	Fine loam	*Pseudotsuga menziesii/ Physocarpus malvaceus*
1.5 km downwind	902	Fine loam	*Pseudotsuga menziesii/ Symphorica albus*
Downwind control (~65 km)	976	Fine loam	*Pseudotsuga menziesii/ Physocarpus malvaceus*

Table 3.2: Foliar injury in March 1997 (n=5 trees; 15 replicates per tree) and March 1998 (n=5 trees; 10 replicates per tree) for Ponderosa pine and Douglas fir (in parentheses years to spill). Values shown are mean foliar injury scores. Categories are : 1) 100 % green; 2) 5 to 25 % chlorotic; 3) >25 % chlorotic; 4) 5 to 25 % necrotic; and, 5) >25 % necrotic. The two control sites are indicated as CU, upwind control, and CD, downwind control. Needle age classes that were not (longer) present on the tree are indicates as "Absent". Sites with higher visual injury compared to all other sites are shown as "*", and apply within each needle age class, species, and sampling date (Chi-square test, P<0.05).

Site / date	Ponderosa pine, needle age class			Douglas fir, needle age class		
	1995 (-1)	1996 (0)	1997 (+1)	1995 (-1)	1996 (0)	1997 (+1)
March '97						
CU	1.2	1.1	Absent	1.6	1.2	Absent
CD	1.8	1.6	Absent	1.6	1.5	Absent
0.8 km	1.4	1.5	Absent	1.7	1.4	Absent
0.2 km	4.2 *	2.3	Absent	2.0	1.6	Absent
50 m	Defoliated	3.9 *	Absent	Defoliated	1.7	Absent
March '98						
CU	1.4	1.3	1.1	1.4	1.4	1.6
CD	1.8	2.2	1.4	1.4	1.4	1.7
1.6 km	2.1	1.7	1.4	1.9	1.4	1.2
0.8 km	1.1	1.3	1.1	2.5	1.8	1.6
50 m	Defoliated	3.1	2.0	Defoliated	1.8	1.2

Table 3.3: Droplet contact angle (degrees, n=10 trees, 2 replicates per tree) for Ponderosa pine and Douglas fir for needle age classes 1995 (-1 yts), 1996 (0 yts), 1997 (+1 yts) and 1998 (+2 yts), measured in August 1998. Letters indicate statistically significant differences within each species and needle age class (nested one-way ANOVA; compare in columns). Differences between needle age classes combined for all sites are shown in the bottom of the table. Values in parentheses indicate one SE.

Species + distance	1995 (-1)	1996 (0)	1997 (+1)	1998 (+2)
Ponderosa pine				
CU	46 (3) a	44 (4)	57 (4)	75 (4) a
CD	51 (4) a	43 (3)	56 (3)	71 (3) a,b
0.8 km downwind	33 (3) b	47 (3)	56 (4)	80 (3) a,b
50 m downwind	Absent	52 (3)	60 (4)	80 (3) b
ANOVA	$F_{3,77}$=11.66, ***	$F_{3,77}$=2.85, *	$F_{3,77}$=0.47, n.s.	$F_{3,77}$=3.15, *
Douglas fir				
Control, upwind	Not measured	66 (4) a	64 (4) a	78 (4)
Control, downwind	Not measured	62 (3) a,b	62 (3) a	78 (4)
0.8 km downwind	Absent	54 (4) b	61 (4) a	73 (3)
50 m downwind	Absent	63 (4) a,b	75 (4) b	81 (4)
ANOVA		$F_{3,77}$=3.87, *	$F_{3,77}$=6.20, ***	$F_{3,77}$=1.24, n.s.
All categories				
Ponderosa pine	43 (2) a	47 (2) a	57 (2) b	77 (2) c
Douglas fir		61 (2) a	66 (2) a	77 (2) b

* $P<0.05$

*** $P<0.001$

n.s not significant

Table 3.4: Total water content (n=4 trees) and relative water content (n=4 trees) of 1995 (-1 yts) and 1996 (0 yts) needles, measured in September 1996. Letters indicate statistically significant differences within each species and needle age class (compare within columns, one-way ANOVA). Values in parentheses indicate one SE.

Species + category	Total water content, gr/gr		Relative water content, %	
	1995 (-1)	1996 (0)	1995 (-1)	1996 (0)
Ponderosa pine				
Control, downwind	1.10 (0.03) a	1.30 (0.06) a	85 (2) a	86 (0) a,c
0.8 km downwind	1.06 (0.02) a	1.21 (0.01) b	81 (1) a	82 (1) b
0.2 km downwind	0.89 (0.04) a	1.30 (0.02) a	79 (0) a	89 (0) a
50 m downwind	0.24(0.03) b	1.42 (0.03) c	52 (6) b	85 (1) b,c
ANOVA	$F_{3,13}$=49.23, ***	$F_{3,13}$=26.62, ***	$F_{3,13}$=12.70, ***	$F_{3,13}$=11.68, ***
Douglas fir				
Control, downwind	1.26 (0.02) a	1.52 (0.02) a	89 (1) a	93 (1)
0.8 km downwind	0.97 (0.03) b	1.32 (0.02) b	88 (1) a	92 (4)
0.2 km downwind	0.98 (0.02) b	1.22 (0.01) b	95 (1) a	91 (1)
50 m downwind	0.17 (0) c	1.76 (0.04) c	69 (10) b	89 (1)
ANOVA	$F_{3,13}$=279.9, ***	$F_{3,13}$=49.01, ***	$F_{3,13}$=8.54, **	$F_{3,13}$=1.38, n.s.

** P<0.01
*** P<0.001
n.s. not significant.

Table 3.5: F_v/F_m ratios (n=5 trees; 2 replicates per tree) of 1995 (-1 yts), and 1996 (0 yts) foliage, measured in May 1997. Letters indicate statistically significant differences within each species and needle age class (t-test/nested one-way ANOVA; compare within columns). Values in parentheses indicate one SE. Needle age classes that were no longer alive are indicated as "Absent".

Category	F_v/F_m, Ponderosa pine		F_v/F_m, Douglas fir	
	1995 (-1)	1996 (0)	1995 (-1)	1996 (0)
Control, upwind	0.810 (0.010)	0.812 (0.006) a	0.792 (0.020)	0.796 (0.015) a
0.8 km downwind	0.809 (0.011)	0.778 (0.013) b	0.794 (0.018)	0.805 (0.007) a
0.2 km downwind	Absent	0.807 (0.011) a/b	Absent	0.736 (0.032) b
50 m downwind	Absent	0.784 (0.017) a/b	Absent	0.778 (0.020) a/b
t-test / ANOVA	T_{18}=0.03, n.s.	$F_{3,37}$=3.76, *	T_{18}=-0.11, n.s.	$F_{3,37}$=4.33, *

* $P<0.05$

n.s. not significant

Table 3.6: Summary of growth measurements over three growing seasons following chlorine gas exposure (values ± 1 SE; in parentheses years to spill). Data are shown for downwind- and upwind control sites (respectively CD and CU; " combined " indicates CD and CU combined), and four sites downwind from the release site. "r" indicates the number of replicates per tree). Letters indicate statistically significant differences within each species and needle age class ($P<0.001$, nested one-way ANOVA or Kruskal-Wallis; annual shoot increment growth was analyzed using a repeated measures analysis). Needle age classes that were no longer alive are indicated as "Absent" and needle age classes that were not measured as "n.m."

Species and site	Needle length (cm)			Annual shoot increment (%)			Buds count (# per branch), Fall		
	1995 (-1)	1996 (0)	1997 (+1)	'95/'94 (-1)	'96/'94 (0)	'97/'94 (+1)	1996 (0)	1997 (+1)	1998 (+2)
Ponderosa pine	n=35 (r =3)	n=35 (r =3)	n=30 (r =2)	n=15 (r =2)	n=15 (r =2)	n=5 (r =2)	n=27 (r = 2)	n=10 (r =2)	n=15 (r =2)
CU	combined	combined	combined	119±17 b	134±14 b	140±22	1.3±0.1 a	1.1±0.1 a	1.1±0.1
CD	10.5±0.2 a	12.1±0.2 a	14.1±0.3 a	73±3 a	63±6 a,c	n.m.	1.2±0.1 a	n.m.	n.m.
1.5 km	15.6±0.7 b	15.7±0.5 b	15.8±0.4 b	99±17 a,b	85±14 a	n.m.	n.m.	n.m.	1.0±0.1
0.8 km	14.6±0.4 b,c	15.0±0.4 b,c	16.1±0.3 b	69±6 a	48±7 c	105±17	2.0±0.1 b	1.7±0.3 a,b	1.2±0.1
0.2 km	14.1±0.7 c	16.0±0.9 b	n.m.	80±11a	73±10 a,c	n.m	2.3±0.1 b	2.2±0.3 b	1.1±0.1
50 m	Absent	14.1±0.7 c	16.1±0.3 b	95±7 a,b	63±7 a,c	104±15	1.2±0.1 a	0.9±0.2 a	1.2±0.1
Douglas fir									
CU	combined	combined	combined	90±4 b	136±13 b	116±17	2.9±0.2 a	1.6±0.4 a	2.2±0.2
CD	2.0±0.05 a	2.2±0.04 a	2.1±0.06	69±4 a	75±6 a	n.m	2.3±0.2 a	n.m.	n.m.
1.5 km	2.0±0.09 a,b	2.1±0.07 a	2.2±0.12	77±6 a	83±7 a	n.m	n.m.	n.m.	1.6±0.2
0.8 km	1.9±0.05 b	2.4±0.07 b	2.1±0.04	64±4 a	72±7 a,c	124±28	5.5±0.3 b	1.1±0.3 a	3.4±0.4
0.2 km	2.1±0.05 a	2.0±0.11 a	n.m.	61±4 a	49±4 c	112±52	3.1±0.2 a	3.4±0.5 b	3.0±0.3
50 m	Absent	2.5±0.09 b	2.2±0.08	91±11 b	83±11a	162±50	2.6±0.2 a	1.2±0.4 a	2.5±0.2

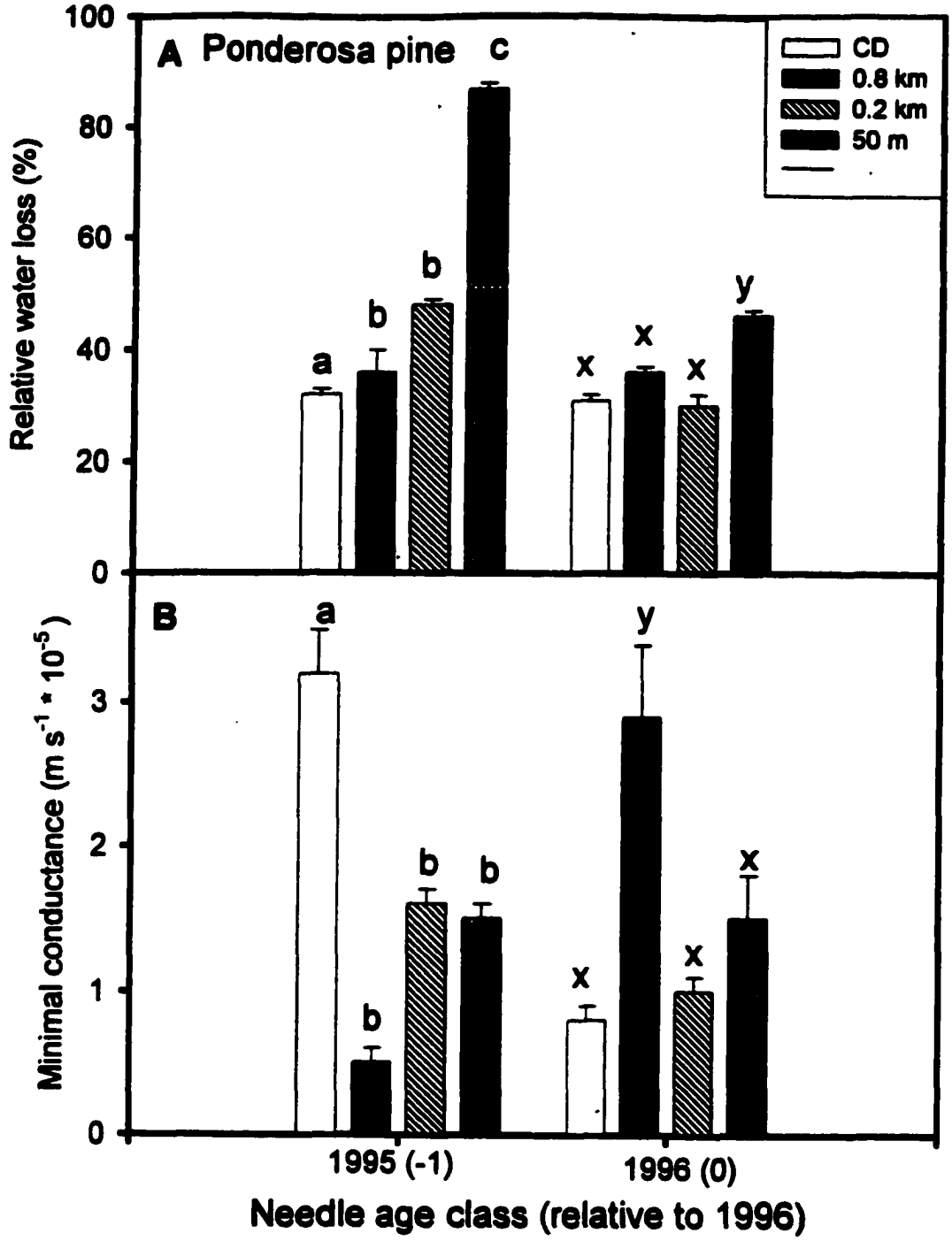


Figure 3.1: Relative water loss (panel A) and $G_{min,H2Ov}$ (panel B) of Ponderosa pine foliage in September 1996 (in parentheses years to spill). Sites shown are the downwind control (CD, white bar), 0.8 km downwind (solid bar), 0.2 km downwind (hatched bar), and 50 m downwind (double hatched bar). Letters indicate statistically significant differences within each species and needle age class (one-way ANOVA, $P<0.001$). Error bars represent 1 SE.

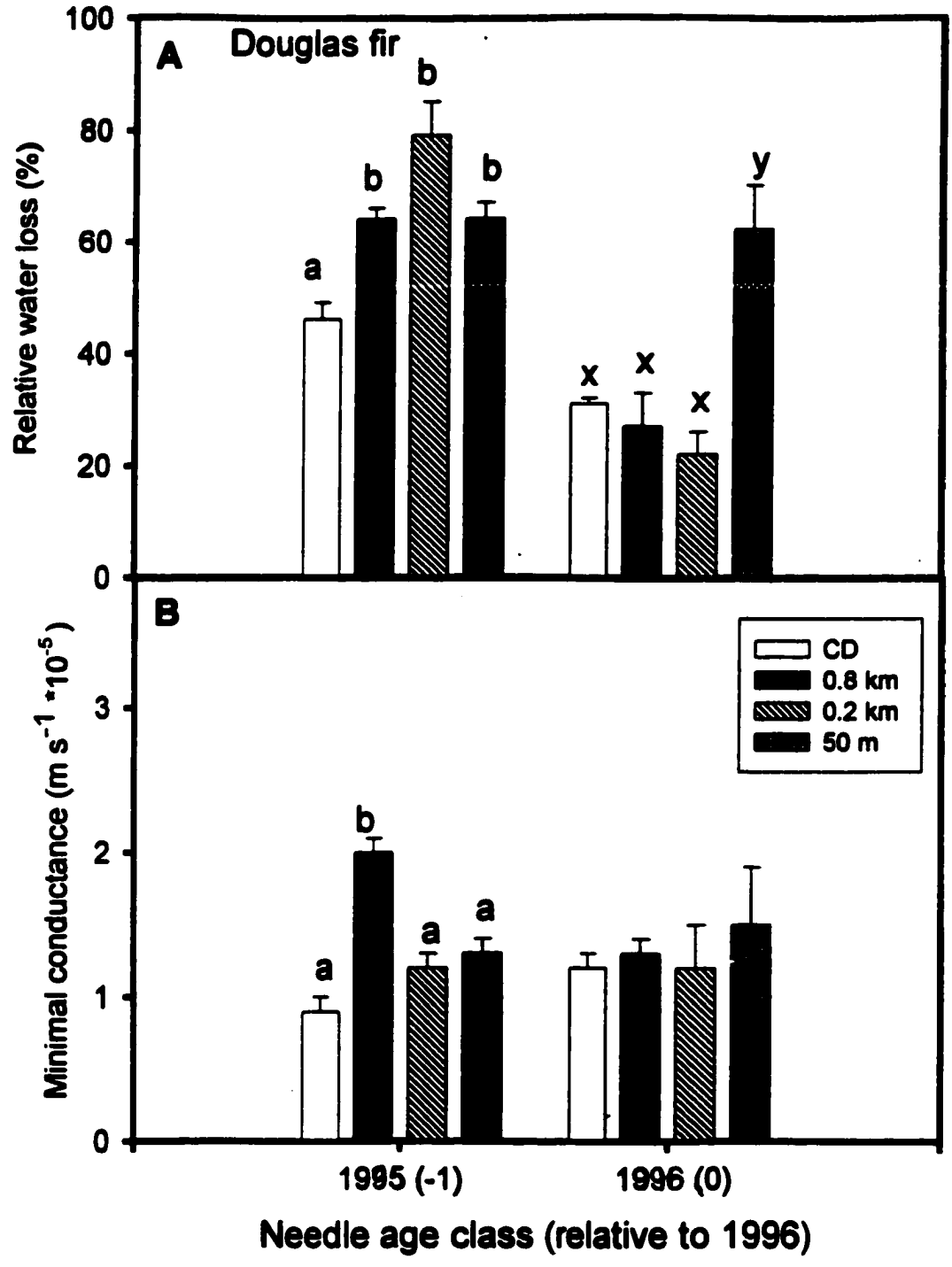


Figure 3.2: Relative water loss (panel A) and $G_{min.H2Ov}$ (panel B) of Douglas fir foliage in September 1996 (in parentheses years to spill). Sites shown are the downwind control (CD, white bar), 0.8 km downwind (solid bar), 0.2 km downwind (hatched bar), and 50 m downwind (double hatched bar). Letters indicate statistically significant differences within each species and needle age class (one-way ANOVA, $P<0.001$). Error bars represent 1 SE.

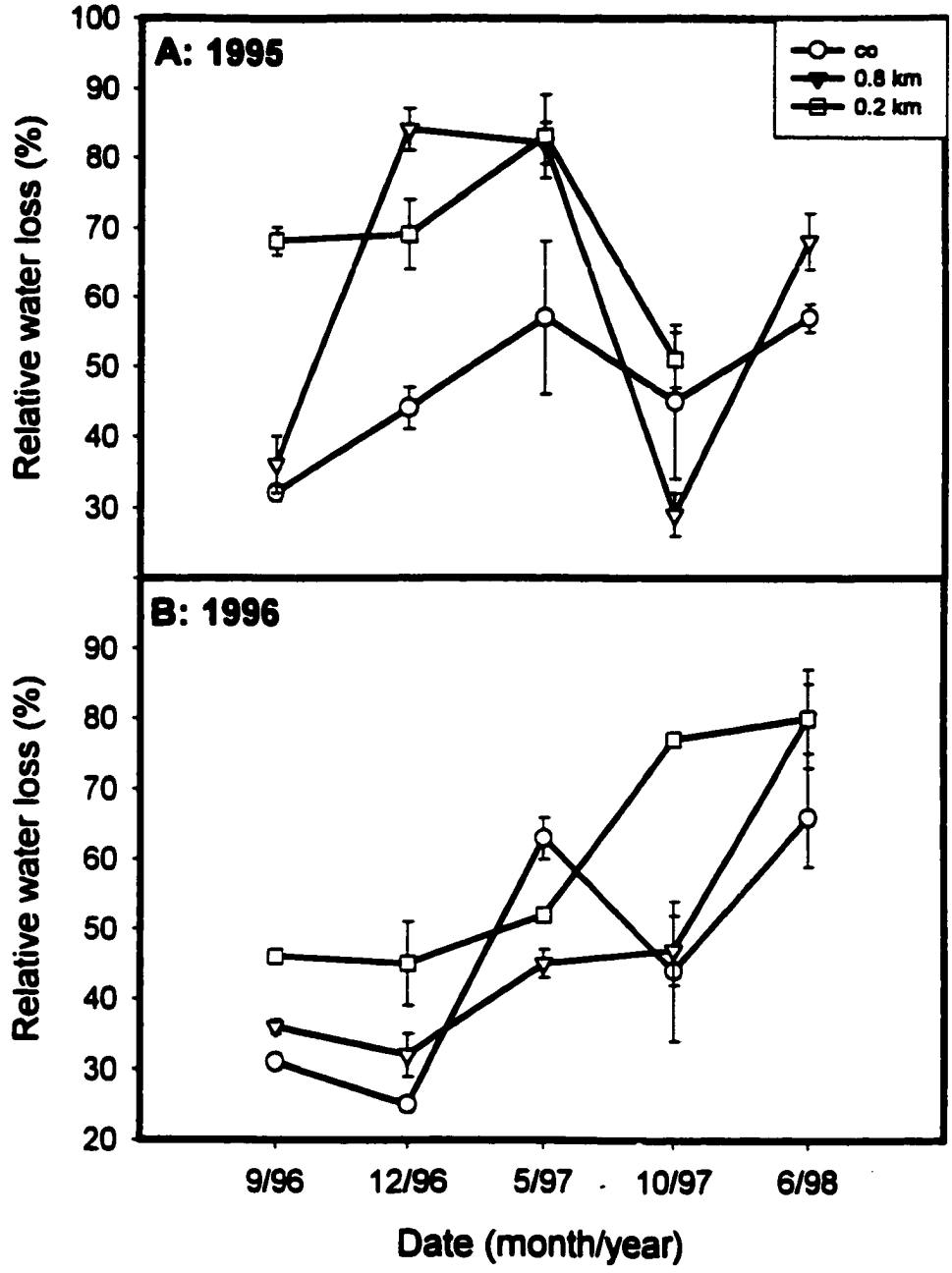


Figure 3.3: Relative water loss of Ponderosa pine foliage over time for 1995 (foliage (-1 yts, panel A) and 1996 foliage (0 yts, panel B). Sites shown are the combined control sites (open circles), 0.8 km downwind (open triangles), and 0.2 km downwind (open squares). "*" indicate sites that are significantly different from the control sites (one-way ANOVA, $P<0.001$; repeated factor: date, $n=5$). Error bars represent 1 SE.

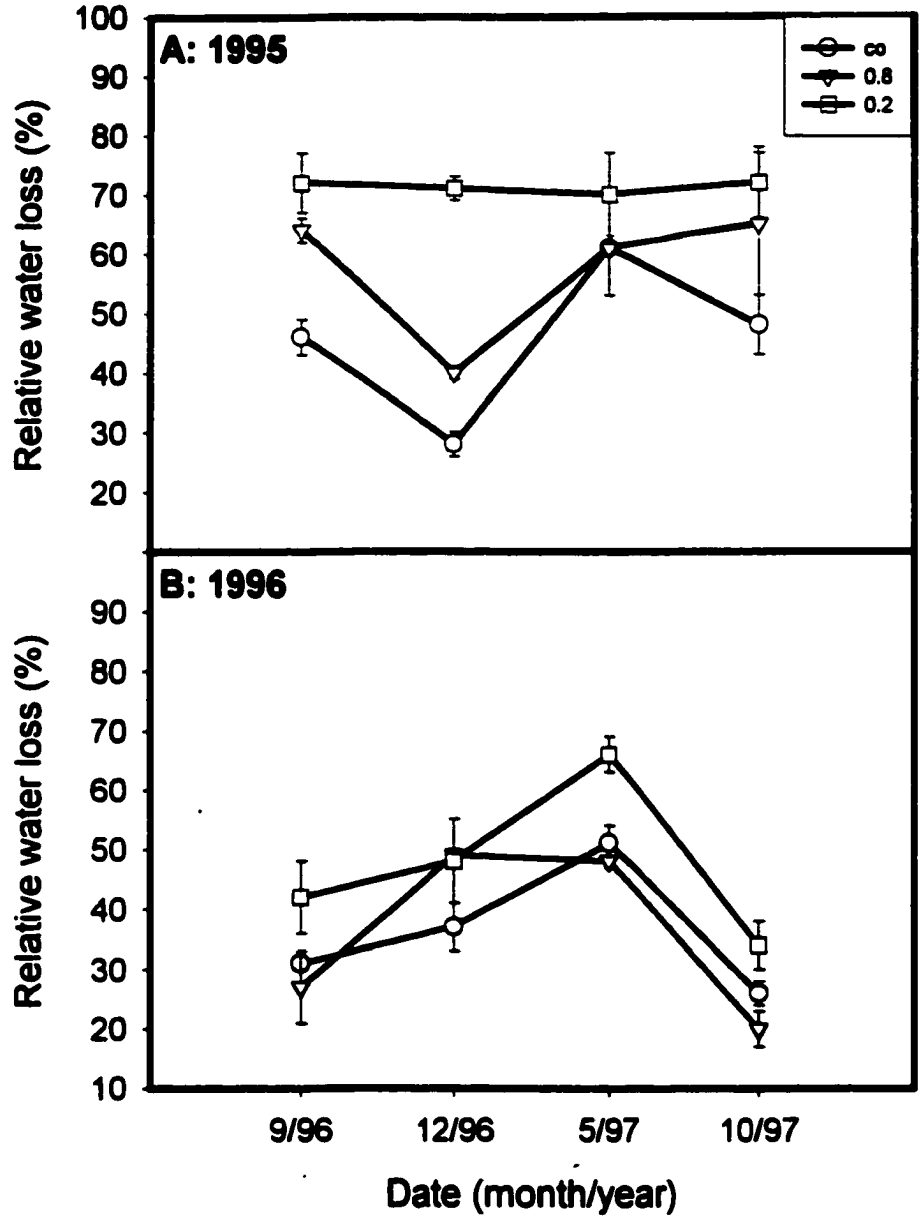


Figure 3.4: Relative water loss of Douglas fir foliage over time for 1995 (foliage (-1 yts, panel A) and 1996 foliage (0 yts, panel B). Sites shown are the combined control sites (open circles), 0.8 km downwind (open triangles), and 0.2 km downwind (open squares). "*" indicate sites that are significantly different from the control sites (one-way ANOVA, $P<0.001$; repeated factor: date, $n=5$). Error bars represent 1 SE.

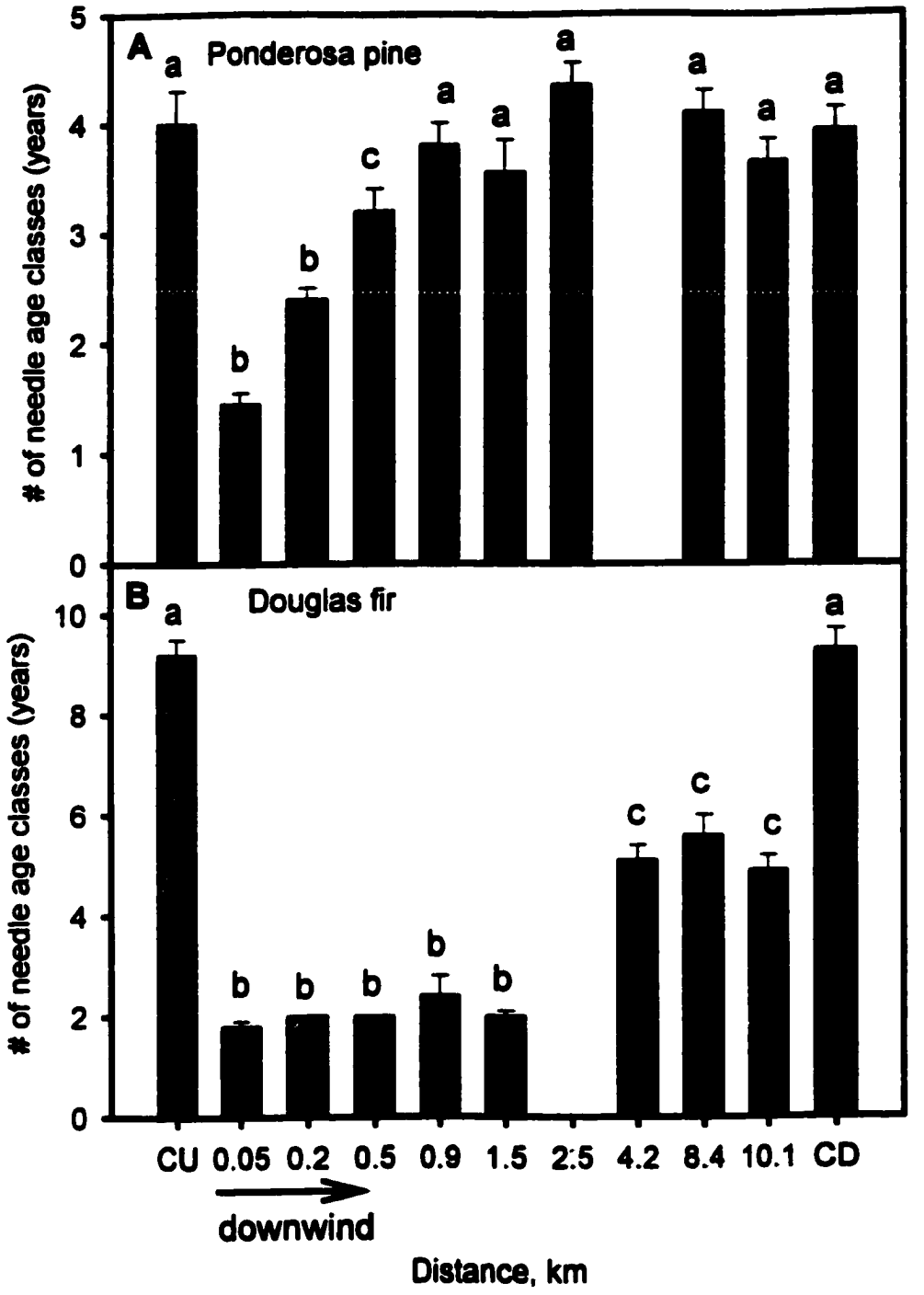


Figure 3.5: Needle age classes present on Ponderosa pine (panel A, n=10) and Douglas fir (panel B, n=10) over a 10 km gradient downwind from the site of gas release, measured in spring 1998 (+2 yts). The two control sites are the upwind control (CU) and the downwind control (CD). Letters indicate statistically significant differences within each species and needle age class (Kruskal-Wallis with Tukey Post-hoc test, P<0.001).

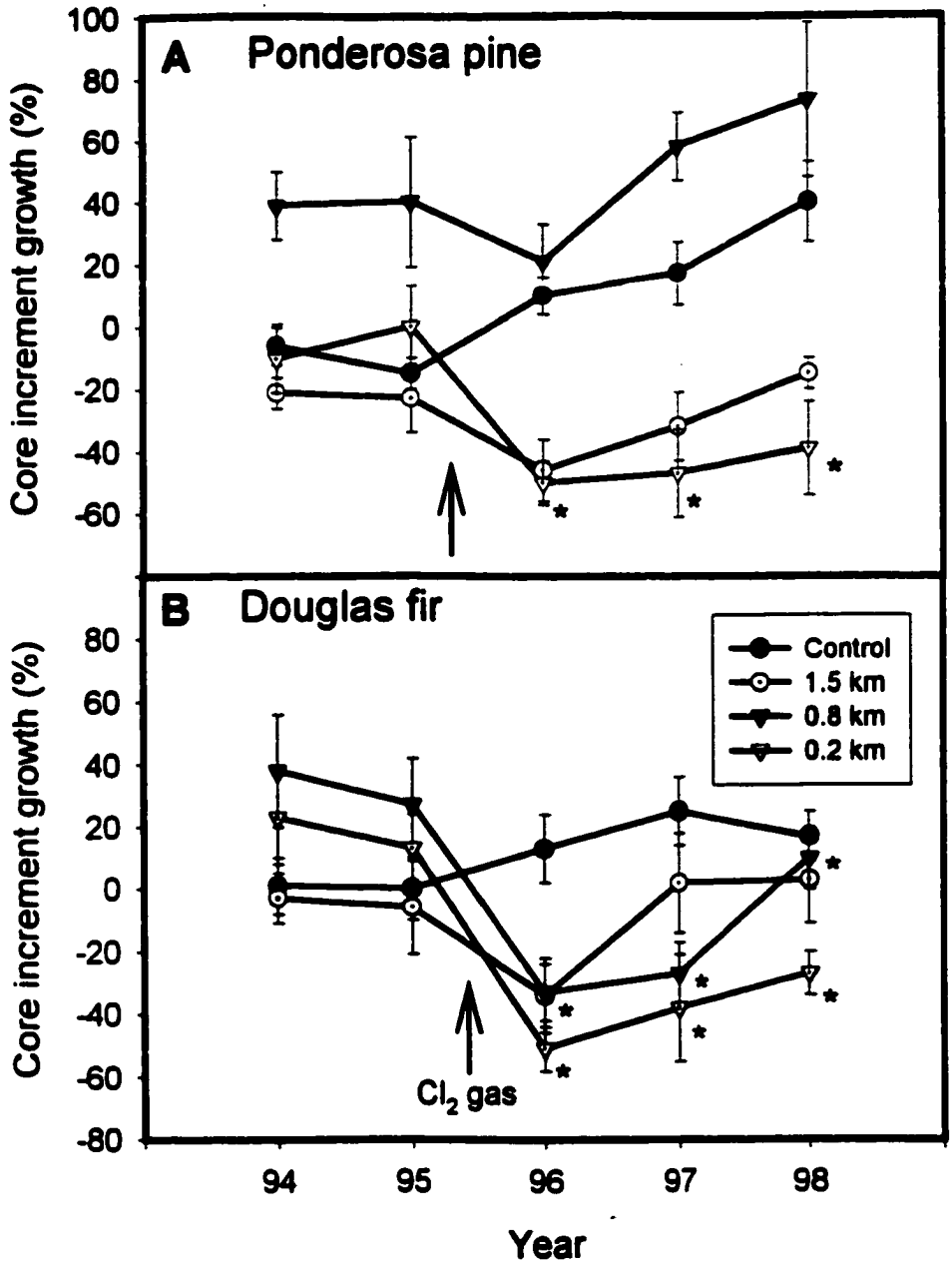


Figure 3.6: Core increment growth of Ponderosa pine (panel A) and Douglas fir (panel B). Shown are the control (CD and CU combined, solid circles), and sites 1.5 km downwind (open circles), 0.8 km downwind (solid triangles), and 50 m downwind (open triangles). * indicates years within a site that are statistically significant different compared to before chlorine gas exposure (n=10 for 1995 and 1996; n=5 for 1997 and 1998; Nested one-way ANOVA, P<0.001; repeated factor: year, n=5).

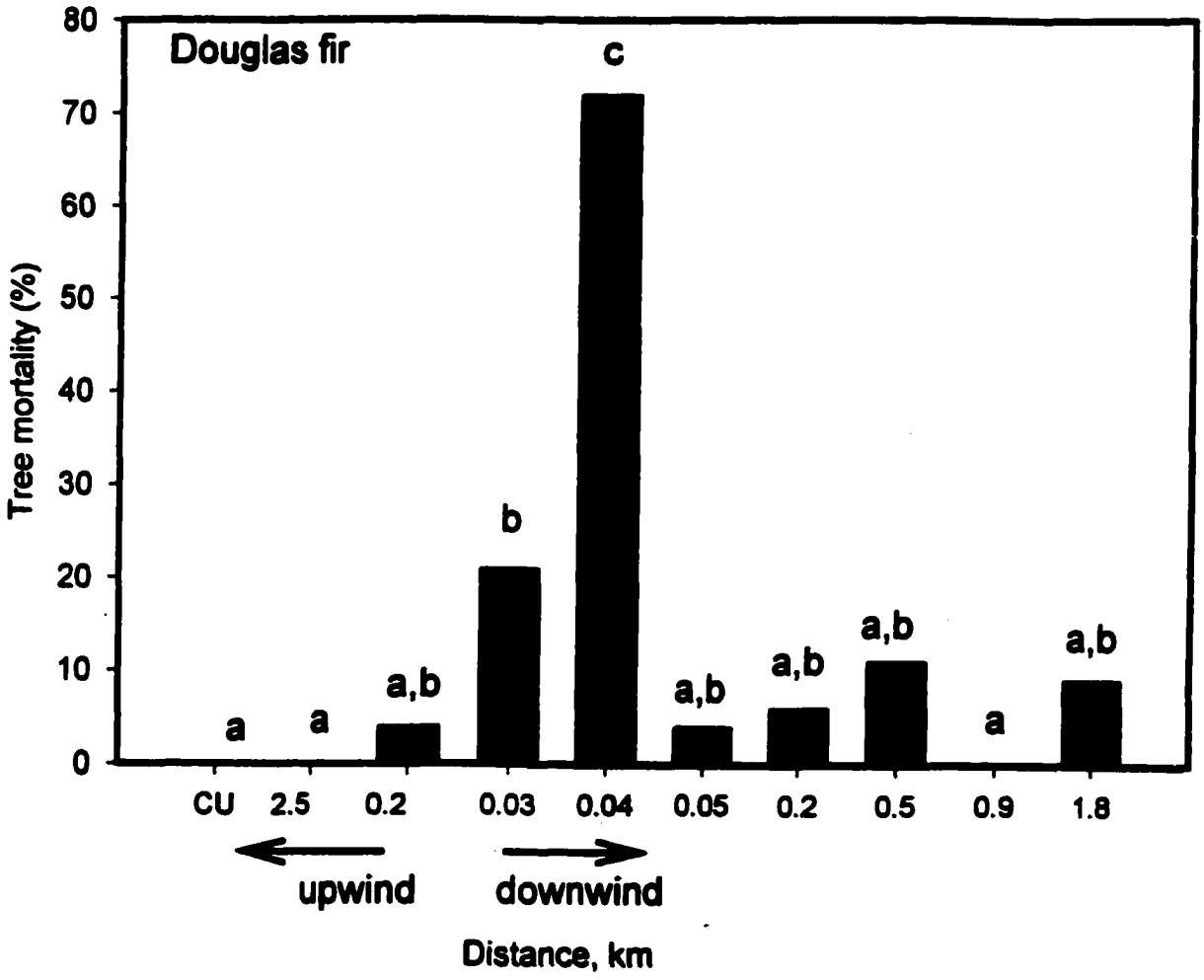


Figure 3.7: Tree mortality for Douglas fir (n=25) over a 1.8 km downwind gradient from the site of gas release and at the upwind control site (CU), measured in May 1999 (+3 yts). Letters indicate statistically significant differences within each needle age class (Fisher's exact test, P<0.001).

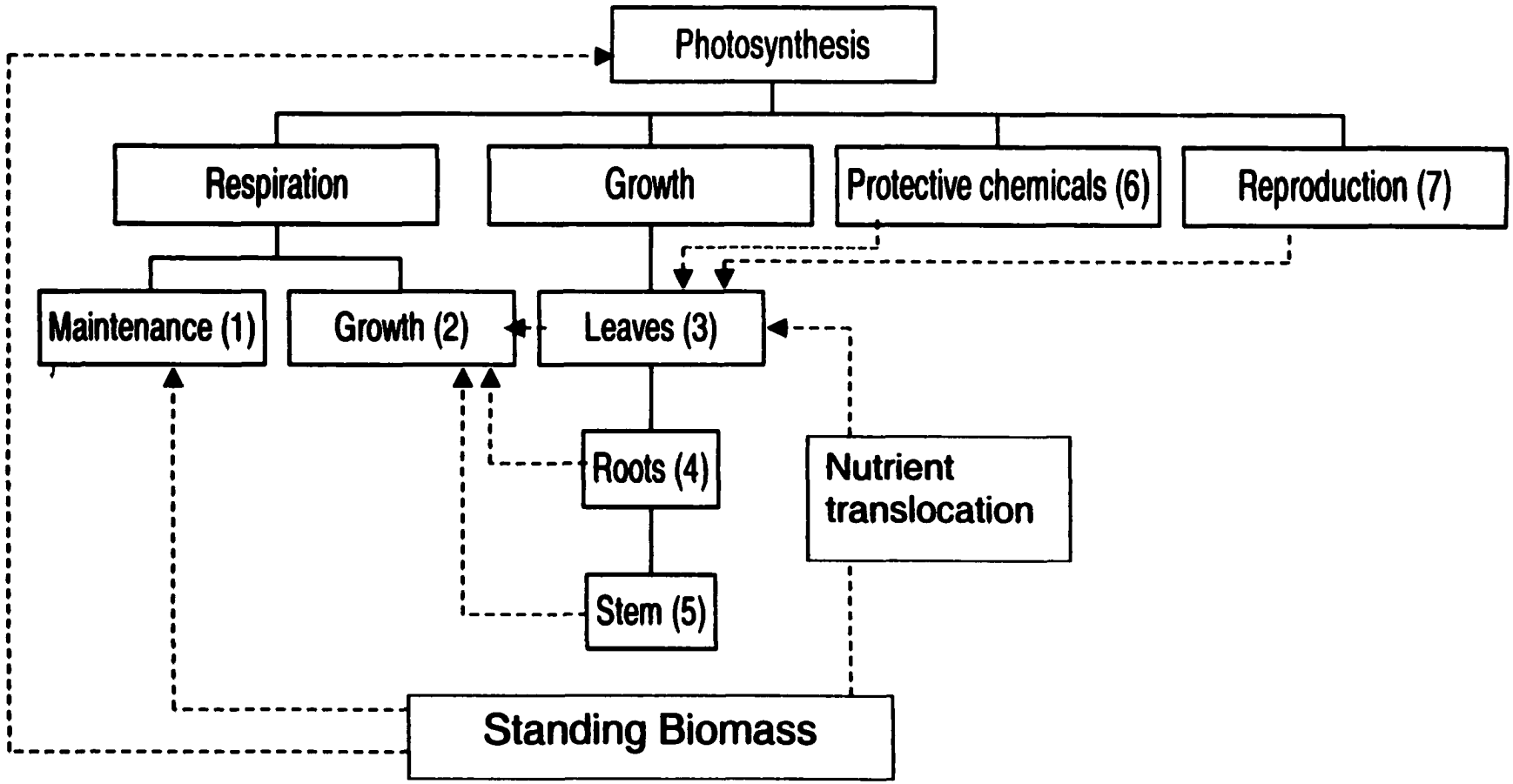


Figure 3.8: Schematic overview of carbon allocation processes in trees. Solid lines – carbon investment; dashed bars – feedback mechanisms; numbers – priority in carbon allocation under normal growing conditions (modified from Running and Gower, 1991; Dewar et al., 1994; Barnes et al., 1998;).

CHAPTER 4

Effects of ozone exposure on leaf wettability, plant, water balance, and growth of Poplar and Douglas fir saplings

ABSTRACT

The effects of ozone exposure were studied for *Pseudotsuga menziesii* (Douglas fir) and two Poplar species, *Populus nigra L. cv. Brandaris* (ozone intolerant) and *Populus euramericana L. cv. Robusta* (ozone tolerant). Using fumigation chambers (T_{day} 20 ^{O}C, T_{night} 17 ^{O}C, RH 70-75 %, light period 16 h/d at ~ 180 µmol m^{-2} s^{-1}), 7 week old saplings were exposed to four different ozone regimes: control (~ 2 µg O_3 m^{-3}), urban ozone concentration (minimum 26 µg O_3 m^{-3} and maximum 82 µg O_3 m^{-3}), and montane ozone concentrations (minimum 61 µg O_3 m^{-3} and maximum 90 µg O_3 m^{-3}) for 6 wk total. Peak ozone concentrations were applied for 6 d (minimum 61 µg O_3 m^{-3} and maximum 335 µg O_3 m^{-3}). Droplet contact angles (CA) and droplet retention angles (RT) of *Populus* leaves decreased over time ($P<0.001$) for all treatments, but under the urban ozone exposure regime the decrease in CA was delayed by 2 to 4 wk ($P<0.001$). Ozone exposure led to increased relative water loss and minimal conductance to water vapor ($G_{min,H2Ov}$) for *P. euramericana* ($P<0.001$), but not *P. nigra*. Although urban ozone exposure increased leaf wettability in Douglas fir (as indicated by lower CA; $P<0.001$), neither water loss or growth of this species was not affected. Ozone exposure led to decreased production of photosynthetic biomass ($P<0.001$) in the Poplar species, especially in the montane ozone treatment, due to fewer new leaves produced ($P<0.001$) and premature leaf abscission ($P<0.001$). Leaf abscission was preceded by foliar injury symptoms characteristic for ozone exposure. Height growth of *Populus* species and Douglas fir were not affected by ozone exposure, although there was a trend toward fewer buds in the ozone treated Douglas fir trees. These results suggest that exposure of Poplar saplings to ozone concentrations common during the growing season can lead to increased water loss and decreased leaf growth for Poplar saplings. This may have consequences for tree vigor and health. However, responses were highly dependent on species and ozone treatment.

INTRODUCTION

Ozone is the most common component of photochemical smog (Cape, 1997). Ozone concentrations considered harmful to public health, crops and natural vegetation are exceeded frequently in many areas of the United States (Smith, 1990; Smith and Lefohn, 1991; Lefohn, 1992) and Europe (Stanners and Bourdeau, 1995; Scheel et al., 1997). And ozone is considered one of the factors contributing to the forest decline phenomenon observed in many regions in western and central Europe (McLaughlin, 1985; Maier-Maercker, 1999; Schmieden and Wild, 1995; Bussotti and Ferretti, 1998; Chappelka and Samuelson, 1998; Skelly et al., 1998). Severe ozone damage to forests has been reported in the western United States (Arbaugh et al., 1998) as well. However, our understanding of the mechanics of ozone damage to forests is still limited, and an unequivocal causal link has not been established yet (Schmieden and Wild, 1995; Chappelka and Samuelson, 1998).

Ozone exposure can result in foliar injury to crops (e.g., Rich, 1964; Hill, Heggestad and Linzon, 1970), deciduous trees (Pääkkonen et al., 1998a, 1998b; Yun and Laurence, 1999), and conifers (Salardino and Caroll, 1998), and has been reported to affect leaf cuticles of both deciduous and coniferous tree species via accelerated wax erosion (Barnes, Davison and Booth, 1988; Bytnerowicz and Turunen, 1994; Mankovska, Percy and Karnosky, 1999), and subsequent premature leaf abscission (Turunen and Huttunen, 1990). Kerfourn and Garrec (1992) reported changes in the chemical composition of cuticular waxes under the influence of ozone exposure, yet amount of cuticular waxes may not be affected (Barnes et al., 1990; Thornton et al., 1993; Cape, Sheppard and Binnie, 1995). For example, ozone exposure decreased cuticular thickness

of Yellow Poplar (McQuattie and Rebbeck, 1994). Ozone-induced changes of plant cuticles may lead to increased cuticular water loss (Delucia and Berlyn, 1983; Kerstiens and Lendzian, 1989b), especially due to changes in the structure of the cuticle (Turunen and Huttunen, 1990). Although cuticular conductance is negligible when stomata are open (e.g., Kerstiens and Lendzian, 1989a), increased cuticular transpiration may predispose trees to summer drought and winter frost conditions (DeLucia and Berlyn, 1984; Hadley and Smith, 1990). However, changes in cuticular properties do not necessarily lead to higher cuticular water loss (Svenningson, 1988; Cape et al., 1995). For example, there were no relationships between droplet contact angles, cuticular transpiration and stomatal injury in *Fagus sylvatica* from healthy and declining stands (Paoletti, Raddi and La Scala, 1998). In other studies, droplet contact angles on conifer foliage decreased with ozone exposure, suggesting that foliage became more wettable (Barnes and Brown, 1990; Barnes et al., 1990b; Turunen and Huttunen, 1990). Increased leaf wettability and permeability of cuticles may increase susceptibility of foliage to infection by fungal plant pathogens (Turunen and Huttunen, 1990).

Ozone exposure also can have adverse effects on physiological processes and growth of plants. Ozone exposure led to decreased photosynthesis in *Populus nigra* (Reichenauer et al., 1997), *Populus euramericana* (Nali et al. 1998), *Populus tremuloides* (Yun and Laurence, 1999), *Fagus sylvatica* (Mikkelsen, 1995) and *Betula pendula* (Pääkonen et al., 1998c and 1998d; Kytoviita et al., 1999). Lower photosynthetic rates also were observed in ozone-exposed *Pseudotsuga menziesii* (Van Hove and Bossen, 1994), *Picea abies* (Führer, Payer and Pfanz, 1993), *Pinus ponderosa* (Takemoto et al., 1997), and *Pinus armandi* (Shan et al., 1996). Lower rates of photosynthesis due to ozone

exposure have been ascribed to several mechanisms, including decreased stomatal conductance (Nali et al., 1998; Pääkkonen et al., 1998b and 1998c; Minnocci et al., 1999; Sober and Sild, 1999), damage to photosynthetic membranes (Mikkelsen, 1995; Reichenauer et al., 1997; Nali et al., 1998), and decreased phloem loading and starch accumulation (Ballach, 1997; Landolt et al., 1997; Grantz and Farrar, 1999). Ozone exposure also has been reported to increase rates of dark respiration in *Populus deltoides* (Reich, 1983), *Betula pendula* (Matyssek et al., 1997), and *Pinus sylvestris* (Skarby, Troeng and Bostrom, 1987).

Decreased photosynthesis and increased respiration costs have been reported to cause lower growth in trees exposed to ozone (Maurer and Matyssek, 1997). Ozone exposure reduced height and stem growth, and photosynthetic and total biomass of poplars (Dickson et al., 1998; Mortensen, 1998; Yun and Laurence, 1999). Reduced photosynthetic biomass was attributed to premature abscission of exposed foliage to ozone (Mikkelsen and Heide-Jørgensen, 1996; Pääkkonen, Holopainen and Karenlampi, 1997; Beare, Archer and Bell, 1999; Yun and Laurence, 1999). Growth reductions due to ozone exposure also have been reported for conifers, both in exposure chambers and open top chambers (Temple, 1988; Karlsson et al., 1995; Shan et al., 1996), as well as in field conditions (Peterson et al., 1995). However, growth effects were not observed for mature *Pinus sylvestris* in the field when exposed to ozone over three growing seasons (Holland et al., 1995).

Many studies on the effects of ozone exposure on plants have used high ozone concentrations characteristic of photochemical smog episodes (e.g., Skeffington and Roberts, 1985; Grantz and Farrar, 1999). These studies addressed ozone effects that may

occur during peak ozone episodes that generally lasted only 1 to 5 days. However, adverse effects of ozone exposure to vegetation may also occur at concentrations characteristic over a whole growing season (April to September in the northern hemisphere), which are considerably lower than peak concentrations. To assess ozone effects that may occur at more commonly observed concentrations, I used ozone concentrations typical for the growing season in both urban regions and at high elevations. A comparison of effects was made for two deciduous and one coniferous tree species. In this study, I addressed the following questions: 1) does ozone exposure affect leaf wettability of intact leaves, and, if so, do these effects occur via direct or indirect mechanisms; 2) do ozone-induced changes to cuticles affect cuticular water loss rates; 3) what are the effects of ozone exposure on plant growth; and 4) do the responses of deciduous and coniferous tree species to ozone differ?

MATERIALS AND METHODS

Plant material

Current year cuttings of *Populus nigra L. cv. "Brandaris"* and *Populus euramericana L. cv. Robusta*, stored in darkness at –2 ^{o}C for 3 months, were transplanted and placed in a growth chamber (T_{day} 21 ^{o}C, T_{night} 12 ^{o}C, relative humidity (RH) 60 %, light period 14 h/d at ~ 425 µmol m^{-2} s^{-1}). After 25 d, the cuttings were planted in pots (10 cm diameter) in a 2:1 mixture of sand and potting soil. After the plants had rooted, they were transplanted to 5-l containers and fertilized with 5 g of OSMOCOTE (15% N, 11% P_2O_5 and 2% MgO). Plants were transported to a second growth chamber for fumigation experiments 50 d after transplanting (T_{day} 20 ^{o}C, T_{night} 17 ^{o}C, RH 70 to 75 %,

light period 16 h/d at ~ 180 ± 10 μmol m^{-2} s^{-1}).

In January 1995, Douglas fir seedlings were potted into 5-l containers and fertilized with 5 g of OSMOCOTE, for growth in a greenhouse (T_{summer} 20-25 ^{O}C, T_{winter} >10 ^{O}C). The plants were ~2 ½ y old when they were transferred to growth chambers for fumigation experiments.

Fumigation treatments

Six exposure chambers (described in more detail by Van Hove et al., 1989) were placed in the growth chamber conditions described in the previous section. Two exposure chambers were assigned to each fumigation treatment, a control treatment (~2 O_3 μg m^{-3}; Figure 4.1), and two ozone treatments. The first ozone concentration treatment, urban exposure, was representative of ozone concentrations during the growing season in many urban and agricultural areas (e.g., Kruppa and Manning, 1988; Wunderli and Gehrig, 1990), with average minimal and maximal ozone concentrations of 26 O_3 μg m^{-3} and 82 O_3 μg m^{-3}, respectively (Figure 4.1). The second ozone treatment, montane ozone exposure, was representative of concentrations observed at high elevations (1600 to 3600 m asl; e.g., Wunderli and Gehrig, 1990; Brace and Peterson, 1998), with minimal and maximal ozone concentrations of 61 and 90 O_3 μg m^{-3}, respectively (Figure 4.1). Concentrations of nitrogen oxides in the exposure chambers were 14 ± 1 ppbv NO and 17 ± 1 ppbv NO_2 in the ozone chambers, and 25 ± 1 ppbv NO and 12 ± 2 ppbv NO_2 in control chambers. Each growth chamber contained 6 poplars, 3 of each species (n=6), and 2 Douglas firs saplings (n=4). Poplars were exposed over a period of 6 wk while Douglas firs were exposed over a period of 23 wk. Poplar experiments in control and urban ozone

treatments were carried out three times (n=18), and the montane ozone exposure experiment was done once (n=6). AOT-40 (sum of hourly ozone concentrations >40 ppbv during the growing season 0900-1700) for the urban and the montane ozone treatment for Poplars were 0.6 ± 0.2 and 1.0 ± 0.2 ppmv.h, respectively. For Douglas fir in the urban ozone treatment, AOT-40 was 2.3 ± 0.2 ppmv.h.

At the end of the final experiment for poplars in the control treatment and for all Douglas firs, saplings were exposed to peak ozone concentrations, representative of photochemical smog episodes (e.g., Smith, 1990). This treatment lasted for 6 d and was characterized by minimal and maximal ozone concentrations of 61 ± 6 and 335 ± 24 O_3 $\mu g\ m^{-3}$, respectively (AOT-40: 6.7 ppmv.h). Ozone concentrations in the control treatment were similar to those shown in Figure 4.1.

Leaf wettability

Droplet contact angles (CA) and droplet retention angles (RT) were used to assess effects of ozone on leaf wettability (Brewer, 1996; Brewer, Smith and Vogelmann, 1991). Six mature poplar leaves per treatment (one leaf per tree; ~5% of leaf biomass) were sampled, with three replicates per leaf on both the adaxial (AD) and the abaxial (AB) leaf surfaces. Leaf wettability parameters were measured on 20 Douglas fir needles (5 per tree) for control and urban ozone treatments, in 4-wk intervals. Both current-year and previous-year Douglas fir foliage were sampled.

Cuticular transpiration

Leaf wettability, cuticular transpiration, and leaf water content of poplar leaves were measured at the start of the experiment, and then at 2-wk intervals. Leaves of the same age were sampled throughout the experiment, to compensate for the effects of leaf age on leaf wettability and cuticular water loss. Cuticular water loss was determined for 6 mature poplar leaves per treatment (one leaf per tree; ~5% of leaf biomass). Upon abscission, leaf area and fresh weight of the leaves were determined. Stems were sealed with paraffin wax to avoid water loss via the stem. Leaves were placed in a drying oven (T 30 ^{o}C, RH 57 ± 2 %) and weighed periodically for up to 5 h to determine water loss. Then leaves were dried for 24 h to determine the dry weight. Total water content (TWC; expressed as gr H_2O per gr dry weight) was derived from the fresh and dry weights of the samples. Minimal conductance to water vapor, $G_{min,H2Ov}$ (Kerstiens, 1996) was derived from data on temperature and relative humidity in the drying oven, and leaf area. Leaf area of Poplar leaves was measured with a leaf area meter (Skye Instruments, Leaf Analysis System, version 2.0) equipped with a digital camera (AEG, CCD Video Camera, model XC 77 CE). To ensure that leaves were of similar age and developmental stage, the same leaf number (i.e., at the same place on the stem) was sampled for each plant within each experiment.

Cuticular transpiration and water content of Douglas fir foliage were measured at 4-wk intervals. After the fresh weight was determined, branches (4 branches per treatment and needle age class) were water-saturated overnight, weighed, sealed with paraffin wax, and weighed again. The samples were dried in a drying oven (T 30 ^{o}C, RH 29 ± 1 %) over a period of 72 h and weighed periodically (Hadley and Smith, 1989).

Samples were left in the oven for another 3 d (T 60 ^{o}C) to determine the dry weight. Relative water loss and $G_{min,H2Ov}$ were calculated from leaf weight and SLA. SLA of Douglas fir foliage was calculated using the glass bead method (Thompson and Leyton, 1971). Total water content (TWC; expressed as gr H_2O per gr dry weight) and relative water content (RWC, expressed as the ratio of fresh weight-dry weight over saturated weight-dry weight) of foliage were derived from the fresh-, saturated-, and dry weights of needle samples.

Growth

Growth parameters in poplar were measured in week 0 (start of experiment), wk 3, and wk 6 (end of experiment). The growth parameters recorded were height (both absolute and relative to initial height), total number of leaves per shoot, number of new leaves per shoot, and number of abscised leaves per shoot. Height growth of the whole tree and the growth leader were recorded for Douglas fir at the start of the experiment (wk 0), and then at 4-wk intervals. At the end of the experiment (wk 23), the number of buds per tree and the percent of buds that had flushed were recorded.

Stomatal density and aperture

Stomatal density and aperture for poplar leaves were determined in wk 3, 6, and 7, for both adaxial and abaxial surfaces of mature leaves (one per tree, same leaves as used for determination of leaf wettability parameters). Prints of surfaces of mature leaves (one per tree) were made using a gel Xantopren L. (Bayer Dental, DIN13913-C2), and an activator, Optisol (Bayer Dental), to harden the gel. Once the gel had hardened, the

imprint of the leaf was covered with a thin layer solution of polystyrene in toluol, forming a clear imprint of the leaf surface. The prints were examined under a microscope (Leitz Wetzlar) connected to a video screen (Philips, LDH 0225/00 and LDH 2122/00). Stomatal density was determined using standard grid laid over the video screen. To study stomatal closure after leaf abscission, prints were made from intact leaves, leaves that had been abscised for 30 min (right before they were placed in the drying oven), and leaves that had been in the oven for 30, 60 and 90 min.

Data analyses

Data were analyzed using SigmaStat (SPSS, 1997). Normally distributed data were analyzed using a one-way analysis of variance (reported as F, P-value), by species and needle age class separately. Pair wise comparisons were made using a Bonferroni post-hoc test or a t-test (reported as T, P-value). The experimental design was a nested analysis of variance. For determination of CA, 18 leaves of similar age were chosen, one per tree, with three replicate measurements per leaf. If there were no significant differences between subsamples, data were pooled by treatment and time ($P<0.05$, Sokal and Rohlf, 1997). A similar sampling procedure was used to test for effects on relative water loss, $G_{min,H2Ov}$, TWC and RWC. For these measurements, I sampled 18 leaves, one leaf per tree, and again data were tested for differences between subsamples (two fumigation chambers per treatment, three replications of the experiment for the control and the urban ozone treatment). Measurements of CA for Douglas for were carried out on four branches (one per tree, $n=4$), with 5 replicate needles per branch. If there were no significant differences between subsamples, data were pooled ($P<0.05$, Sokol and Rohlf,

1997).

Analysis of growth data also was based on a nested experimental design. Plant height growth was measured for all shoots of poplars (2 or 3 shoots per plant). When no differences were found between shoots, i.e., within plant differences, data were pooled according to the criteria mentioned earlier. Leaf growth data were recorded for the whole plant as the sum of the individual shoots of each plant.

Data that did not meet the normality requirements were analyzed using a Kruskal-Wallis ANOVA on ranks (reported as H, P-value), or a Kolmogorov-Smirnov test (non-parametric test; reported as ksz, P-value). This was the case for RT for all species, relative water loss data for Poplars, and leaf abscission data for poplars. As before, 18 poplar leaves were chosen (one per plant) for determination of RT and water loss, with three replicates per leaf. Subsamples were pooled for statistical analysis if there were no significant differences between subsamples ($P<0.05$, Sokol and Rohlf, 1997). Because each plant was measured several times over the course of the experiments, repeated measures techniques (RM) were applied as appropriate.

RESULTS

Foliar injury

Visual injury due to ozone exposure was observed on both poplar species. Foliar injury developed first as a dull, light green appearance on leaves, followed by development of small chlorotic and necrotic spots (Figure 4.2). The severity of chlorosis and necrosis increased over time and resulted in premature leaf abscission. Leaves dropped by themselves or from the slightest physical contact. The first signs of chlorosis

and necrosis were observed in plants 3 to 5 wk after exposure to urban ozone concentrations. However, plants exposed to montane ozone concentrations showed chlorosis and necrosis as early as 1 ½ wk after exposure had started. Older foliage was most susceptible to foliar injury. *P. euramericana* was less susceptible to injury than *P. nigra*, especially when exposed to peak ozone concentrations. Control plants did not show any of these injury symptoms.

Douglas fir foliage exposed to urban ozone concentrations was not visibly injured. However, after exposure to peak ozone concentrations, Douglas fir needles became chlorotic, especially the older age classes, but did not defoliate.

Leaf wettability

There were significant effects of ozone exposure on CA. In general CA decreased over time for both species and both sides of the leaf in poplars on control and exposed leaves ($F_{4,68}$=9.68 to $F_{4,68}$=37.62, P<0.001; Figure 4.3), suggesting that leaf surfaces generally became more wettable over time. CA was higher for the adaxial surface compared to the abaxial surface (Figure 4.3), for both *P. nigra* ($F_{2,118}$=4.42, P=0.036) and *P. euramericana* ($F_{2,118}$=9.63, P=0.002). There was a 2 to 4 wk delay in the decline of CA in the urban ozone treatment compared to the other treatments, although CA did eventually decline to angles similar to the other treatments ($F_{3,57}$=9.22 to $F_{3,57}$=11.71, P<0.001; Figure 4.3). RT decreased after 2 wk in the exposure chambers for both *P. nigra* and *P. euramericana* (H_3=167.41 and H_3=257.94, P<0.001; Figure 4.4), and was higher for the adaxial surface compared to the abaxial surface for *P. nigra* (H_2=30.88,

$P<0.001$; Figure 4.4). There were no significant differences in RT due ozone exposure compared to control leaves (RM, $P>0.05$).

CA of Douglas firs were significantly higher for current-year foliage compared to previous-year foliage ($F_{2,258}=23.19$, $P<0.001$; Figure 4.5). Moreover, urban ozone exposure led to decreased CA in Douglas fir foliage compared to controls, starting after 4 to 8 wk of exposure (Figure 4.5). This was the case for both current- and previous-year foliage ($F_{2,258}=10.29$ and $F_{2,258}=31.29$ respectively, $P<0.001$; Figure 4.5). There were no effects of needle age class or ozone exposure on RT for Douglas fir foliage.

Cuticular transpiration

Relative water loss was higher for *P. nigra* exposed to montane ozone concentrations after 2 and 4 wk compared to controls ($H_2=6.51$, $P=0.039$ and $H_2=36.00$, $P<0.001$ respectively; Table 4.1), but differences were lost by 6 wk of exposure. $G_{min,H2Ov}$ for *P. nigra* in the montane ozone treatment was higher than the other treatments after 4 wk of exposure only ($H_2=19.66$, $P<0.001$; Table 4.1). Effects of ozone exposure on water loss for *P. euramericana* were more evident and consistent than for *P. nigra*. Relative water loss for *P. euramericana* was higher for plants exposed to ozone after 2, 4 and 6 wk ($H_3=18.60$ to $H_3=39.37$, $P<0.001$; Table 4.1). The highest relative water loss generally was observed for the montane ozone exposure, corresponding to the plants that received the highest ozone dose. *P. euramericana* exposed to ozone had higher $G_{min,H2Ov}$ compared to controls from 2 to 6 wk ($H_3=6.37$ to $H_3=21.46$, $P<0.001$; Table 4.1). Relative water loss and $G_{min,H2Ov}$ over the first 30 min were generally higher for plants exposed to ozone ($F_{3,69}=3.86$, $P=0.03$ to $F_{3,69}=21.27$, $P<0.001$; data not

shown). Higher relative water loss and $G_{min,H2Ov}$ did not lead to decreased foliar water content (TWC) in either poplar species (RM, $P>0.05$), which may have been due to ample water supply during the experiment. Overall, *P. nigra* had lower TWC (0.72 ± 0.004) than *P. euramericana* (0.74 ± 0.004; $T_{72}=3.82$, $P<0.001$). Exposure to peak ozone concentrations did not affect relative water loss, $G_{min,H2Ov}$, or TWC for Poplars. When plants were exposed to an 8-d drought period following 6 wk of ozone exposure, ozone-exposed plants had a higher incidence of foliar drought injury, evidenced by higher defoliation of leaves.

Relative water loss and $G_{min,H2Ov}$ in Douglas fir increased over the course of the experiment ($F_{2,46}=7.70$ to $F_{2,46}=10.35$, $P<0.001$; Table 4.2), and were significantly higher in previous-year foliage compared to current-year foliage ($F_{2,94}=43.26$, $P<0.001$; Table 4.2). However, exposure to urban ozone concentrations generally did not affect water loss from Douglas fir foliage. Exceptions were in wk 20 and 23, when previous-year foliage of control trees had higher relative water loss than foliage of trees exposed to ozone ($F_{2,14}=11.41$ and $F_{2,14}=7.22$, $P<0.05$; Table 4.2). There were no differences TWC and RWC of Douglas fir foliage.

Growth

Ozone exposure did not significantly affect height growth of poplars (Table 4.3), although *P. euramericana* showed a trend towards decreased height growth in the urban ozone treatment (Table 4.3). Interestingly, *P. euramericana* saplings in the montane ozone treatment, which received the highest ozone dose, had the highest growth rates (Table 4.3). Ozone exposure led to decreased leaf growth for both poplar cultivars. After

3 and 6 wk of ozone exposure, both *P. nigra* and *P. euramericana* had fewer leaves ($F_{2,34}$=28.87 and $F_{2,34}$=3.75, P<0.001 respectively; Figure 4.6), especially in the urban ozone treatment for *P. nigra* (Figure 4.6). However, there were no differences in the number of leaves between *P. nigra* exposed to montane ozone concentrations and control plants. The reduction of the number of leaves on plants exposed to ozone seems to be related to two factors. First, trees exposed to urban ozone concentrations produced fewer new leaves than control trees, especially for *P. nigra* (RM, $F_{3,42}$=8.44, P<0.001; Figure 4.6). Second, ozone exposure led to premature leaf abscission for poplar in wk 3 as well as wk 6 (RM, $F_{3,42}$=129.74 and H_3=48.78, P<0.001 respectively; Figure 4.6). Leaf abscission was significantly higher in the montane ozone treatment compared to the urban ozone treatment (Figure 4.6).

Leaf size of mature *P. nigra* leaves was lower after 4 wk of fumigation in both ozone treatments (Table 4.4). However, this trend was not statistically significant (RM, P>0.05), and there were no differences in leaf size at the end of the experiment (Table 4.4). Leaf size of *P. euramericana* in the urban ozone treatment decreased by ~27% in wk 4 and 6 of fumigation ($F_{2,36}$=3.73, P=0.033; Table 4.4). Stomatal density in new leaves was lower after ozone exposure on both adaxial and abaxial surfaces of *P. nigra* ($F_{3,107}$=14.70 and $F_{3,57}$=6.57 respectively, P<0.001; Table 4.4). However, ozone exposure did not affect stomatal density of *P. euramericana*. Stomatal density was higher on the abaxial surface for both cultivars (Table 4.4).

Height growth of Douglas fir saplings was not affected by ozone exposure. There were no differences in absolute tree height, length of the growth leader (RM, P>0.05; Table 4.5), or relative growth rates (RM, P>0.05). After 23 wk of exposure to urban

ozone concentrations, Douglas fir saplings formed 20 % fewer buds than controls (means 125 ± 33 and 159 ± 33 buds per tree, respectively). However, this difference was not statistically significant (T_{14}=0.95, P=0.380). The majority of buds flushed during the last month of fumigation (90 to 95 %), and the percentage of buds that flushed was not affected by ozone exposure treatment.

DISCUSSION

The results of this study suggest that ozone concentrations commonly observed during a growing season, in both urban and montane environments, can induce increased cuticular water loss, foliar injury, and growth reductions in photosynthetic biomass in poplar saplings. However, the intensity of these effects depends on the species. These findings have implications for tree water balance and growth of these poplars. In general Douglas fir was not affected to the same extent as poplars, and visual injury was observed only after exposure to peak ozone concentrations. Nonetheless, cuticular changes in Douglas fir were apparent after ozone exposure, and these may have implications for ozone uptake and gas exchange of wet foliage (Chapter 5).

Leaf wettability

Adaxial and abaxial surfaces of both Poplar species became more wettable over time (Figure 4.3). This may influence gas exchange processes, and pollutant deposition on these leaves (Chapter 5). Observed values of CA agreed well with values for *Fagus sylvatica* (101^{o}-108^{o}; Paoletti et al., 1998), and were generally in the range of values observed for montane herbaceous plant species (Brewer and Smith, 1997). Exposure to

urban ozone concentration delayed this decrease by 2 to 4 wk (Figure 4.3), suggesting that ozone exposure affected the development of epicuticular waxes in young poplar leaves. However, CA's for leaves exposed to higher montane ozone concentrations did not differ from control leaves. RT also decreased over time in a pattern similar to CA, but ozone did not affect RT of poplar species (Figure 4.4). Both CA and RT were higher for the adaxial surface than for the abaxial surface, typical of species in a broad range of habitats (Brewer et al., 1991; Brewer and Smith, 1997), and for hypostomatous leaves (Smith and McLean, 1989).

Overall observed CA values for Douglas fir agreed well with those observed for other conifers (Barnes and Brown, 1990; Barnes et al., 1990a, 1990b; Stazewski et al., 1998), which ranged from 73^{o} to 113^{o}. CA's of Douglas fir exposed to urban ozone were lower than those of controls starting after 4 wks of fumigation (Figure 4.5), suggesting that ozone exposure affected cuticles in Douglas fir. This trend was observed for both current- and previous-year foliage, and agreed well with ozone effects on CA reported for *Picea abies*, when exposed to similar ozone for two to three growing seasons at concentrations similar to those used in this study (Barnes and Brown, 1990; Barnes et al., 1990a, 1990b). Interestingly, ozone exposure of *Picea abies* in open-air fumigation systems over four growing seasons did not affect CA values (Cape et al., 1995). CA of Douglas fir decreased with increasing needle age (Figure 4.5), a trend that was also observed for *Picea abies* (Barnes et al., 1990b), and spruce and pine stands in Poland (Stazewski et al., 1998).

The observed changes in CA and RT on the cuticle may have implications for cuticular conductance to water vapor, $G_{min,H2Ov}$ (Turunen and Huttunen, 1990). Higher

$G_{min,H2Ov}$ may lead to increased susceptibility to periods of drought stress, both in summer (Mengel, Hogrebe and Esch, 1989), and in cold winter conditions (Barnes and Davison, 1988). Moreover, increased leaf wettability, as observed for Douglas fir, may lead to higher incidence of water droplets and water films on leaf surfaces. Water on leaves can increase ozone uptake, and decrease rates of photosynthesis and respiration (Chapter 5).

Tree water balance

Plants of *P. nigra* in both ozone treatments had increased relative water loss during the first 4 wk of ozone exposure (Table 4.1). However, $G_{min,H2Ov}$ of ozone-exposed *P. nigra* leaves was only higher in week 4. Interestingly, relative water loss and $G_{min,H2Ov}$ of ozone exposed *P. euramericana* (the species considered to be more ozone tolerant) were higher compared to controls over the entire fumigation period (Table 4.1). These data suggest that ozone exposure led to increased cuticular water loss of poplar foliage, and that responses are species dependent. A possible explanation for increased water loss is the erosion of epicuticular waxes, because erosion of the wax layers can lead to increased permeability of cuticles to water vapor (Turunen and Huttunen, 1990). However, adverse effects of ozone exposure on isolated cuticles have not been reported (Kerstiens and Lendzian, 1989b; Kerstiens, 1995). This may be attributed to the possibility that it is very difficult to detect indirect effects of ozone exposure on cuticles using isolated cuticles. The changes in cuticular properties responsible for increased $G_{min,H2Ov}$ in ozone treated foliage could be related to effects on the developmental process of the cuticle via indirect ozone effects on developing epidermis cells. Observed values of

$G_{min,H2Ov}$ for the poplar species were at the higher end of the range observed for deciduous leaves (Kerstiens, 1996).

Relative water loss and $G_{min,H2Ov}$ for Douglas fir increased over time for both control and ozone-exposed foliage, but were not altered by urban or peak ozone concentrations (Table 4.2). Observed $G_{min,H2Ov}$ for Douglas fir agreed well with values reported in the literature (1-12 m s^{-1} *10^{-5}; e.g., Kerstiens, 1996). Interestingly, in wk 20 and 23, previous-year foliage of control trees had higher relative water loss than foliage for trees exposed to ozone. A possible explanation of this observation was increased relative water loss and $G_{min,H2Ov}$ in the first hour of the drying experiment for controls ($F_{7.00}$ and $F_{7.37}$, $P<0.05$; Table 4.2). Water loss was higher for previous-year foliage than for current-year foliage (Table 4.2). Higher $G_{min,H2Ov}$ with increasing needle age has been reported for other conifers as well (Barnes et al., 1990), and typically is explained by erosion of cuticles (Turunen and Huttunen, 1990; Günthardt-Görg, 1994) and changed wax composition (Kerfourn and Garrec, 1992). Furthermore, relative water loss and $G_{min,H2Ov}$ of Douglas fir also increased over the course of the experiment (Table 4.2). This appeared to be a chamber effect, as the same trend was found for all treatments, including the control treatment, and for both needle age classes.

These data suggest that poplar leaves exposed to ozone, especially *P. euramericana*, were more susceptible to water loss via the cuticle, as well as via stomata (which had been presumed to be closed). Thus, a potential role for decreased rates of stomatal closure in plants exposed to ozone cannot be excluded. $G_{min,H2Ov}$ has both cuticular and stomatal components. The method to determine $G_{min,H2Ov}$ is based on the assumption that stomata are fully closed. However, some stomata were open on the

adaxial (~25 %) and abaxial (~90 %) surfaces in intact leaves on trees. After leaf abscission, stomata closed rapidly. After 30 to 60 min of drying the number stomata closed averaged 94% to 98% (± 1). Thus, stomata were mostly closed during the drying experiments, especially during the time period that was considered for $G_{min,H2Ov}$ (Table 4.1).

Ceulemans, Hinckley and Impens (1989) reported that stomata closed within 90 min of leaf abscission, but that stomatal closure often was incomplete, especially on the abaxial leaf surface. In my study, stomatal aperture was about 0.1 μm (± 0.3) after 60 min in the oven, suggesting that the relative contribution of cuticular conductance to total $G_{min,H2Ov}$ may have been about 10 to 22 % (Kerstiens, 1996). Moreover, all cases of significantly higher relative water loss and $G_{min,H2Ov}$ for poplar and Douglas fir coincided with significantly higher water loss and $G_{min,H2Ov}$ within the first 30 min (in Poplars) to 60 min (Douglas fir) of drying. This suggests that ozone exposure may have decreased the rate of stomatal closure for abscised leaves. In general, ozone exposure can cause damage to stomata (Barnes et al., 1988; Maier- Märcker and Koch, 1995; Paoletti et al., 1998), lower rates of stomatal closure (Barnes et al., 1990a; van Hove and Bossen, 1994; Maier-Märcker and Koch, 1995; Maier-Märcker, 1999), and increase susceptibility to drought stress in regions with high ozone loads (McLaughlin, 1985; Maier- Märcker and Koch, 1995; Schmieden and Wild, 1995; Skarby et al., 1998). Thus, although increased water loss due to ozone exposure may have been caused partially by increased permeability of the cuticle to water vapor, increased water loss via stomata cannot be excluded as a causal mechanism.

Growth

Ozone exposure did not affect height growth of poplar species (Table 4.3), but significantly lowered production of photosynthetic biomass (Figure 4.6). Plants in both ozone treatments had fewer leaves, especially *P. nigra*, due to decreased new growth of leaves and premature leaf abscission (Figure 4.6). However, responses depended on species and ozone treatment. Moreover, leaf abscission was highest in the montane ozone treatment, which received the highest ozone dose. Photosynthetic biomass of *P. euramericana* was further decreased due to the formation of smaller leaves in the urban ozone treatment. Leaf abscission was preceded by foliar injury, characteristic of ozone exposure (Figure 4.2; Hill et al., 1970; Matyssek et al., 1997; Pääkkonen et al., 1998a; Yun and Laurence, 1999). Increased leaf abscission due to ozone exposure has been attributed to premature leaf senescence (Mikkelsen and Jørgensen, 1996; Pääkkonen et al., 1997), and has been reported for *Populus deltoides* (Reich, Lassoie and Amundson, 1983), *Populus tremuloides* (Yun and Laurence, 1999), *Betula pendula* (Matyssek et al., 1997; Pääkkonen et al., 1997), and *Fagus sylvatica* (Mikkelsen and Heide-Jørgensen, 1996). Other ozone effects on growth reported for deciduous trees include reduced vertical growth, and lower shoot and root growth (Reich et al., 1983; Cooley and Manning, 1987; Landolt et al., 1997; Dickson et al., 1998; Mortensen, 1998). Lower leaf, stem, and root growth under ozone exposure also have been reported for *Betula pendula* (Pääkkonen et al., 1998a), *Fagus sylvatica* (Pääkkonen et al., 1998b), and *Populus tremuloides* (Yun and Laurence, 1999).

Height growth of Douglas fir saplings was not affected by ozone exposure at urban levels. There was a trend toward fewer buds on exposed trees, but this trend was

not significant. The absence of ozone effects on growth of Douglas fir suggests that conifers are less susceptible to ozone than deciduous trees (e.g., Kytoviita et al., 1999), and this result may be attributed to lower stomatal conductance, and thus, lower ozone uptake (Reich, 1987). However, because conifers maintain foliage for several years, the cumulative ozone dose may eventually exceed a critical threshold, which may then lead to foliar injury and growth reductions. Exceedance of a critical ozone dose may have caused the severe foliar injury of Douglas fir observed after exposure to 6 d at peak ozone concentrations (AOT-40: 9.0 ppmv.h). While growth reductions in conifers due to ozone exposure have been reported for *Pinus ponderosa* (Takemoto et al., 1997), *Picea abies* (Karlsson et al., 1995), and *Pinus jeffreyi* (Temple, 1988), evidence of these growth reductions occurred only after ozone exposure over 2 to 3 growing seasons (Temple, 1988; Karlsson et al., 1995; Takemoto et al., 1997). This suggests that deleterious effects ozone exposure may carry-over to the subsequent growing seasons in some species. For example, deleterious effects of ozone exposure on photosynthesis and growth have been reported for *Pseudotsuga menziesii*, *Betula pendula*, and *Vitis vinifera* up to two growing seasons after ozone exposure was stopped (Soja et al., 1997; Langebartels et al., 1998; Oksanen and Saleem, 1999).

Yet the data can be contradictory depending on the species; ozone exposure over three growing seasons of *Pinus sylvestris*, *Picea abies* and *Picea sitchensis* saplings did not result in growth reductions (Holland et al., 1995). The duration of ozone exposure in my study may not have been long enough to induce growth effects in Douglas fir. Moreover, there may be differences in sensitivity to ozone exposure between seedlings, saplings, and mature trees. This is important because there are reports that large, mature

conifers generally were more susceptible to ozone than younger trees due to lower stomatal conductance (e.g., Kolb et al., 1997). However, in *Quercus rubra* large trees had higher stomatal conductance, which resulted in higher ozone uptake and foliar injury (Kolb et al., 1997).

Ozone exposure led to decreases in stomatal density in new leaves by 10 - 20 % for *P. nigra*, but not for *P. euramericana*. This is contrary to reported increases in stomatal density for *Betula pendula* (Pääkkonen et al., 1997, 1998a) and *Olea europaea* (Minnocci et al., 1999) exposed to ozone. A possible explanation for decreased stomatal densities in *P. nigra* is that plants were slightly water stressed during the fumigations, and it is known that drought stress can induce lower stomatal densities in leaves of *Betula pendula* (Pääkkonen et al., 1998a, 1998b, 1998c). Since ozone-exposed plants had higher $G_{min,H2Ov}$ than control plants, ozone exposure may have led to higher sensitivity to drought stress and responses of stomatal density to drought.

CONCLUSION

The results of this work suggested that ozone exposure may lead to higher water loss through the cuticle, and loss of photosynthetic biomass of poplars. These effects may have implications for tree water balance and growth. However, responses were highly species dependent. Moreover, the strongest and most consistent effects of ozone exposure were found for *P. euramericana*, the more ozone tolerant species. Thus, "ozone tolerance" may depend on the response variable considered. Future research needs to address the effects of ozone in field conditions and for trees of different sizes because tree responses to ozone depend on environmental circumstances as well as tree size.

Moreover, future research is needed to better understand the role of ozone-exposed cuticles in water loss from foliage. Progress in this field is dependent on the development of methods that can distinguish between cuticular water loss and minimal opening of the stomata on intact leaves. The assessment of ozone effects in field conditions is complicated by the species-specific responses.

TABLE 4.1: Cuticular water loss of poplar leaves for control plants, and plants exposed to urban and montane ozone concentrations. Data are relative water loss after 5 hours of drying (± 1 SE), and $G_{min,H2Ov}$ from 1 to 5 hours (± 1 SE). Significant differences based on ozone treatment are indicated by letters and apply within species and week (nested one-way ANOVA, $P<0.001$; repeated factor: hours, $n=8$, and week, $n=3$).

Species / week	Relative H_2O loss after 5 hours, %		
	Control (n=18)	O_3,urban (n=18)	O_3,mtn (n=6)
P. nigra			
Week 2	56 (6) a	59 (6) a,b	70 (8) b
Week 4	57 (6) a	59 (7) a,b	81 (1) b
Week 6	71 (3)	74 (2)	80 (2)
P. euramericana			
Week 2	50 (4) a	57 (5) b	77 (2) c
Week 4	49 (5) a	60 (5) b	79 (1) c
Week 6	64 (4) a	79 (1) b	76 (2) b
	$G_{min,H2Ov}$, 1-5 hrs, m s^{-1} *10^{-5}		
	Control	O_3,urban	O_3,mtn
P. nigra			
Week 2	17.0 (1.1)	18.0 (1.0)	15.9 (1.5)
Week 4	12.4 (1.2) a	11.3 (1.1) a	18.2 (3.0) b
Week 6	23.4 (1.4)	22.2 (1.0)	23.9 (2.6)
P. euramericana			
Week 2	13.4 (0.7) a	15.6 (1.0) b	17.1 (1.5) b
Week 4	14.4 (0.9) a	19.2 (1.5) b	27.0 (2.9) b
Week 6	17.5 (1.2) a	19.6 (1.4) a	22.1 (2.8) a,b

TABLE 4.2: Cuticular water loss of Douglas fir foliage for control and urban ozone treatment of two needle age classes. Data are relative water loss after 36 hours of drying (± 1 SE; n=4; 2 replicates per tree), and $G_{min,H2Ov}$ from 2 to 36 hours (± 1 SE; n=4; 2 replicates per tree). Significant differences based on ozone treatment indicated by letters and apply within needle age class and week (nested one-way ANOVA, P<0.05; repeated factor: hour, n=8, and week, n=6).

Age class / time	Relative H_2O loss at 36 hours %		$G_{min,H2Ov}$, 2-36 hrs $m\ s^{-1} * 10^{-5}$	
	Control	O_3-urban	Control	O_3-urban
Current-year				
Week 0	38 (4)	38 (4)	2.9 (0.5)	2.9 (0.5)
Week 4	84 (6)	68 (5)	6.1 (1.7)	4.4 (0.4)
Week 8	80 (3)	70 (6)	4.8 (0.8)	4.2 (0.5)
Week 12	87 (4)	72 (11)	6.6 (1.4)	5.6 (1.3)
Week 16	88 (3)	82 (7)	7.0 (1.1)	6.7 (1.4)
Week 20	95 (1)	87 (4)	7.1 (0.8)	7.5 (0.9)
Week 23	92 (1)	85 (3)	7.4 (0.7)	6.9 (1.1)
Previous-year				
Week 0	27 (3)	27 (3)	1.3 (0.1)	1.3 (0.1)
Week 4	36 (3)	39 (3)	1.8 (0.2)	1.5 (0.2)
Week 8	34 (2)	34 (4)	1.4 (0.1)	1.6 (0.2)
Week 12	54 (7)	47 (11)	2.5 (0.3)	2.7 (0.8)
Week 16	53 (11)	56 (2.7)	2.7 (0.5)	3.9 (1.3)
Week 20	92 (2) a	63 (10) b	5.6 (0.6)	3.6 (0.6)
Week 23	88 (6) a	66 (5) b	5.3 (0.9)	4.0 (0.7)

TABLE 4.3: Height growth of poplar saplings (~2 shoots per plant) during ozone exposure, expressed as absolute growth and growth relative to starting height (week 0). Treatments were control (n=18; 2 replicates per tree), urban ozone exposure (n=18; 2 replicates per tree), and montane ozone exposure (n=6, 2 to3 replicates per tree). Values in parentheses indicate 1 SE. Values indicated as "n.a." could not be calculated.

Species and week	Absolute plant height cm			Relative height growth % of week 0		
	Control	O_3,urban	O_3,mtn	Control	O_3,urban	O_3,mtn
P. nigra						
Week 0	27 (2)	29 (2)	31 (2)	n.a.	n.a.	n.a.
Week 3	52 (2)	51 (3)	61 (3)	108 (74)	89 (10)	103 (12)
Week 6	67 (3)	72 (3)	79 (3)	185 (23)	180 (21)	170 (22)
P. euramericana						
Week 0	19 (1)	19 (1)	17 (2)	n.a.	n.a.	n.a.
Week 3	43 (2)	41 (2)	47 (3)	147 (18)	127 (14)	190 (14)
Week 6	69 (3)	66 (3)	75 (4)	314 (35)	271 (20)	376 (30)

TABLE 4.4: Effects of ozone exposure on leaf size and stomatal density for poplars. Leaf sizes are means (± 1 SE) for controls (n=18), urban ozone exposure (n=18), and montane ozone exposure (n=6). Stomatal densities (in week 6 and 7) are shown for the adaxial (AD, n=18; 6 replicates per tree) and the abaxial leaf surface (AB, n=18; 3 replicates per tree). Significant differences are indicated by letters and apply within (by rows) species and week (nested one-way ANOVA, P<0.05; repeated factor: week, n=3).

Factor		*P. nigra Brandaris*	
	Control	O_3,urban	O_3,mtn
Leaf size (cm^2)			
Week 2	46 (5)	55 (6)	58 (5)
Week 4	75 (9)	65 (8)	42 (15)
Week 6	69 (7)	61 (6)	61 (8)
Stomatal density (# mm^{-2})			
Adaxial (AD)	99 (2) a	89 (2) b	80 (30) c
Abaxial (AB)	201 (4) a	204 (6) a	175 (6) b
AD/AB	0.49	0.44	0.46
		P. euramericana Robusta	
	Control	O_3,urban	O_3,mtn
Leaf size (cm^2)			
Week 2	58 (5)	56 (7)	47 (8)
Week 4	82 (6) a	61 (6) b	83 (8) a
Week 6	94 (8) a	68 (7) b	96 (11) a
Stomatal density (# mm^{-2})			
Adaxial (AD)	141 (3)	133 (4)	133 (6)
Abaxial (AB)	224 (4)	207 (5)	220 (6)
AD/AB	0.63	0.64	0.60

TABLE 4.5: Height growth of Douglas fir during ozone exposure, expressed as total tree height and length of the growth leader. Treatments were control and urban ozone exposure (± 1 SE, n=4).

Time	Tree height, cm		Growth leader, cm	
	Control	O_3-urban	Control	O_3-urban
Week 0	48.7 (4.6)	51.1 (2.4)	9.2 (1.0)	11.5 (0.5)
Week 4	49.7 (4.6)	51.3 (2.5)	9.8 (1.1)	12.0 (0.5)
Week 8	50.5 (4.6)	52.4 (2.4)	10.6 (1.2)	13.1 (0.5)
Week 12	51.2 (4.8)	53.0 (2.4)	10.8 (1.1)	13.3 (0.6)
Week 16	51.3 (4.8)	53.4 (2.6)	10.9 (1.2)	13.3 (0.6)
Week 20	52.2 (2.8)	53.8 (1.1)	11.3 (1.4)	13.8 (0.8)
Week 23	55.3 (3.5)	54.3 (0.3)	14.7 (2.5)	14.5 (1.6)

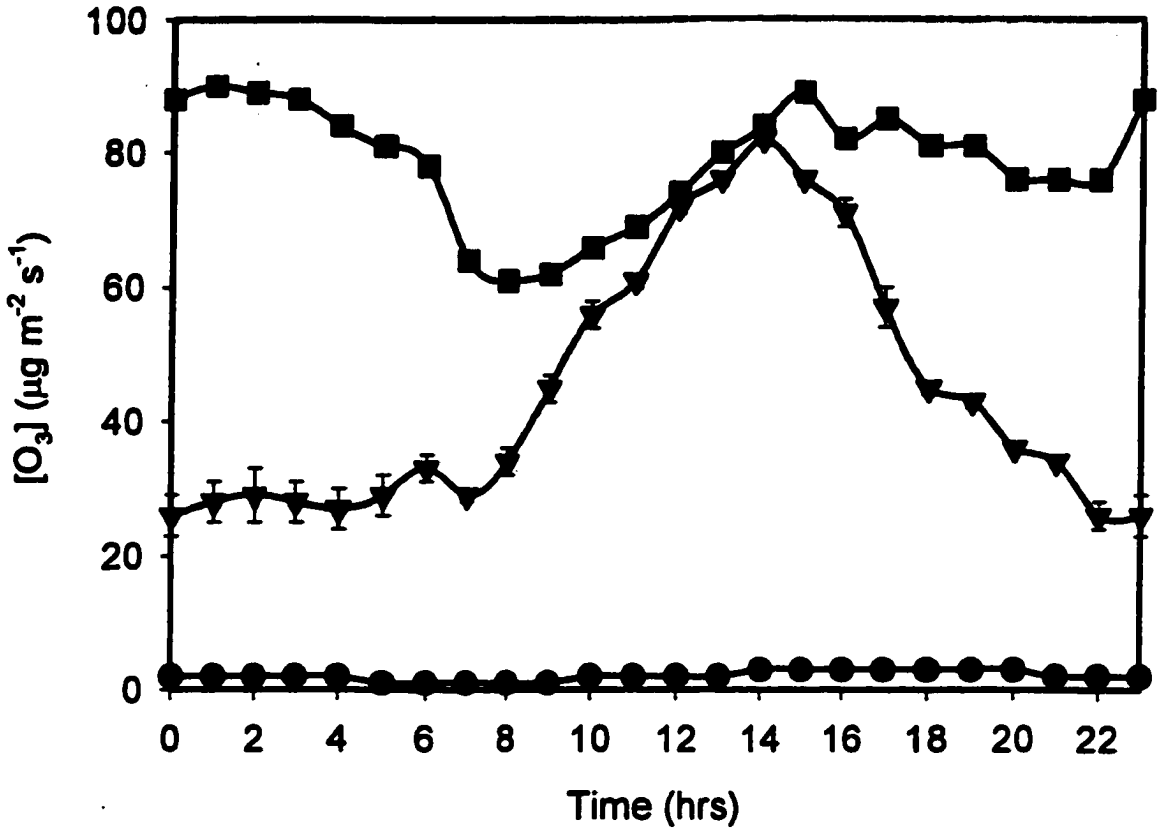


FIGURE 4.1: Diurnal course of ozone concentrations in fumigation treatments. Shown are controls (circles), urban ozone exposure (triangles), and montane exposure (squares). Errors bars represent 1 SE.

Panel A

Panel B

Figure 4.2: Visual leaf injury after 4 wk of ozone exposure. Shown are healthy and chlorotic leaves necrotic for *P. nigra* (A), and healthy (background), chlorotic (lower foreground), and necrotic leaves (upper foreground) for *P. euramericana* (B).

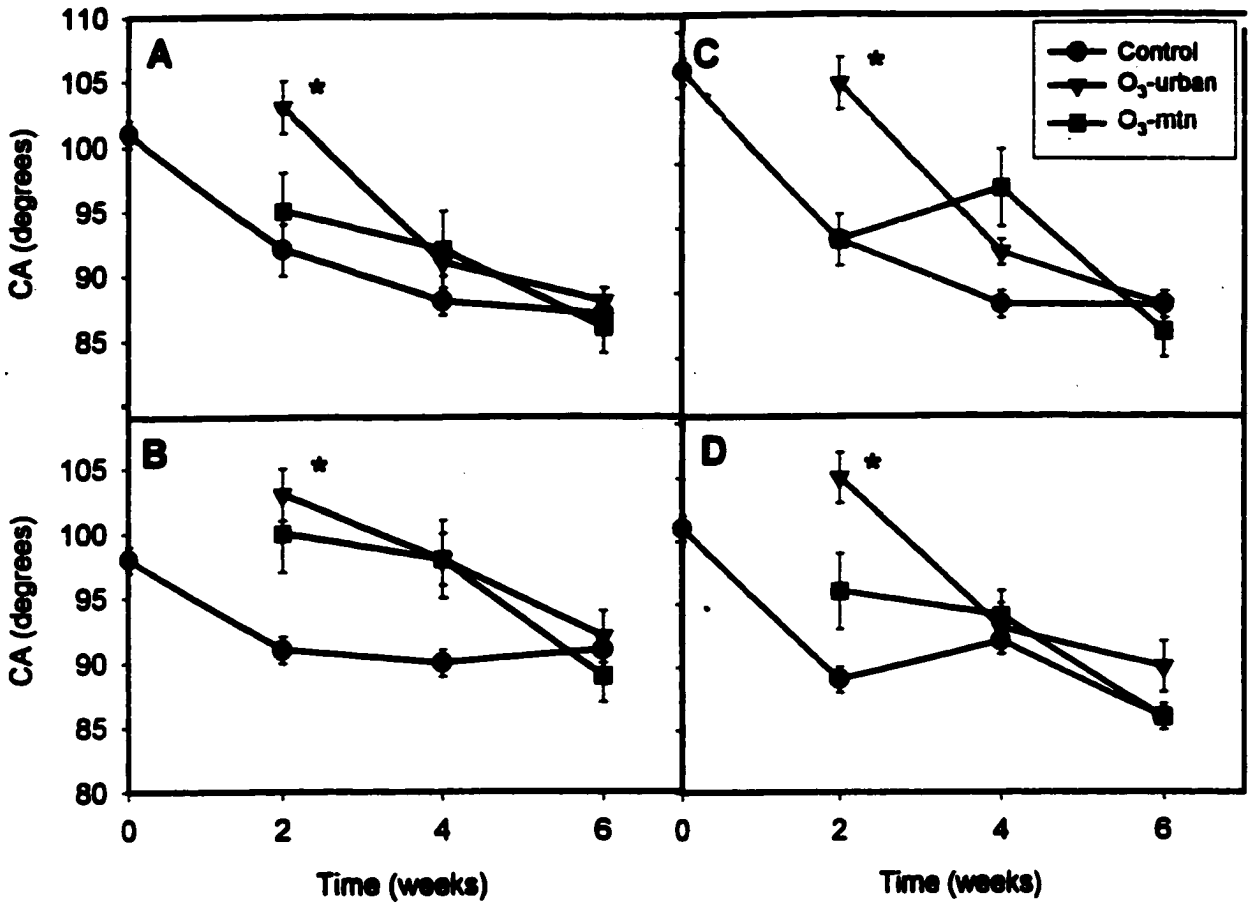


FIGURE 4.3: Droplet contact angles of poplar leaves, for controls (circles, n=18; 3 replicates per tree), urban ozone exposure (triangles, n=18; 3 replicates per tree), and montane ozone exposure (squares, n=6; 3 replicates per tree). Shown are values for *P. nigra*, adaxial surface (A) and abaxial surface (B), and *P. euramericana*, adaxial surface (C) and abaxial surface (D). Error bars represent one SE, and significant differences between treatments are indicated by "*"(nested one-way ANOVA, P<0.001; repeated factor: week, n=4).

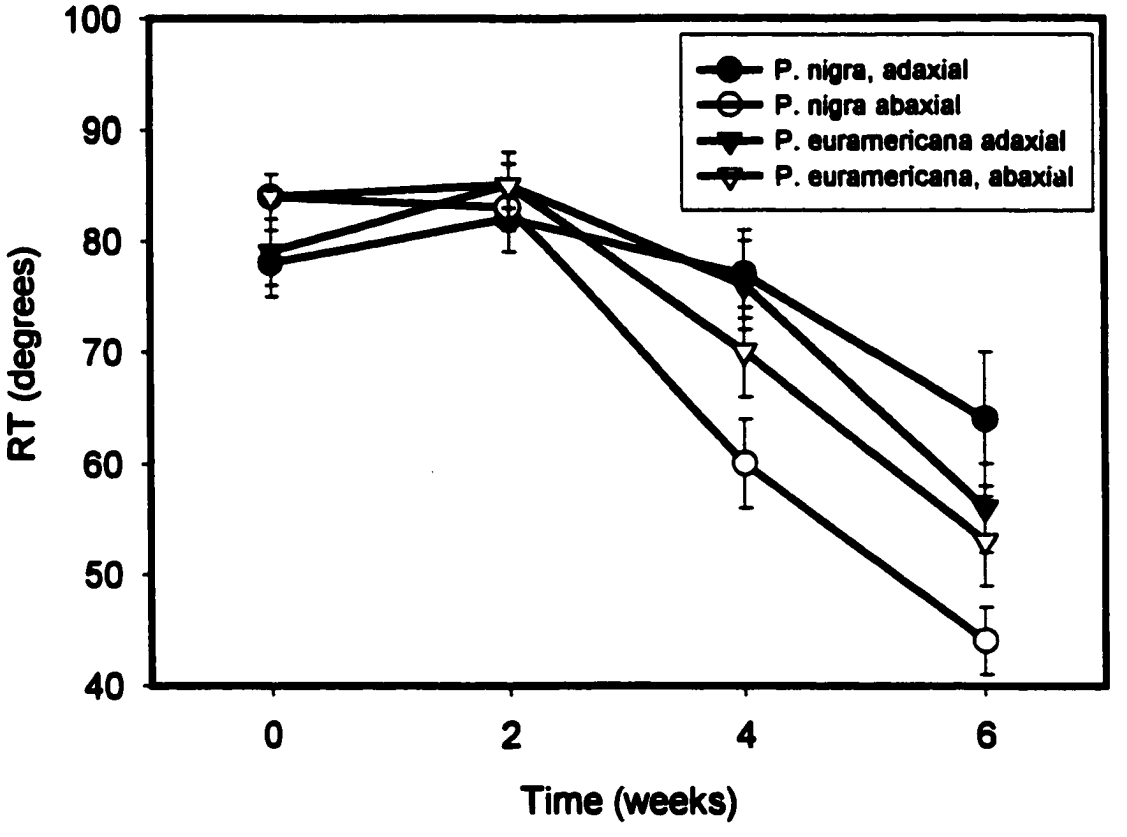


FIGURE 4.4: Droplet retention angles of poplar leaves. Data shown are for *P. nigra*, adaxial surface (solid circles) and abaxial surface (open circles), and *P. euramericana*, adaxial surface (solid triangles), and abaxial surface (open triangles). Errors bars represent 1 SE. The effect of ozone treatment was not significant, but the effect of week was significant (nested one-way ANOVA, $P<0.001$; $n=30$; 3 replicates per tree; repeated factor: week, $n=4$).

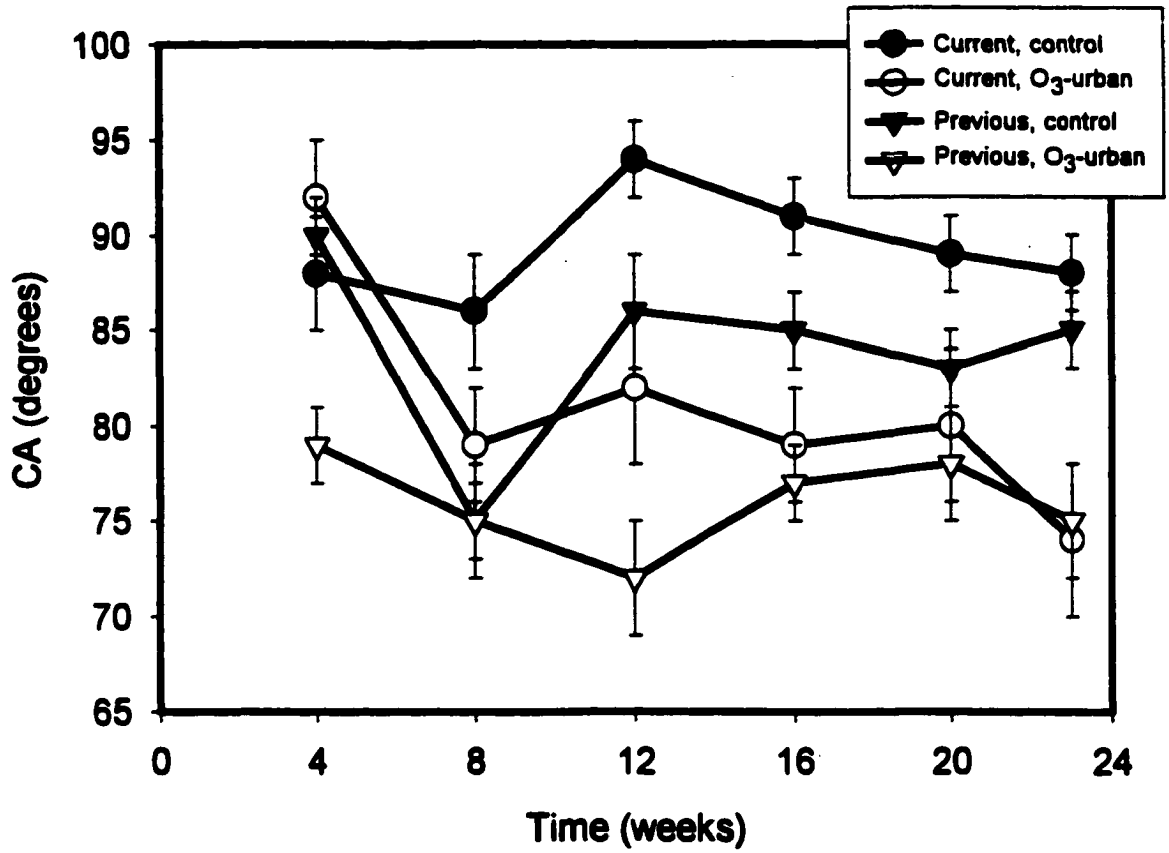


FIGURE 4.5: Droplet contact angles of Douglas fir foliage (n=4). Values shown are for current-year foliage, control (solid circles) and urban ozone exposure (open circles), and previous-year foliage, control (solid triangles) and urban ozone exposure (open triangles). Error bars represent 1 SE. (nested one-way ANOVA, $P<0.001$; 5 replicates per tree; repeated factor: week, n=6).

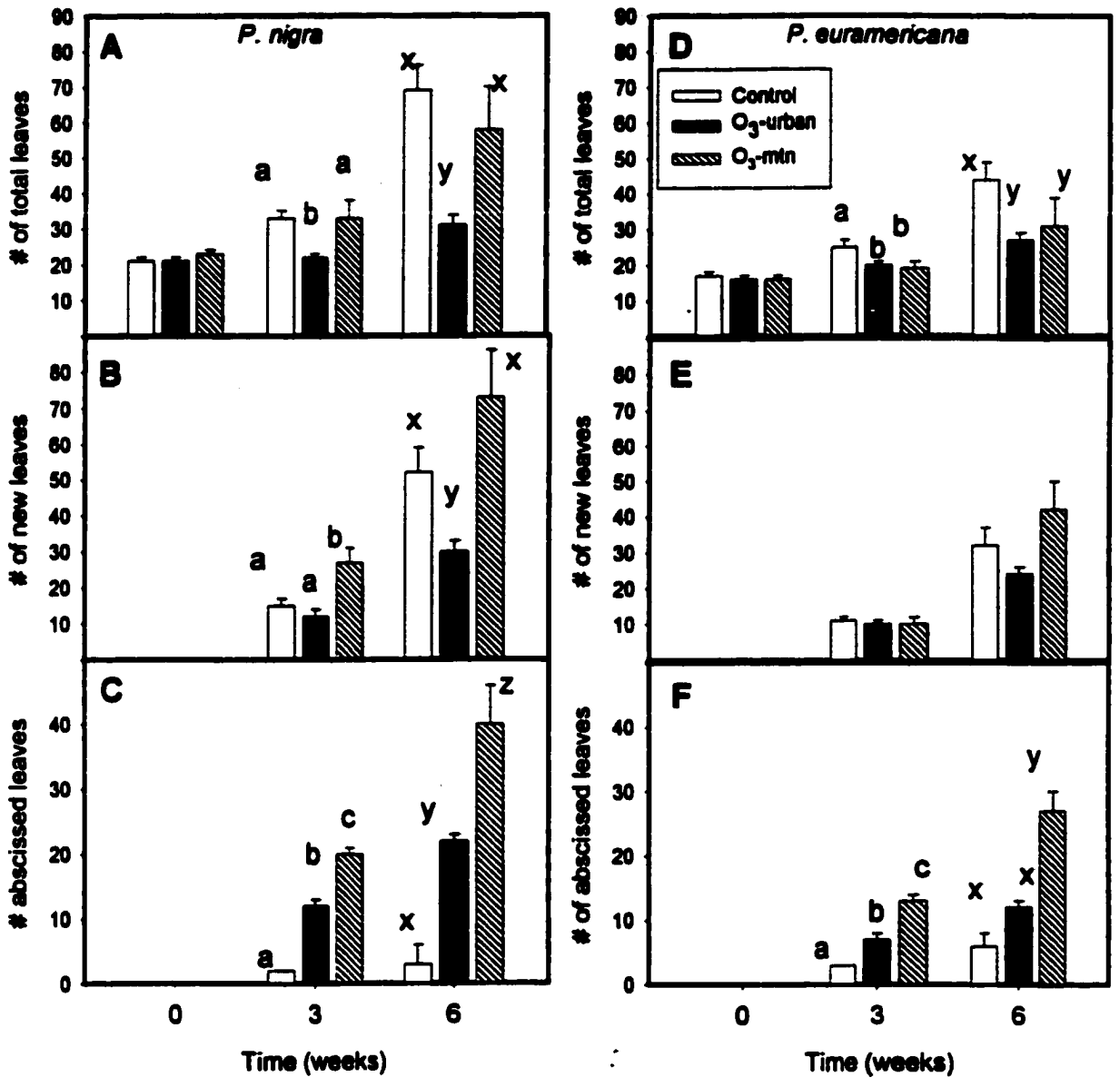


FIGURE 4.6: Leaf growth of poplar exposed to different ozone treatments. The ozone treatments were control (open bars, n=18), urban ozone exposure (solid bars, n=18), and montane ozone exposure (hatched bars, n=6). Shown are the total number of leaves per shoot (A), number of new leaves per shoot (B), and number of abscised leaves per shoot (C) for *P. nigra* as well as *P. euramericana* (D, E and F). Error bars represent 1 SE. (one-way ANOVA, P<0.001÷ repeated factor: week, n=4; comparisons within weeks).

CHAPTER 5

Interactions between leaf wetness, ozone uptake, photosynthesis, and dark respiration in poplar leaves

ABSTRACT

Leaf surface wetness events such as dew, rain and mist are common in field conditions and affect many processes over leaf surfaces. Moreover, the chemical composition of leaf surface moisture influences plant interactions with atmospheric pollutants. We evaluated the influence of leaf wetness on ozone deposition to *Populus nigra brandaris* leaves in light (PAR 420 ± 7 μmol m^{-2} s^{-1}) and dark conditions. We also examined the effects of leaf wetness on photosynthesis and dark respiration. Leaves were sprayed with ionic solutions simulating dew, acid rain, and acid mist (pH 6.2, 4.5 and 3.8, respectively). Background ozone concentrations in the leaf cuvettes averaged 102 ± 1 μg O_3 m^{-3}. Ozone deposition was highly correlated with rates of net photosynthesis (r^2=0.87, P<0.001). Leaf wetness led to increased ozone deposition by 15% in light conditions and by 170 to 240 % in dark conditions. The influence of leaf surface wetness decreased with lower pH. Leaf wetness decreased maximum net photosynthesis by ~16 percent (P<0.001) and CO_2 emission in dark conditions by 60 (pH 6.2), 82 (pH 4.5) and 100% (pH 3.8) (P<0.001). However, it was uncertain to what extent observed changes in CO_2 emissions were caused by lower dark respiration of wet leaves, or by chemical reactions of CO_2 in the aqueous phase. Ozone deposition onto wet leaves was modeled incorporating two counteracting processes, decreased ozone uptake due to lower stomatal conductance, and increased ozone deposition into the water on the leaf surface (r^2=0.86, P<0.001). In addition to significantly changed ozone deposition and CO_2 gas exchange (especially in dark conditions), the effects of leaf wetness are highly dependent on pH and chemical composition of the water present on the leaves.

- INTRODUCTION

Ozone is a nearly ubiquitous air pollutant that negatively affects many aspects plant growth (Rich, 1964; Cooley and Manning, 1987; Darrall, 1989; Turunen and Huttunen, 1990; Chappelka and Freer-Smith, 1995; Shan et al., 1996; Arbaugh et al., 1998 and it is widely considered to be one of the causal factors of forest decline in this century (McLaughlin, 1985; Maier-Märcker and Koch, 1995; Schmieden and Wild, 1995; Chappelka and Samuelson, 1998). In many regions with high ozone levels, acid precipitation in the form of rain and mist also adversely impacts vegetation through influences on photosynthesis and respiration (McLaughlin, Tjoelker and Roy, 1993), water relations (Mengel, Hogrebe and Esch, 1989; Igawa et al., 1997), and growth (Tomlinson and Tomlinson, 1990; Erisman et al., 1998). The co-occurrence of acid precipitation and ozone may be particularly problematic at higher elevations because nighttime ozone concentrations at high elevation are considerably higher than at lower elevations (Wunderli and Gehrig, 1990). In addition to the stresses imposed by more severe climatic conditions at high elevations, the health of forests may be affected adversely by high doses of ozone and acid deposition (McLaughlin, 1985; Smith and Lefohn, 1991; Thornton et al., 1993).

Leaf surface wetness in nature can occur as dew, rain and mist droplets. For example, dew is a common phenomenon during the growing season in many regions of the world. In agricultural fields, dew can last from 8 to 16 hours per day for exposed and shaded leaves respectively (Pedro and Gillespie, 1982). Fuentes et al. (1994) reported that the canopy of a deciduous forest in Ontario, Canada, remained wet due to dew and rainfall at least 50% of the time during the growing season. Leaf surface wetness due to

rain and dew also is observed frequently in montane and subalpine environments, especially in exposed habitats (Brewer and Smith, 1994 and 1997). The surface of conifer needles may be wet up to 72% of the time, both in the form of rain and thin water films (Burkhardt, 1994; van Hove and Adema, 1996). Furthermore, the formation of water films on leaf surfaces can be enhanced by the presence of small atmospheric particles (Burkhardt, 1994).

Leaf surface wetness in the form of droplets and water films affects the deposition rates of atmospheric gases and particles. For example, leaf surface wetness increased deposition rates of NH_3 and SO_2 (van Hove and Adema, 1996; Andersen et al., 1999) and NO_2 (Weber and Rennenberg, 1996) compared to dry surfaces. Leaf wetness also influences ozone deposition to plant surfaces, but reports in the literature have not been conclusive as to whether rates of ozone deposition are increased or decreased. Grantz et al. (1995) reported that there was a significant non-stomatal component to ozone uptake by a grape canopy, and that the importance of this component increased when the canopy was wet. Others have reported decreased ozone deposition on wet leaves compared to dry leaves. For example, leaf surface wetness decreased ozone deposition to an amphistomatous cotton canopy (Grantz et al., 1997), which was attributed to a decrease in stomatal conductivity over wet leaves, as the water droplets clogged up the stomata. Fuentes and Gillespie (1992) reported increased ozone deposition (by up to 40%) after applying simulated rain to red maple leaves in leaf chambers. Similarly, ozone deposition to a deciduous forest was considerably higher when the canopy was wet due to dew or rain (Fuentes et al., 1992 and 1994). Increased ozone deposition to wet leaves has been ascribed to chemical reactions with the water present on the leaf (e.g., Grantz et al.,

1995). Although the solubility of ozone in water is relatively low, it readily oxidizes many inorganic and organic compounds present in dew, rain or fog (Hoigne, 1988; Oke, Smith and Zhou, 1998), as well as many biological compounds essential to plants (Chameides, 1989; Alscher et al., 1997; Pell, Schlagnhaufer and Arteca, 1997). Therefore, the effect of leaf surface wetness on ozone deposition to a canopy will depend on the chemical consistency of the aqueous phase. Moreover, the solubility of ozone in water depends on pH and chemistry of the aqueous phase (Hoigne, 1988).

Leaf surface wetness in the form of droplets and water films also influences gas exchange of CO_2 between plants and their environment. For example, the presence of dew on the leaf surface led to decreases in net photosynthesis of soybean and pond lilies by 15% and 20%, respectively (Brewer and Smith, 1994 and 1995). Smith and McClean (1989) reported a decline of 80 % in CO_2 uptake by glabrous leaves of alpine plant when wetted by dew field and experimental conditions. Lower photosynthesis over a growing season may lead to lower production of leaf area, aboveground biomass, and seed biomass (Brewer and Smith, 1994). Photosynthetic gas exchange of *Phaseolus vulgaris* decreased up to 40 % when leaves were wetted with simulated rain, primarily by decreasing stomatal opening (Ishibashi and Terashima, 1995). Reduced rates of photosynthetic gas exchange over wet leaves can be attributed to the fact that CO_2 gas diffuses almost 10,000 times slower through water than through air (Monteith and Unsworth, 1990). In some cases the effect of leaf wetness depends on the surface of the leaf that is wetted. When simulated rain was applied to the adaxial surface of red maple leaves only, photosynthesis increased by 30%, due to increased stomatal conductance (Fuentes and Gillespie, 1992). In this case stomatal pores were on the abaxial side of the

leaf.

Because leaf surface wetness influences gas exchange between leaves and the environment, and also plays a role in deposition of atmospheric pollutants, I addressed the following questions: 1) what is the effect of leaf surface wetness on ozone deposition in both light and dark conditions; 2) what mechanisms account for observed effects of leaf surface wetness on ozone deposition rates; 3) how does leaf surface wetness influence net photosynthesis and dark respiration; and 4) how do pH and the chemistry of the aqueous phase on wet leaf surfaces influence gas exchange?

MATERIALS AND METHODS

Plant material

Current year cuttings of *Populus nigra L. cv. "brandaris"* that had been stored in darkness at –2 ^{O}C for 3 mo were transplanted to a growth chamber (T_{day} 21 ^{O}C; T_{night} 12 ^{O}C; RH 60 %; light period 14 h/d at ~ 425 μmol m^{-2} s^{-1}). After 25 d, the cuttings were transplanted into pots (10 cm diameter) using a 2:1 mixture of sand and potting soil. After the plants had rooted, they were transplanted a final time to 5.0-l containers and fertilized with 5 g of OSMOCOTE (15% N, 11% P_2O_5 and 2% MgO). Fifty days after transplanting, plants were moved to a second growth chamber (T_{day} 24 ^{O}C; T_{night} 20 ^{O}C; RH 70 %; light period 12 h/d at ~ 170 μmol m^{-2} s^{-1}). After 6 to 8 wks, individual plants were randomly chosen for fumigation and leaf wetness experiments.

Fumigation and wetness treatments

Individual leaves were fumigated in leaf chambers (2.6 l internal volume) while they remained attached to the plant (described in more detail by van Hove et al., 1988). Background ozone concentrations in empty control chambers averaged 102 $\mu g\ O_3\ m^{-3}$ (± 1 SE), and did not differ in light or dark conditions. Fumigated leaves were held in a leaf chamber for 5 to 7 d and received 12 h/d at 419 $\mu mol\ m^{-2}\ s^{-1}$ (± 7 SE). At this light intensity, plants achieved about 83 % of their maximum photosynthetic rate. Temperatures of dry leaves in light and dark conditions were 22.1°C (± 0.2) SE and 21.7°C (± 0.2 SE) respectively.

Gas exchange of O_3, CO_2, and H_2O was measured over both dry and wet leaves, and in light and dark conditions. Leaf wetness events were simulated by opening the cuvette, spraying both the upper and lower site of the leaf, and immediately closing the cuvette. Equilibrium of gas concentrations in the cuvettes after opening and closing was established within minutes, so that measured changes in O_3, CO_2, and H_2O concentrations could be fully attributed to leaf surface wetness. Leaves were sprayed with three types of water solutions: deionized water (pH 6.2) which was similar to dew (Erisman and Hey, 1991); simulated acid rain (pH 4.5) with a solute concentrations of 50 $\mu mol\ l^{-1}\ SO_4^{2-}$, 30 $\mu mol\ l^{-1}\ NO_3^-$, and 50 $\mu mol\ l^{-1}\ NH_4^+$; and simulated acid mist solution (pH 3.8) with a solute concentrations of 109 $\mu mol\ l^{-1}\ SO_4^{2-}$, 161 $\mu mol\ l^{-1}\ NO_3^-$, and 260 $\mu mol\ l^{-1}\ NH_4^+$. The pH and ion concentrations of the acid rain and mist solutions were similar to those reported for ambient conditions (Cape and Unsworth, 1988; Hertz and Bucher, 1987; Laj et al., 1997; Sheel et al., 1997b). Both acid solutions were prepared by mixing appropriate amounts of deionized water and a 95-97 % H_2SO_4 stock solution, in which

NH_4NO_3 crystals were dissolved. The size of the sprayed droplets was normally distributed with a mean diameter of 0.81 mm (± 0.48 stdev; n=300), which was slightly smaller than droplet sizes observed in the field on wheat leaves after rain events (Brain and Butler, 1985). Each treatment was repeated with four plants and for one leaf per plant. Leaves were wetted up to twice a day, at ~1200 h and ~2130 h.

Measurements

Ozone concentrations at chamber inlets and outlets were measured every 15 to 30 min with a UV (254 nm) ozone analyzer (Mon Labs 8810). Similarly, CO_2 and H_2O_v concentrations at chamber inlets and outlets were monitored with infrared gas analyzers (ADC 225 Mk 3; detection limit for CO_2<$1*10^{-5}$ µmol m^{-3}). CO_2 analyzers were calibrated using a calibrated gas cylinder, and H_2O_v using an air stream with a known amount of water vapor. The temperature of the air and the leaf in the chamber were measured using copper constantan thermocouples. The leaf area was measured with a Sky Instruments Leaf analysis system, equipped with a CCD Video camera (AEG, model XC 77 CE).

Modeling analyses

Gas exchange of CO_2, H_2O and O_3 over the leaf surface was calculated using the gas concentrations of the incoming and outgoing air in the chambers. Data on CO_2 and H_2O exchange were used to assess the nature of direct relationships between ozone uptake, CO_2 uptake, and stomatal conductance. These relationships were used to gain more insight into the mechanisms responsible for the effect of leaf surface wetness on

ozone uptake. Calculations were based on the resistance analogy with electrical resistances (Figure 5.1). The total gas flux was derived from:

$$F = \frac{f(C_{in} - C_{out})}{A} \qquad (5.1)$$

In which: F = gas flux (mass m^{-2} s^{-1})

f = air flow through the chamber (m^3 s^{-1})

C_{in} = gas concentration incoming air (mass m^{-3})

C_{out} = gas concentration outgoing air (mass m^{-3})

A = two sided leaf area (m^{-2})

The leaf total resistance of a gas, r_t (s m^{-1}), was determined by:

$$r_t = \frac{C_{out} - C_{leaf}}{F} \qquad (5.2)$$

In equation 5.2, C_{leaf} represents the gas concentration in the leaf chamber (mass m^{-3}). The H_2O_v concentration in the stomatal cavity was calculated based on the saturated water vapor pressure at T_{leaf}. The ozone concentration in the leaf was assumed to be zero (Laisk, Kull and Moldau, 1989). The total resistance for ozone (Figure 5.1) is described by:

$$r_t = r_b + \left[\frac{1}{(r_s + r_i)} + \frac{1}{(r_c + r_{ic})}\right]^{-1} \qquad (5.3)$$

Where: r_b = boundary layer resistance (s m^{-1})

r_s = stomatal resistance (s m^{-1})

r_i = internal resistance, stomatal pathway (s m^{-1})

r_c = cuticular resistance (s m^{-1})

r_{ic} = internal resistance, cuticular pathway (s m^{-1})

The boundary layer resistance of ozone and CO_2 were derived from the boundary layer resistance to water vapor, $r_{b,v}$, using the relationship:

$$r_{b,g} = r_{b,v}\left(\frac{D_g}{D_v}\right)^{-0.66} \qquad (5.4)$$

In equation 5.4, $r_{b,g}$ represents the boundary layer resistances to CO_2 or ozone. D_g is the diffusion coefficient for CO_2 or O_3, and D_v is the diffusion coefficient for H_2O_v, with values of 25, 25 and 15 $mm^{-2}\ s^{-1}$ respectively. The estimated $r_{b,v}$ was 41 $s\ m^{-1}$ based on evaporation rates of saturated filter paper in the shapes of leaves. The stomatal resistance to O_3 and CO_2 was derived from the stomatal resistance to H_2O_v, according to:

$$r_{s,g} = r_{s,v}\left(\frac{D_g}{D_v}\right) \qquad (5.5)$$

The stomatal resistance to water vapor was estimated using the Penman equation for a hypostomatous leaf (Monteith and Unsworth, 1990). Thus, the internal and the cuticular resistance to O_3, r_i and r_c, can be estimated by rewriting equation 5.3 in the form:

$$(r_t - r_b)^{-1} = (r_s + r_i)^{-1} + (r_c + r_{ic})^{-1} \qquad (5.6)$$

When $(r_t - r_b)^{-1}$ is plotted against $(r_s + r_i)^{-1}$, a straight line should result, with slope=unity and intercept $=(r_c + r_{ic})^{-1}$. Since r_t, r_b, and r_s are known, r_i and $(r_c + r_{ic})$ can be derived from the fitted curve. Finally, the deposition rate of O_3 to the leaf, $V_d O_3$, was calculated as the inverse of $r_{t,O3}$, i.e., $V_d O_3 = r_{t,O3}^{-1}$. It should be noted that the term "ozone uptake" is used to cover both ozone uptake and deposition, because it was not possible to distinguish between the two processes over wet leaf surfaces.

Leaf wetness effects on gas exchange were described using three parameters, the maximum effect, the total effect, and the duration of the effect (Figure 5.2). The

maximum effect was defined as the maximum change in gas exchange in wet conditions as a percentage of the gas exchange in dry conditions. In the case of CO_2, this maximum effect will be lower than the effect in dry conditions (Figure 5.2) because CO_2 uptake is decreased over wet leaves. The total effect on gas exchange was defined as the total integrated effect of leaf wetness on gas exchange in wet conditions as a percentage of the effect in dry conditions. The total wetness effect is shown in Figure 5.2 as the hatched area between the actual net photosynthesis curve and the lower limit of the 95% confidence interval (CI) during dry conditions. Total net photosynthesis in dry conditions equals the dry signal integrated over the same time period as the wet signal. Finally, the duration of the leaf wetness effect was calculated as the time period between the onset of leaf wetness and the first gas exchange value that was within the 95% CI during dry conditions (Figure 5.2). Note that to calculate the total effect on transpiration (E) and O_3 deposition, the upper limit of the 95% CI was used (V_d,O_3), because wetting the leaf increased E and V_d,O_3.

Modeling ozone deposition rates to wet leaf surfaces

The two processes that contribute to the changes of V_d,O_3 over wet leaf surfaces, the observed changes in net photosynthesis and a constant to correct for ozone uptake by the aqueous phase, were used to describe the effect of leaf wetness on maximum V_d,O_3 in light conditions according to:

$$V_d,O_{3,max,wet} = \left(V_d,O_{3,max}, f(psn, wet)\right) * Correction_{wet} \qquad (5.7)$$

In equation 5.7, maximum V_d,O_3 in wet conditions was estimated from the observed net photosynthesis rate in wet conditions (linear regression; r^2=0.81 to 0.87), multiplied by a

factor to correct for increased ozone deposition to the aqueous phase ($Correction_{wet}$). This correction factor was calculated from the observed increase of V_d,O_3 in dark conditions. The total V_d,O_3 was predicted according to:

$$V_d,O_{3,total,wet} = V_d,O_{3,total,dry} * \left(\frac{V_d,O_3,f(psn,wet)}{V_d,O_{3,dry,observed}} \right) * Correction_{wet} \quad (5.8)$$

Equation 5.8 describes the leaf wetness effect on total V_d,O_3 as a function of the total V_d,O_3 observed in dry conditions, multiplied by correction factors for the decrease due to lower stomatal conductance and the increase due to chemical reactions of ozone with the aqueous phase. Equation 5.8 assumes that the total influence of leaf wetness effect on V_d,O_3 due to reduced stomatal conductance is proportional to the reduction of the maximum V_d,O_3. This reduction was calculated as the ratio of the maximum V_d,O_3 in wet conditions (derived from net photosynthesis in wet conditions) to the observed V_d,O_3 in dry conditions.

Data analysis

Data were analyzed using SigmaStat (SPSS Inc., 1997). Data that met the requirements for normal distribution were analyzed using one-way analysis of variance (reported as F, P-value) with a Bonferroni post-hoc test, or a t-test (reported as T, P-value). The experimental design was a nested analysis of variance. Four leaves were tested in light and dark conditions, with four or five replicate measurements per leaf. Subsamples were not statistically different and were pooled for analysis ($P<0.05$; Sokal and Rohlf, 1997). Because several measurements were carried out on each leaf, the data also were tested with a repeated measures analysis. However, there was no significant

effect of subject or number of days in the cuvette on any of the response variables.

Data that did not meet the normality requirements were analyzed using a Kruskal-Wallis ANOVA on ranks (reported as H, P-value). This was the case for the duration of leaf wetness effects on CO_2 exchange over leaves.

RESULTS

Ozone deposition

In dry conditions $V_{d,}O_3$ was highly correlated with net photosynthesis and dark respiration (r^2=0.87, P<0.0001, n=483), and with transpiration (r^2=0.81, P<0.0001, n=483). In light conditions, 87% of ozone uptake by dry leaves was accounted for by stomatal uptake, and 13% by ozone deposition to the cuticle. In wet conditions, the cuticular component increased to 36% of total ozone uptake by leaves. Leaf wetness in light conditions increased maximum $V_{d,}O_3$ by 15% (T_{56}=2665, P<0.001), and total $V_{d,}O_3$ by 6% (Figure 5.3). There were no differences based on the pH of the solution. In dark conditions, leaf wetness increased maximum $V_{d,}O_3$ up to 237% (T_{48}=827, P<0.001), and total $V_{d,O3}$ by 150% (Figure 5.3). The effect on maximum $V_{d,}O_3$ decreased with decreasing solution pH, although this trend was not statistically significant. In light conditions, the duration of leaf wetness effects on $V_{d,}O_3$ (27 ± 2 min; Figure 5.3) was about 10 times shorter than effects on photosynthesis (Figure 5.4) and 5 times shorter than the effect on evapotranspiration (Figure 5.5). This can be explained by the chemical equilibrium between O_3 in the gaseous and the aqueous phase. This equilibrium was reached well before the water had evaporated from the leaf surface. This was confirmed by control measurements with petri dishes filled with the same solutions that were used to

wet leaves, resulting in a chemical equilibrium after 210 min (± 40 SE), while water was still present in the dish after 480 min.

Over dry leaf surfaces, there was a strong linear relationship between $(r_t-r_b)^{-1}$ and $(r_s+r_i)^{-1}$ (r^2=0.76, P<0.001). The internal residual resistance to ozone, r_i, was estimated to be -0.11s mm^{-1}, and the cuticular resistance, r_c+r_{ic} (indicated by the intercept with the y-axis) was 1.07 s mm^{-1}. Assuming that r_i equals r_{ic}, r_c was estimated to be 1.18 s mm^{-1}.

Photosynthesis

In light conditions, leaf wetness decreased maximum photosynthesis by 16% (T_{56}=4.62, P<0.001) and total net photosynthesis by 6% (Figure 5.4). Solution pH did not affect the magnitude of the response of net photosynthesis to leaf wetness, nor the duration of the leaf wetness effect (Figure 5.4). In dark conditions, leaf surface moisture led to decreases in respiration rates of 60 to 100% (T_{48}=2542, P<0.001; Figure 5.4). Interestingly, respiration rates were more affected at lower pH than at higher pH values (F_{56}=16.56, P<0.001). Leaf wetness effects on total respiration showed a similar pattern (F_{38}=8.69, P<0.001), but to a lesser extent than the maximum decrease in the respiration rate. This can be attributed partially to the shorter duration of the leaf wetness effect at lower pH (H_3=20.55, P<0.001; Figure 5.4).

Evapotranspiration

The evapotranspiration rates over dry leaves were highly correlated with net photosynthesis (r^2=0.85, P<0.0001, n=90). In light conditions, leaf wetness increased maximum evapotranspiration rates, E_{max}, by 20% (T_{56}=2684, P<0.001), and total

evapotranspiration, E_{total}, by 11% (T_{56}=861, P<0.001; Figure 5.5). The solution with the lowest pH resulted in the largest increase, but the effect lasted for the shortest period of time (Figure 5.5). In dark conditions, leaf wetness increased E_{max} up to 15 times, and E_{total} up to 9 times (Figure 5.5, pH 3.8). However, since stomates tended to be closed at night, the measured H_2O_v flux was mainly attributed to evaporation rather than transpiration in dark conditions. Effects on E_{max} and E_{total} increased with lower solution pH, but the duration of the effects decreased with lower solution pH. This may have been due to slightly smaller droplet sizes with lower pH, because evaporation rates increase as droplet sizes decrease (Butler, 1985, 1986). Leaf surface wetness effects on net photosynthesis and respiration lasted longer than the effect on evapotranspiration, by factors of 2.6 and 1.8, respectively.

Modeling ozone uptake by wet leaf surfaces

The model results suggest that leaf surface wetness decreased $V_{d,}O_3$ due to lower stomatal conductance and increased $V_{d,}O_3$ due to chemical reactions with the aqueous phase. The effects of stomatal conductance on $V_{d,}O_3$, estimated from the linear relationship between photosynthesis and $V_{d,}O_3$ (r^2=0.87, P<0.001), increased with lower pH in light conditions (Table 5.1). However, in dark conditions, when stomates were closed, leaf surface wetness and solution pH did not have consistent effects on $V_{d,}O_3$ (Table 5.1). Changes in $V_{d,}O_3$ due to chemical reactions with the wet leaf surface were increased with lower solution pH in light conditions, and were highest in dark conditions (Table 5.1). The combination of these two processes resulted in a slight increase of $V_{d,}O_3$ in light conditions, compared to dry leaf surfaces, and a considerable increase in dark

conditions (Figure 5.3).

Equation 5.7 predicted the maximum V_d,O_3 in wet conditions quite well, with values within 10% of observed values (Table 5.2). However, the relationship between predicted and observed values was only statistically significant for the solution of pH 6.2, indicating that the model performance decreased with increasing pH of the solution (Table 5.2). Predictions of the total effect of leaf wetness on V_d,O_3 in wet conditions were within 18% of observed values. However, there was a trend towards lower predicted values with decreasing pH, suggesting that the pH and chemical composition of the solution affected the performance of the model. However, the correlations between predicted and observed values were very high and not affected by solution pH.

DISCUSSION

Leaf surface wetness had significant effects on gas exchange rates of O_3 and CO_2 over poplar leaves, especially in dark conditions. In light conditions, leaf wetness increased maximum V_d,O_3 by 7% and decreased maximum CO_2 uptake by 16%. In dark conditions leaf surface wetness decreased respiration 60 to 100%, depending on solution pH, while maximum V_d,O_3 increased 2.7 to 3.4 times. The observed effect of leaf surface wetness on V_d,O_3 was the result of two counteracting processes, lower O_3 uptake via the stomata, and higher ozone deposition to wet leaf surfaces. Chemical reactions of O_3, CO_2 and O_2 with the aqueous phase have an important influence on leaf wetness effects on gas exchange.

Ozone deposition

There were strong correlations between ozone deposition, V_d,O_3 and net photosynthesis. Similarly, strong correlations have been reported between O_3, CO_2 and H_2O fluxes in field conditions (Mitic et al., 1999). This can be explained by the fact stomatal conductance is the most important factor that determines ozone uptake by plants (Neubert et al., 1993; Wieser and Havranek, 1993; Wang et al., 1995; Fredericksen et al., 1996; van Hove et al., 1999). This is especially true for dry poplar leaves, where 87 % of V_d,O_3 was explained by stomatal uptake. The contribution of the cuticle to total ozone uptake increased from 13% in dry conditions to 36% in wet conditions. This is in agreement with results by Grantz et al. (1995), who reported a considerable non-stomatal component to V_d,O_3 in natural canopies, which increased when the canopy was wet due to rain or dew.

In light conditions, V_d,O_3 averaged 0.50 cm s^{-1} (± 0.01 SE) and 0.57 (± 0.01 SE) in dry and wet leaf conditions, respectively. These values agree well with ozone deposition rates observed in both laboratory (Fuentes et al., 1994) and field conditions (Fuentes et al., 1994; Grantz et al., 1995; Massman and Grantz, 1995; Padro, 1996; Zhang, Padro and Walmsley, 1996; Grantz et al., 1997; Massman et al., 2000). In dark conditions V_d,O_3 to dry leaves averaged 0.13 cm s^{-1} (± 0.01 SE), which was similar to values reported for leaves with closed stomata (0.14 cm s^{-1} ± 0.10 SE; Kerstiens and Lendzian, 1989a). Zhang et al. (1996) reported similar values of V_d,O_3, ranging from 0.10 to 0.20 cm s^{-1}, for deciduous forest, grape and cotton canopies during dry nighttime conditions. Average observed values of V_d,O_3 over wet poplar leaves in the dark were

~0.36 cm s^{-1} (± 0.01 SE), which agrees well with observed nighttime ozone deposition rates to wet cotton and grape canopies, which ranged from 0.25 to 0.60 cm s^{-1} (Grantz et al., 1995 and 1997). Although ozone deposition to plant surfaces at night was considerably lower than during the day, nighttime ozone deposition caused similar or more foliar injury compared to damage during the daylight conditions (Musselman, Tamara and Minnick, 2000).

The 15% increase of in V_d,O_3 in light conditions is in the lower range of values observed in field conditions, which vary from 1.1 to 2.4 times greater after dew, and 1.4 to 3.0 time greater after rain (Fuentes and Gillespie, 1992; Fuentes et al., 1992 and 1994; Grantz et al., 1995). In darkness, maximum V_d,O_3 increased by a factor of 2.7 to 3.4 due to leaf wetness. This agreed well with observed increases of V_d,O_3 due to dew in the field, varying from 1.1 (Grantz et al., 1995), to 3 - 6 times as much (Fuentes et al., 1992 and 1994). When the increase in V_d,O_3 was expressed as a percentage of the predicted V_d,O_3 based on the decrease in net photosynthesis, values from this study on poplars agreed very well with the literature, both in light and dark conditions (Table 5.1).

In light conditions, the pH and ion concentrations of the sprayed solution did not affect response of V_d,O_3. However, in dark conditions the effect of leaf surface wetness on V_d,O_3 decreased with lower pH. The percent change of V_d,O_3 due to leaf wetness was proportional to the concentration of NH_4^+ ions in the solution, suggesting that ozone reacted with NH_4^+. Ozone is a strong oxidant, (Hoigne, 1988; Oke, Smith and Zhou, 1998), and readily oxidizes NH_4^+ to form N_2, NO, N_2O, and NO_2. Increased rates of chemical reactions of ozone with the water solution due to the presence of NH_4^+ ions also appeared to shorten the time to needed to establish a chemical equilibrium with the

aqueous phase. This decreased the synergistic effect of leaf surface wetness on V_d,O_3, in agreement with shorter half-life times of ozone in the aqueous phase in solutions with higher concentrations of compounds that can be oxidized (Graham, 1997). However, although the chemical composition of the solution influenced the response of maximum V_d,O_3, there were no differences in the effects on total V_d,O_3.

In light conditions, the influence of leaf surface wetness on V_d,O_3 was 4 to 5 times shorter than the effect on evapotranspiration and the time period in which water droplets could be visually observed on the leaf surface, suggesting that a chemical equilibrium was established with the aqueous phase well before water had evaporated from the leaf.

Photosynthesis

Net photosynthetic rates in dry conditions (10.8 μmol m^{-2} s^{-1} ± 0.3 SE) were in the lower range of maximum photosynthetic rates reported for *Populus nigra brandaris* (Reichenauer et al., 1997) and other *Populus* clones (Ceulemans and Impens, 1983). Dark respiration rates, -0.5 μmol m^{-2} s^{-1} (± 0.01 SE), also were lower than those reported in the literature (Ceulemans and Impens, 1983). The lower rates of maximum net photosynthesis and dark respiration observed in my study can be explained by lower light intensities (170 - 420 μmol m^{-2} s^{-1}) compared to Ceulemans and Impens (1400 μmol m^{-2} s^{-1}; 1983). The observed decrease in net photosynthesis due to leaf wetness is similar to data reported by Brewer and Smith (1994 and 1995) and Ishibashi and Terashima (1995), where leaf surface wetness from rain and dew decreased net photosynthesis by 15 to 20% and 30 to 40% respectively.

A potential confounding factor in the interpretation of these results is that the methods did not allow to distinguish between interactions of CO_2 with the aqueous phase and effects of leaf wetness on stomatal opening. However, Ishibashi and Terashima (1995) reported that the decrease in CO_2 uptake observed after simulated rain could be attributed to stomatal closure, injury to photosynthetic membranes and to some extend to a limitation of CO_2 diffusion in the water film. Similarly, spray-misted leaves showed a decline in photosynthetic gas exchange up to 80 % for alpine plant species with wettable leaf surfaces. Moreover, experiments with metal models in the shape of poplar leaves showed that wetting of these leaves led to a small increase of CO_2 deposition (data not shown). Thus, reactions of CO_2 with the water present on a real leaf would have led to increased apparent photosynthesis, which is opposite of what we observed. Thus, it appears that the decrease in CO_2 uptake observed in light conditions could be attributed to lower photosynthesis, which may have been caused by stomatal closure, as well as limitations to CO_2 diffusion in the water film (Ishibashi and Terashima , 1995). Moreover, since reactions of CO_2 in the aqueous phase tended to increase apparent CO_2 deposition rates, the observed effects on photosynthesis may have been underestimated by ~3 %.

Dark respiration

Interestingly, in my study, CO_2 exchange by leaves that were wet during the night was reduced by 60 to 100 %, suggesting that leaf surface wetness strongly decreased rates of dark respiration of poplar leaves. A possible explanation of this phenomenon is the limited diffusion of O_2 gas into the leaf over wet leaf surfaces; O_2 has a diffusion

coefficient through water that is similar to that of CO_2, ~10,000 times slower through water than through air (Monteith and Unsworth, 1990). The effect of leaf wetness on gas exchange in dark conditions could be estimated within 5% of observed values (r^2=0.96) based on the percent change of CO_2 exchange rates during the day, the absolute gas fluxes in light and dark conditions, and the diffusion coefficients of CO_2 and O_2 in air and water. This also suggested that dark respiration rates of wet leaves were limited by O_2 diffusion into the leaf. Since the overall gas flux at night is considerably lower than during the day, the reduction of O_2 uptake at night will have a relatively larger impact on plant respiration than the reduction of CO_2 uptake during the day has on net photosynthesis.

However, as mentioned before, spraying metal leaf models led to a small increase on CO_2 deposition, suggesting a chemical interaction CO_2 with the aqueous phase. Thus, reduced CO_2 emission from wet leaves in dark conditions may be partially due to chemical reactions of CO_2 in the water phase. Future research will need to address the causal mechanisms of the response of CO_2 emission to leaf wetness in dark conditions. To what extend is this observation caused by CO_2 gas reacting with the aqueous phase? If this phenomenon is indeed caused by lower dark respiration rates of plants, what are the consequences for plant metabolisms? Impacts of leaf wetness on nighttime respiration have received little attention. This is a particularly important area for study given the potentially higher repair costs for leaves damaged by ozone exposure. For practical purposes I will continue to refer to the effects of leaf wetness on CO_2 emission in the dark as respiration in the remainder of this paper.

The effect of leaf wetness on respiration increased with lower solution pH. This

may be attributed to reaction of O_2 with the NH_4^+ ions present in these solutions because of the reaction of O_2 with NH_4^+ (to form N_2, N_2O and NO_2). An alternative explanation depends on the reaction of CO_2 gas with the aqueous phase, which may have caused lower emissions of CO_2 gas from a leaf. However, at low pH, CO_2 tends to gas out of the solution on the leaf, leaving carbonic acid mainly present in the un-dissociated form of H_2CO_3. Therefore, one would expect that the effect of leaf surface wetness would decrease with lower pH. Since the opposite was observed, lower respiration rates of wet leaves were probably caused by limited O_2 diffusion. Interestingly, the duration of leaf wetness effects on dark respiration also decreased with lower pH (Figure 5.4). This may be related to increased degassing of CO_2 from the aqueous phase with lower pH.

The duration of leaf surface wetness effects on net photosynthesis and dark respiration were considerably longer than the effects on evapotranspiration. In light conditions, the effects on net photosynthesis lasted about 2.5 times longer than both the effects on evapotranpiration and the visual presence of water droplets on the leaf surface. The effects on dark respiration lasted about 1.8 times longer than the effects on evapotranspiration. These results suggest that after droplets have disappeared from the leaf surface, a residual water film could remain on the leaf surface, and continue to influence the exchange of CO_2 over the leaf surface. This is in agreement with results by Burkhardt (1994), who reported that water films are present on conifer needles more than twice as long as visible water can be observed on the surface. The thickness of water films on leaf surfaces increases with decreasing vapor pressure deficit in the air (Hove and Adema, 1996).

Modeling ozone deposition to wet leaf surfaces

In light conditions ozone deposition to wet leaf surfaces was influenced by two counteracting processes: a decrease in V_d,O_3 due to decreased stomatal conductance and an increase in V_d,O_3 due to enhanced ozone deposition to the wet cuticle (e.g., Grantz et al., 1995 and 1997). The model combining these two factors, equation 5.7, predicted both maximum and total V_d,O_3 during leaf wetness events reasonably well (within 10% of the observed values; Table 5.2). However, correlation coefficients between observed and predicted V_d,O_3 decreased with lower solution pH, suggesting that chemical reactions of O_3 and CO_2 with the solutions decreased model performance. This approach may prove useful to model V_d,O_3 over wet leaf surfaces in light conditions, using observational data on photosynthesis, V_d,O_3 over dry leaf surfaces in light conditions and V_d,O_3 over wet leaf surfaces in dark conditions. Another, more accurate approach would be to derive estimates of V_d,O_3 over wet leaves from the relationships between photosynthesis, stomatal conductance to H_2O_v, and stomatal conductance to ozone. An advantage of the latter approach would be that ozone deposition to the cuticle and uptake by stomata can potentially be estimated separately. However, this approach did not work too well for my data, and estimates of ozone deposition to the cuticle varied widely.

CONCLUSION

Ozone deposition increased when leaf surface were wetted with simulated dew, rain or mist. In light conditions, this increase was low, but in dark conditions it was substantially higher compared to dry conditions. Two counteracting processes explain the observed increase in ozone deposition due to leaf surface wetness. First, droplets and

water films present on leaf surfaces may interfere with stomata, thus decreasing stomatal conductance and V_d,O_3. Second, leaf surface wetness increased ozone deposition to the cuticle, the non-stomatal component of V_d,O_3. Models combining these processes performed well in predicting V_d,O_3 to wet leaves in light conditions. This study also showed that leaf surface wetness led to significant decreases in photosynthetic gas exchange over leaf surfaces. Moreover, CO_2 emissions in dark conditions decreased considerably for wet leaves. At this point it uncertain to what extent observed changes in CO_2 emissions are caused by actual changes in dark respiration of plants, or by chemical reactions of CO_2 in the aqueous phase. There was a strong interaction between leaf wetness effects on exchange of ozone and CO_2 over leaves with the pH and chemical composition of the solution present on the leaf surface. More work is needed to better understand the effects of leaf surface wetness and the chemical composition of the aqueous phase, on the exchange of gaseous air pollutants and CO_2 gas over natural canopies and crops. It is important to incorporate leaf wetness effects into ozone deposition models to predict ozone deposition to plant canopies more accurately. Finally, little is known about effects of leaf wetness in respiration rates in the dark. My research suggested that dark respiration could be reduced considerably due to leaf surface wetness. This may be particularly important for ozone exposed leaved, that may have higher repair costs than healthy leaves. Given the potential impacts of leaf surface wetness on respiration rates at night, more research is needed in this area.

TABLE 5.1: Relative contributions of effects of stomatal conductance, $G_{stomata}$, and chemical reactions at the leaf surface to the overall effect of leaf surface wetness on ozone deposition to poplar leaves. Data are shown for light and dark conditions, and solutions of pH 6.2, pH 4.5 and pH 3.8 (n=4 leaves with 4 to 5 replicate measurements per leaf, ± 1 SE). Predicted $V_{d,O3}$ over wet leaves was derived from the CO_2 flux. The percent change in $V_{d,O3}$ over wet leaves due to stomatal closure (indicates as $G_{stomata}$) is calculated as the ratio of $V_{d,O3}$-(wet, predicted) over $V_{d,O3}$-(dry, Observed), and the flux to the wet leaf surface as the ratio of $V_{d,O3}$-(wet, observed) over $V_{d,O3}$-(wet, predicted).

Conditions	Ozone deposition rate, $V_{d,O3}$ (cm s^{-1})			Change in $V_{d,O3}$ (%)	
	Dry, observed	Wet, observed	Wet, predicted	$G_{stomata}$	Surface
Light					
pH 6.2	0.46 ± 0.03	0.53 ± 0.04	0.36 ± 0.01	80 ± 3	145 ± 6
pH 4.5	0.48 ± 0.03	0.53 ± 0.03	0.33 ± 0.01	71 ± 4	161 ± 10
pH 3.8	0.56 ± 0.02	0.64 ± 0.02	0.32 ± 0.01	58 ± 1	201 ± 8
Dark					
pH 6.2	0.09 ± 0.01	0.28 ± 0.03	0.08 ± 0.01	101 ± 12	345 ± 36
pH 4.5	0.12 ± 0.01	0.37 ± 0.02	0.08 ± 0.01	67 ± 4	483 ± 34
pH 3.8	0.15 ± 0.01	0.39 ± 0.02	0.15 ± 0.01	114 ± 29	421 ± 52

TABLE 5.2: Predicted V_d,O_3 to wet leaves in light conditions as a percentage of the observed V_d,O_3 for solutions of pH 6.2 (n=4, 5 replicates per leaf), pH 4.5 (n=4, 4 replicates per leaf) and pH 3.8 (n=4, 5 replicates per leaf) (± 1 SE). Statistics shown are the correlation coefficients using linear regression analysis between predicted and observed values of V_d,O_3 (r^2, P-value).

Factor	Predicted/Observed V_d,O_3, % (r^2, P-value)		
	pH 6.2	pH 4.5	pH 3.8
Maximum V_d,O_3	100 ± 4	99 ± 6	91 ± 3
	0.86, P<0.001	0.40, P=0.088	0.35, P=0.130
Total V_d,O_3	118 ± 4	103 ± 5	98 ± 3
	0.99, P<0.001	0.99, P<0.001	0.98, P<0.001

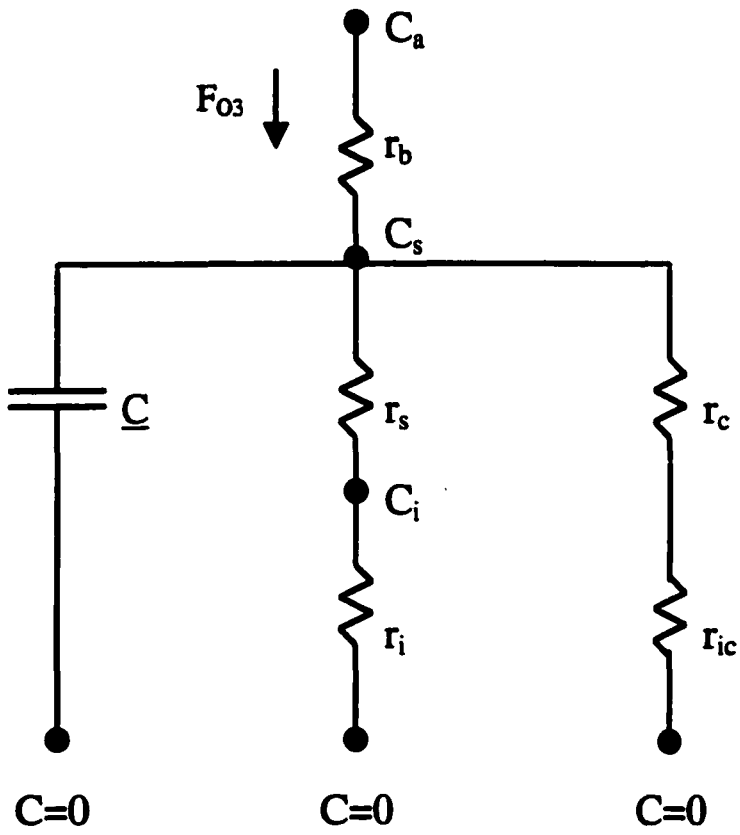


Figure 5.1: A resistance analysis of ozone uptake by one side of a leaf. F_{O3} represents total ozone flux into the leaf. C_a, C_s and C_i represent ozone concentrations in the ambient air, at the leaf surface and in the substomatal cavity of the leaf. The uptake resistances are the boundary layer resistance, r_b, stomatal resistance, r_s, internal resistance via the stomatal pathway, r_i, cuticular resistance, and r_c, internal resistance via the cuticular pathway, r_{ic}. The capacity, $\underline{C}$, represents adsorption of ozone to the leaf surface.

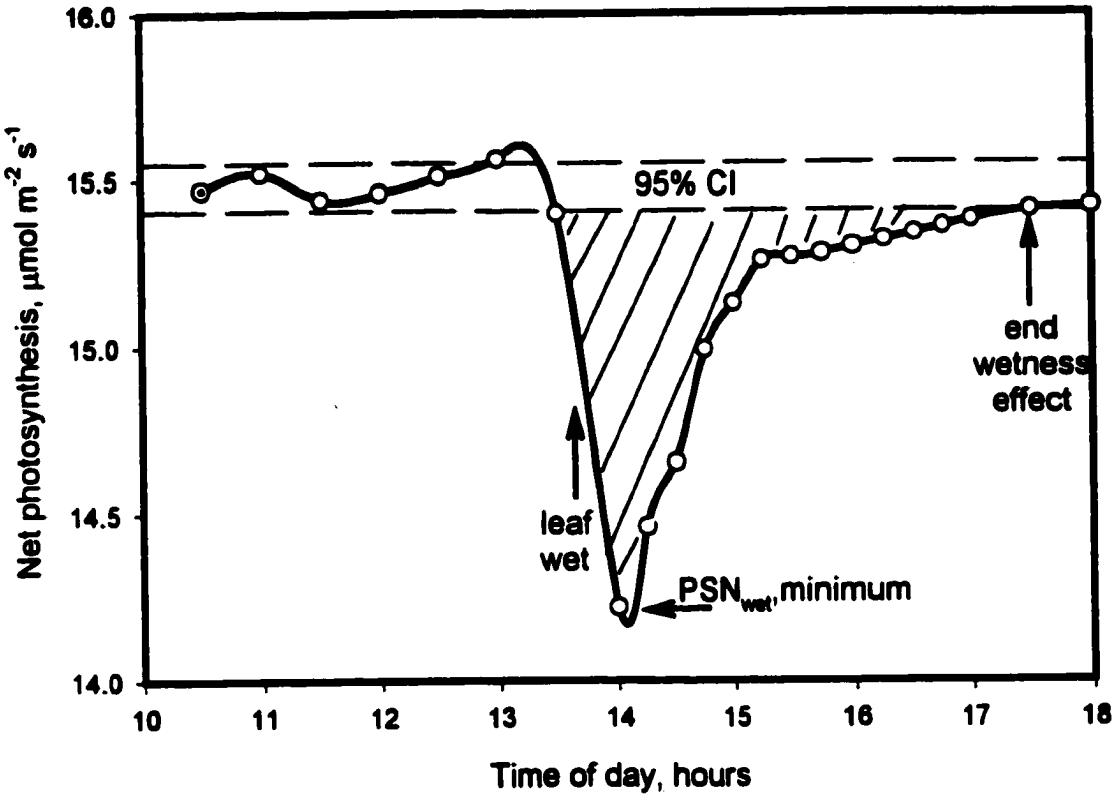


Figure 5.2: Response of net photosynthesis (PSN) to leaf surface wetness. Measurements are indicated as open circles and the line represents a fitted curve. The dashed lines indicate the limits of the 95 % confidence interval (95 % CI) of PSN in dry conditions. Arrows indicate when the leaf was wetted, the maximum effect on PSN and when PSN values returned to dry background values. The total effect of leaf wetness on PSN is shown as the hatched area between the PSN curve and the lower limit of the 95 % CI. The duration of the leaf wetness effect is calculated as the time between when the leaf was sprayed and when PSN values returned to the lower limit of the 95 % CI of PSN in dry conditions.

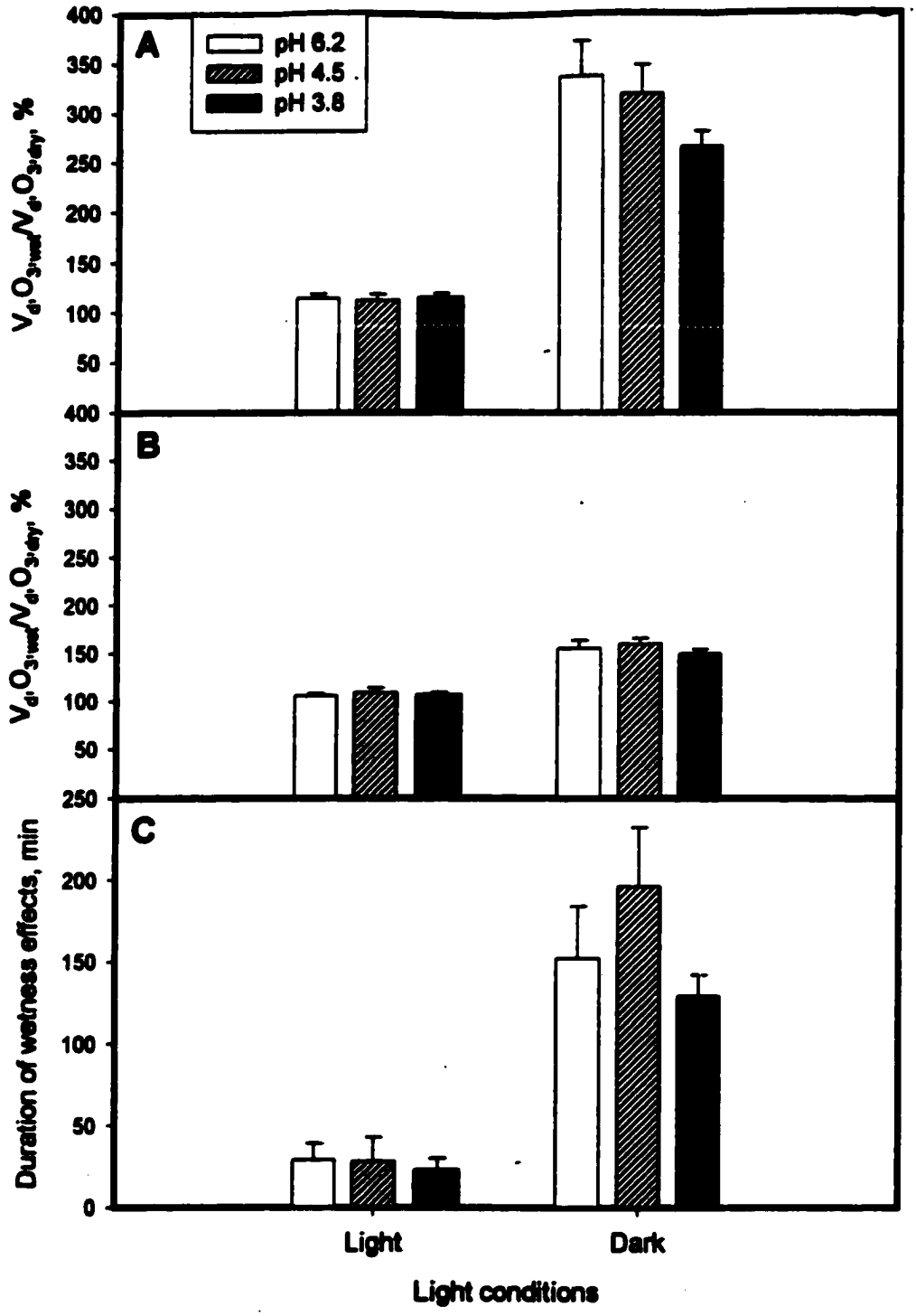


Figure 5.3: Ozone deposition rates in wet conditions, $V_d,O_{3,wet}$, as a percentage of dry conditions, $V_d,O_{3,dry}$, for maximum O_3 deposition (A) and total O_3 deposition (B), and the duration of the wetness effect (C). Solution pH values were 6.2 (white bar; n=4 with 5 replicas per leaf), 4.5 (hatched bar; n=4 with 5 replicas per leaf), and 3.8 (double hatched bar; n=4 with 5 replicas per leaf).

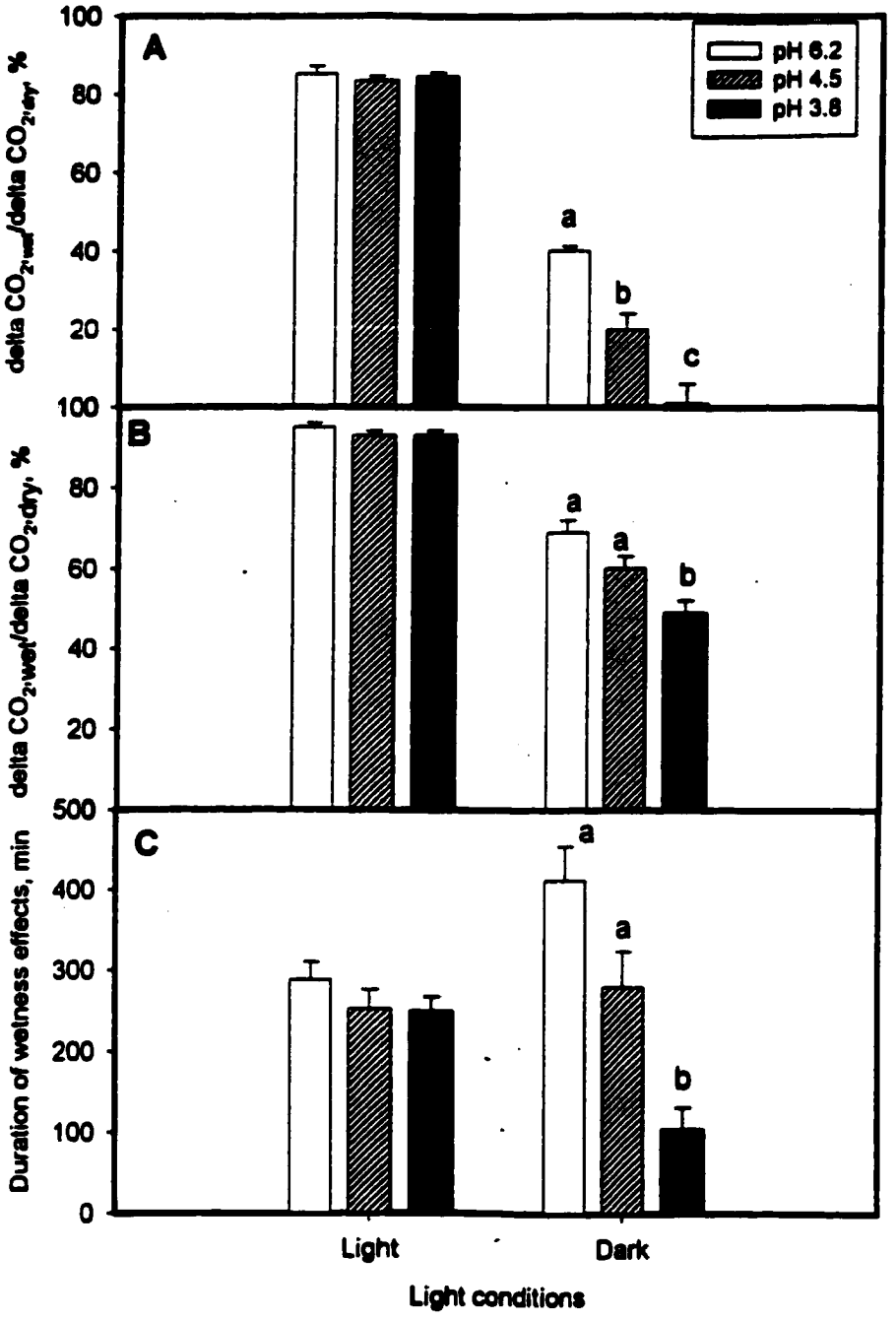


Figure 5.4: CO_2 gas exchange rates in wet conditions, $\Delta CO_{2,wet}$, as a percentage of dry conditions, $\Delta CO_{2,dry}$, for maximum CO_2 exchange (A) and total CO_2 exchange (B), and the duration of the wetness effect (C). Solution pH values were 6.2 (white bar; n=4 with 5 replicas per leaf), 4.5 (hatched bar; n=4 with 5 replicas per leaf), and 3.8 (double hatched bar; n=4 with 5 replicas per leaf). Letters indicate statistically significant differences between water treatments within the same light conditions (nested one-way ANOVA; subsamples pooled; $P<0.001$).

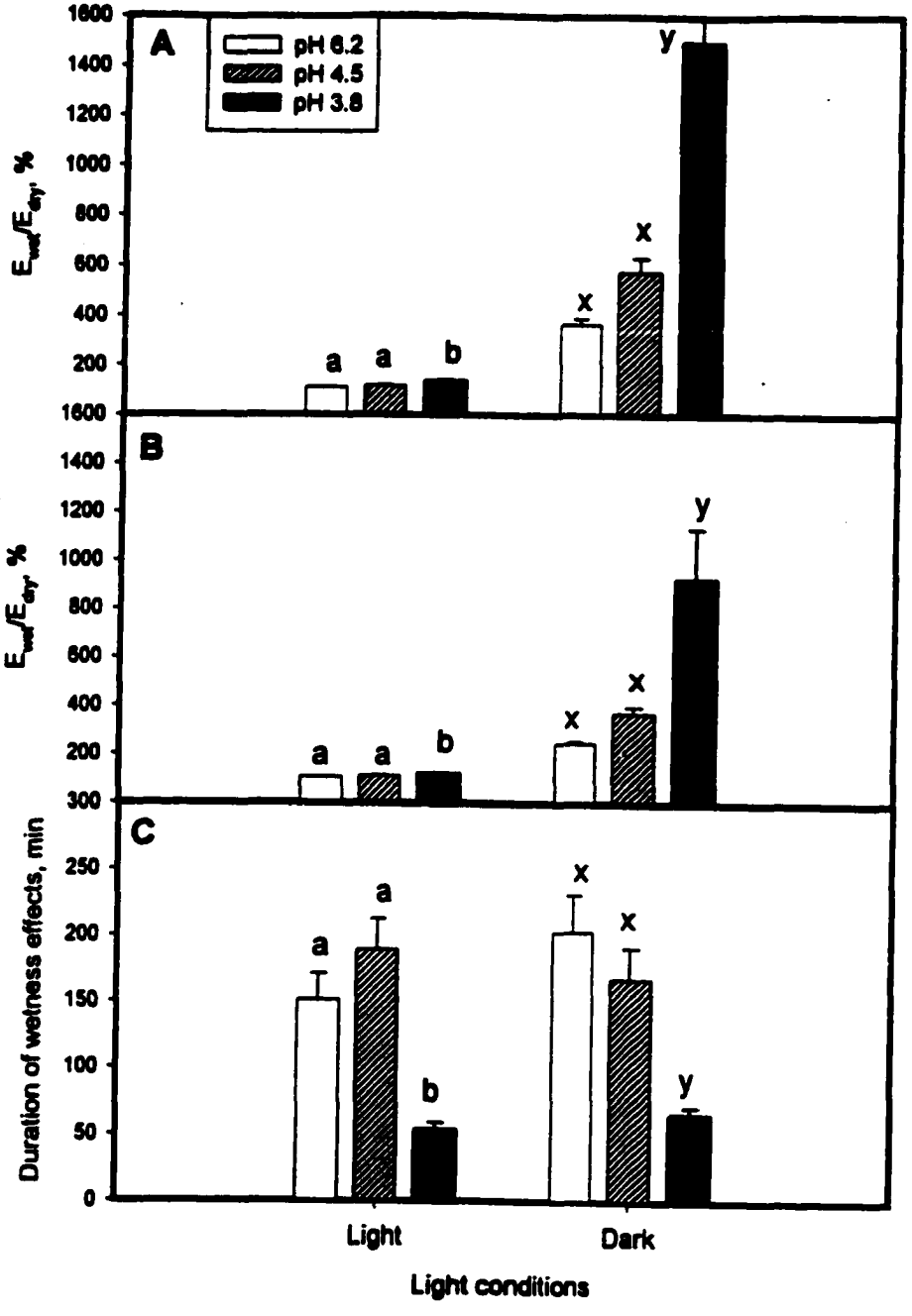


Figure 5.5: Evapotranspiration rates in wet conditions, E_{wet}, as a percentage of dry conditions, E_{dry}, for maximum H_2O_v exchange (A) and total H_2O_v exchange (B), and the duration of the wetness effect (C). Solution pH values were 6.2 (white bar; n=4 with 5 replicas per leaf), 4.5 (hatched bar; n=4 with 5 replicas per leaf), and 3.8 (double hatched bar; n=4 with 5 replicas per leaf). Letters indicate statistically significant differences between water treatments within the same light conditions (nested one-way ANOVA; subsamples pooled; $P<0.001$).

CHAPTER 6

Air pollution and forest growth: Impacts on local and regional scales

Humans have a long history of causing air pollution and since the industrial revolution at the end of the 1800's, not only has the production volume of goods increased, but emissions of pollutants into the environment have increased dramatically as well. Especially in densely populated urban areas, the effects of air pollution have been felt. For example, in London in the 1950's, the combination of stagnant winter weather conditions and high levels of emissions from industry and coal-heated houses led to severe air pollution and hundreds of deaths due to lung diseases.[1] These winter episodes of high levels of air pollution are referred to as SMOG, an abbreviation of the words "smoke" and "fog". In a region formerly known as Eastern Europe, chronic exposure to smog episodes similar to those of London has led to a higher incidence of respiratory diseases such as asthma, and shorter life expectancies compared to less polluted regions in (Eastern) Europe.[2] Interestingly, the first effects of air pollution on plants also were reported in the late 1950's and our knowledge of these effects has increased since then as well. This is due to more advanced technology and research equipment for detecting influences, but it is also a consequence of the increased volume of pollutants that is emitted into the air each year. The ways in which air pollution affects vegetation vary from acute severe impacts, to chronic and more subtle effects. Moreover, impacts may be felt from local to regional and global scales. There are many different types of air pollutants and many avenues for exposure. Two common chemicals, chlorine gas and ozone, illustrate the diversity of impacts as well as scales of influence that air pollutants

have on plants. These two air pollutants have similar effects on plants, but very different types of exposure.

Air pollution on a local scale: Effects of chlorine gas on vegetation

Chlorine gas, an element consisting of two chloride atoms (Cl_2), is used on a large scale in our society. If we would stop using chlorine gas today, the results would certainly be felt: no more white paper for books and printers, no more "whiter than white" diapers and sheets, certain medicines would not exist, and can you imagine life without PVC plastics?[3] Moreover, swimming pools would no longer have that familiar smell that we all try to wash off after a swim, and there could be hazards for biological infections from unsanitary water. Drinking water may not be safe for that same reason.[4]

So, as a society, we certainly have benefited from many applications of chlorine gas. However, this has not been without risk, because chlorine gas is highly toxic to humans. Health effects of chlorine gas exposure vary from eye, nose and throat irritation, to severe lung damage and even death, depending on the concentration and the exposure period.[5] The long-term effects of chlorine gas on health are less well documented, and consist of long-term respiratory problems and damage to the central nervous system. For example, subjects exposed to chlorine gas have complained of symptoms such as lightheadedness, extreme fatigue, sleep problems, irritability, loss of concentration and memory, mood swings, decreased alcohol tolerance, loss of appetite, and depression.[5,6] Most human exposures to chlorine gas are accidental of character. But are these accidents frequent enough to worry about? Yes. A study in 1996 reported 138 accidental releases involving chlorine gas over a period of 3 years in 9 states in the USA (an average of

about 5 accidents per state per year!). Of these releases, about 25 percent involved human injuries, and about 30 percent led to evacuations.[7] And considering that the production volume of chlorine gas is expected to increase over the next decade,[3] we can expect the risk of accidents involving chlorine gas to increase as well.

So why is chlorine gas so toxic? In contact with water, chlorine gas forms hydrochloric acid (HCl) and hypochloric acid (HOCl). Hypochloric acid is also known as household bleach, the substance that we buy to get our laundry just a little whiter, and to keep our bathrooms free from germs. We are also familiar with the warning labels attached to bleach bottles. Given that hydrochloric acid is an even stronger acid than bleach, it is logical that exposure to such strong acids can readily damage our health. It is this characteristic of chlorine gas, the formation of highly acid solutions, that makes chlorine gas toxic to vegetation as well as well as humans.

What do we know about the effects of chlorine gas on plants? Most reports on chlorine gas effects on plants have focused on visible injury symptoms. These symptoms consist of bleaching of leaf tissues, called chlorosis, and the death of leaf tissues. The latter is called necrosis, and occurs in a streaky pattern in grasses, and as brown spots on leaves of herbs and shrubs. In coniferous trees, such as pines and firs, necrosis starts at the tip of the needle and eventually extends to the needle base. Once leaves or needles are completely brown (or dead), they are generally dropped by the plant, resulting in a bare appearance, and considerably lower leaf material than healthy plants.[8] The amount of chlorine gas that is taken up by plants, as well as the extent of leaf injury, depends on the concentration and duration of exposure, as well as environmental and weather conditions.[9]

Effects of chlorine gas exposure on plants have only been explored in short-term studies. Thus, the acute effects of chlorine gas on plants are reasonably well understood, but long-term effects have hardly been studied at all. What might some of these long-term effects be? How long after exposure do these effects persist? To begin answering these questions, we began a study that lasted 3 years after a major chlorine spill. Our study began after a train derailment released about 60 tons of chlorine gas into the atmosphere near Alberton, MT, in April 1996. This release was the second largest railroad accident involving chlorine gas in US history, and led to the evacuation of about 1000 people, and medical treatment of over 350 people.[10] The chlorine gas, in combination with ample atmospheric moisture, led to the formation of a highly acidic cloud. Moreover, the dispersion of the chlorine cloud was limited due to low wind speed, which led to highly concentrated chlorine gas cloud that persisted for several days in a very small region. Moreover, the spill happened in a narrow mountain valley, which enhanced the adverse effects of the spill on humans, since there was only one escape route available to local residents. In addition to the tragic toll on human health, this derailment also exposed a coniferous forest, consisting mainly of Douglas fir (*Pseudotsuga menziesii*) and Ponderosa pine (*Pinus ponderosa*) to the toxic gas cloud. These two species of conifers are characteristic for this region of the Rocky Mountains.

So, what were some of the effects of this chlorine release on these conifers? The visible symptoms one month after exposure were similar to those reported after other spills. Douglas fir and Ponderosa pine showed bleached leaves (chlorosis) and leaf death (necrosis). How about changes to conifer needles that could not be seen with the naked eye? Both conifer species showed changes in the characteristics of their exposed needle

surfaces. Leaf and needle surfaces of most plants species are covered by a waxy layer, the cuticle, which protects plants against excessive water loss and infection by pathogens, such as bacteria, viruses and fungi.[11] Chlorine gas changed these waxy layers. For example, water droplets tended to spread out more on Douglas fir needle surfaces that were exposed to chlorine gas compared to surfaces that were not. Interestingly, this was also the case for exposed foliage that did not show signs of injury to the naked eye, and for foliage that flushed out after the chlorine gas had subsided. What does this mean? First, foliage was affected even when there was no visible injury. Second, foliage on exposed trees that was hidden in winter buds also was affected, suggesting that chlorine gas can have both indirect and direct negative influences in plants.

This leads us the question of whether or not these cuticular changes have implications for other aspects of tree health. As mentioned earlier, cuticles form a barrier that help prevent excessive water loss from foliage. Our experiments showed that needles on trees exposed to chlorine gas lost more water through the cuticle. Moreover, directly exposed foliage of both conifer species had lower total water content in the leaf tissue compared to needles that were not gassed by chlorine. During the daytime, the influence of this damage is relatively low since most of the water lost by trees is through pores in the leaf surface, called stomata. However, during the night, these stomatal pores are closed, and the exposed trees lost water predominantly through the cuticle. In summer, when western Montana often experiences drought conditions, loosing precious water through a damaged cuticle may predispose trees to drought stress and subsequent death of needles. This was confirmed by observations in the field during the summer, when foliage on exposed trees showed signs of drought damage, such as brown needles.

Eventually many needles fell off the trees. In comparison, trees at places the chlorine cloud did not reach did not have these drought symptoms. The drought susceptibility of exposed trees remained higher up to 1 to 2 ½ years after they were exposed to chlorine gas.

As mentioned earlier, conifer foliage near the site of chlorine gas release became necrotic, and subsequently dropped off the tree. This may have had an important effect on tree growth because trees need leaves for photosynthesis. How does photosynthesis work? Plant leaves use sunlight and atmospheric carbon dioxide to produce sugars, which are used in the leaf or exported to other plant tissues. Eventually these sugars are broken down into carbon dioxide and water when the plant uses them for energy. Breaking sugars down provides plants with the energy needed to maintain their living tissues (also called respiration), grow, and reproduce. Plants that lost a lot of leaves may not produce enough energy to survive, grow and produce seeds. So what can be expected when a conifer that carries needles produced over several years (four years for Ponderosa pine and nine years for Douglas fir) looses most of its needles? They lose their ability to photosynthesize, or in other words, to acquire energy from their environment. Thus, we expected the loss of foliage to result in lower growth rates for trees exposed to chlorine gas.[12] This is exactly what was observed. Although branch and needle growth were not affected, exposed trees had lower stem growth (up to ½ mile downwind from the gas release) and produced fewer cones, and therefore, fewer seeds. This pattern can be explained by thinking about how these long-lived trees might allocate the energy that they take in to different tissues and functions. Just as people may prioritize how they spend their limited resources, such as time and money, trees prioritize how they spend the

sugars they produce.[13] In general, shoot and leaf growth are high priorities for energy allocation in these trees, and we did not see big changes in how many leaves were produced on defoliated trees compared to healthy trees. However, stem growth and seed production may be lower priorities in any given year. And the damaged trees allocated less energy to these processes compare to healthy trees. In other words, the trees exposed to chlorine gas did not grow very much and they did not produce very many seeds.

Finally we observed higher mortality, especially for Douglas firs, within 50 yards of the area where railroad cars derailed and released the toxic clouds of chlorine gas. There are two possible explanations for the death of these trees. First, the trees may have been killed directly by chlorine gas exposure. Second, the defoliated trees only had a small fraction of needles left and it took these needles two months after the chlorine gas exposure to appear. This may not have been enough needles to meet the energy demands of the tree.[14] That is, more carbon was used than the tree could acquire via photosynthesis. This can be compared with a savings account at the bank. When more money is taken out (i.e., energy requiring processes) than comes in via deposits and interest (photosynthesis), eventually this will lead to a negative balance (no more energy to spend on survival so the tree dies). This process may have been promoted by two factors: 1) the needles were not as efficient in photosynthesis on trees exposed to chlorine gas, and/or 2) there were high costs to repair tissues damaged by chlorine gas exposure. The first factor translates into lower inputs to the bank account balance, for example, lower interest rates or fewer deposits. The second factor is equivalent to higher withdrawals to pay for repair costs. The same factors that may have contributed to tree

mortality also may be (partially) responsible for the observed decline in stem growth, and seed and pine cone production.

To summarize, we can say that adverse effects of chlorine gas exposure on conifers are not restricted to acute visible injury of foliage. Chlorine gas exposure was shown to have long-term effects on physiological and growth processes of these conifers. The main causes of these effects were higher susceptibility to environmental stress factors, such as drought stress, and lower mass of needles on the trees. Interestingly, Douglas fir and Ponderosa pine responded differently to chlorine gas exposure. The differences may have been due to differences in how long these trees keep their needles, how much different years of needles contribute to whole tree photosynthesis, and drought tolerance. Over time, these conifers will probably replace the needles that were lost due to chlorine gas exposure, and adverse effects will probably disappear over time. Projections using tree growth models indicate that this recovery may take at least 4 to 7 years in western Montana. However, tree responses were mediated by climate (relatively dry summer), site characteristics (relatively dry sites) and species (even within conifers there were different responses. Thus, these factors need to be considered in case of future chlorine exposures.

Regional air pollution: Effects of ozone on vegetation

An example of an air pollutant that influences plants on a regional scale is ozone. During the last several years, ozone has received a lot of press coverage. However, most of the news has focused on decreasing ozone concentrations in the stratosphere, the atmospheric layer above the layer that we breathe and where the weather takes place. So,

most people will be familiar with what is referred to as the "ozone hole", a decline of ozone in the earth's stratosphere. Scientists predict that the loss of stratospheric ozone will lead to higher levels of harmful UV-B radiation at the earth's surface, and cause adverse effects on public health and vegetation.[15, 16] However, another environmental problem relating to ozone takes place in the troposphere, the layer of the atmosphere that we live in. In the troposphere, ozone concentrations have increased over the last decades, and they are expected to increase even more over the next decades as well.[17]

Tropospheric ozone is formed from two chemical ingredients, nitrogen oxides and volatile organic hydrocarbons (VOC's). The main emission sources of these pollutants are exhausts from motor vehicles, power generation, and solvent use.[18] Ozone production requires sunlight. Thus, ozone pollution generally is a problem in the spring and summer, especially in urban areas with a lot of traffic exhaust and industrial emissions. The skylines of Los Angeles or Mexico City, showing a thick brown layer of pollution near the surface with limited visibility, are excellent images of conditions with high levels of ozone.

So one may wonder, if too little ozone in the upper atmosphere is bad, shouldn't more ozone in the lower atmosphere be a good thing? Unfortunately, this is not the way it works. Human exposure to tropospheric ozone causes irritation to eyes and nose, lowers lung performance, and can worsen asthmatic symptoms.[19] Ozone levels considered harmful to human health are exceeded frequently in many areas of Europe and the United States.[2, 20] Ozone levels are high not only in urban regions, but also in agricultural and forested regions, because ozone and its chemical ingredients can be transported over hundreds of miles. Thus, if you go for a hike in the mountains on a nice sunny summer

day near the Los Angeles basin, you may not be totally safe from experiencing discomfort from high ozone levels.[21]

Like chlorine gas, ozone also has negative effects on plants, and ozone concentrations observed in many parts of Europe and the United States are high enough to cause these negative effects.[2, 20] The adverse effects of ozone on vegetation were first discovered in the LA Basin in the 1950's, where visible injuries to leaves, as well as reduced yield and quality, were observed for several crop species.[18, 22, 23] Since then, a steady stream of reports on adverse ozone effects on plants has hit the press. Ozone influences many processes that affect plant growth. These include damage to plant membranes that can reduce photosynthesis, higher repair cost for injured plant tissues, premature loss of leaves, and lower growth.[24, 25, 26] Exposure to ozone also changes how plants interact with their physical environment. For example, plants exposed to ozone may be more susceptible to freezing temperatures and drought stress.[27, 28]

When ozone-exposed plants encounter the stresses of drought, they cannot completely close their stomatal pores. Thus, the plants tend to be susceptible to excessive water loss.[28] While we understand how ozone affects stomata, relatively little is known about ozone effects on plant cuticles. As mentioned earlier, the cuticle is waxy layer on leaf and needle surfaces that protects plants against excessive water loss and infection by pathogens, such as bacteria, viruses and fungi.[11] Does ozone affect plant cuticles in way that water loss through the cuticle increases? Could damage to the cuticle influence the overall water balance of plants through the course of a year? To better understand how ozone affects plants and their cuticles, we set up several laboratory experiments. We used two species of poplars (*Populus nigra brandaris* and *Populus euramericana Robusta*)

and Douglas fir (*Pseudotsuga menziesii*) trees in these experiments. For 6 weeks, we exposed Poplar species to ozone concentrations that these plants might experience on a daily basis during a season when they are growing in the forest. Interestingly, ozone had relatively little effect on the cuticles of these poplars. For example, a typical water droplet spreads out just the same way on leaves, whether or not plants were exposed to ozone. Is there a relationship between how water spreads on a leaf surface and water loss across the cuticle? In the case of poplars, it would be a very poor relationship. Why? The two species of poplars behaved in different ways. Leaves exposed to ozone from one poplar species (*P. euramericana*) lost significantly more water than leaves that did not get exposed. Yet, there were no differences in how water spread out on the leaf surface, suggesting that the cuticle was not badly damaged. Interestingly, some scientists have suggested that one of the possible mechanisms contributing to the decline in forest health that has been observed in Europe and the United States is water loss through these damaged plant surfaces.[28,29,30] So what happened with the other poplar species? Leaves of *P. nigra* exposed to ozone lost just as much water as leaves that never came in contact with ozone. This interesting result suggests that the way a plant responds to ozone depends on the type of species.

Many scientists reported that ozone also can interfere with how leaves grow.[26] Our experiments confirmed these reports. Ozone exposure caused considerable injury to poplar leaves, which was easily detected by the naked eye, including the presence of many chlorotic and necrotic spots. At some point, these leaves became so fragile that they simply dropped off the tree by themselves, or broke off with the slightest touch. What is the effect on the tree? The effect is similar to the effect we described for leaves exposed

to chlorine gas. There are fewer leaves on the tree, and less leaf area to capture sunlight for photosynthesis. This effect was enhanced by fewer new leaves on ozone-exposed trees.

Douglas fir, a conifer, and poplars are very different in terms of how long they keep their leaves. Poplars are deciduous, meaning they lose their leaves every year when conditions for growth are no longer favorable. Conifers, in comparison, tend to keep their leaves for many years. So how might conifers differ in their response to ozone compared to deciduous trees? We exposed Douglas fir (*Pseudotsuga menziesii*) trees and poplar trees to the same levels of ozone, but Douglas firs were exposed to ozone for 23 weeks, nearly four times longer than poplars. This is a much higher ozone dose for the Douglas firs, so you might expect that the conifers would suffer greater damage than the poplars. However, that is not what we found at all. Ozone exposure did not affect how much water was lost through the cuticles of Douglas fir and it did not affect growth either! Needle injury you could actually see only occurred at extremely high ozone levels. Douglas fir is similar to other conifers in being more tolerant to ozone than deciduous trees.[31] But remember that conifer needles can live many years, and when they get exposed to ozone year after year, the negative influence of ozone may eventually show up.[32] While exposed conifers did not loose more water, ozone exposure did seem to affect the cuticles of Douglas fir after four weeks of exposure, because water spread out in different ways, depending on whether or not the needles were in contact with ozone. This observation supports what we learned about poplars - the negative effects of ozone on cuticles are independent of the way ozone influences how much water the leaves loose. And changes in how water behaves on a leaf surface may have significant impacts on how plants

interact with air pollutants. This is critical for predicting how pollutants get into a leaf to do damage.

Water droplets on leaf surfaces: implications for pollution uptake

Both chlorine gas and ozone exposure increased the wettability of conifer needles, that is, water droplets spread out more over these exposed leaf surfaces compared to needles that were not exposed. Why is this relevant for understanding how air pollution and plants interact? Conditions leading to wet leaves are very common in nature; for example, crop plants and forests can be wet from dew or rain more than 50 percent of the day during the growing season.[33, 34] The surface of conifer needles may be wet up to 72 percent of the time.[35] Many types of air pollution can react with water on leaves and have a big influence on how much of the pollutant lands on and gets into the leaf. We set up another experiment to address the effects of leaf surfaces wetness on ozone deposition. We did this using a special chamber where we could study dry and wet poplar leaves when they were exposed to ozone.

What did we learn? Ozone uptake by poplar leaves was 1.5 to 2 times greater during the day when leaves were wet and pores were open for photosynthesis. Moreover, during the night, when stomatal pores are usually closed, leaf surface wetness increased ozone uptake 3.5 to 5 times compared to dry leaves! So day or night, the presence of dew, rain, or mist droplets on leaf surfaces can lead to considerably higher ozone uptake by plant leaves. Oddly enough, you would expect more ozone to get into a leaf when the stomatal pores on the leaves are open compared to when they are shut. How can we explain this counterintuitive result? There were two counteracting processes. The good

news is that leaf surface wetness tends to cause stomatal pores to close during the day. This alone led to a 20 to 40 percent reduction in the uptake of ozone. The downside is that when stomata close, plants cannot get as much carbon dioxide, so photosynthesis is slower. But that is not all the bad news; ozone deposition is higher on wet leaf surfaces, so the amount of ozone that plants take up also increases. Consequently, wet plant leaves tend to receive a higher dose of ozone than dry leaves, which increases adverse effects of ozone exposure.

This experiment brought up new questions for future research. The amount of ozone deposited to a wet leaf surface was highly dependent on the chemical composition of the water on the leaf. More ozone was deposited when the water on the leaf was slightly acidic compared to neutral. More research is needed using water with acidities similar to those in nature to understand how leaf surface wetness might affect ozone deposition in natural conditions. Moreover, ozone deposition on wet conifer needles may be different from that on wet deciduous leaves, such as poplar leaves.

Chlorine gas and ozone: summary of effects and possible solutions

Although chlorine gas and ozone are very different in their chemistry, how plants are exposed to them, type of exposure as well as duration, both pollutants can change how leaves interact with water. Chlorine exposure increased the susceptibility of conifers to drought stress. Ozone exposure did so only for poplars. Both pollutants had negative effects on leaf growth, although the effect of chlorine gas on conifer needles was more severe than the ozone effects on poplars. Acute chlorine gas exposure did have significant long-term effects of conifers. We expect the same result for ozone effects on conifers, but

we need more long-term studies. Nonetheless, our experiments helped us to clarify some of the mechanisms that may play a role in how ozone damages plants.

This leads us to what some scientists, farmers and forest managers consider the million dollar question: what can we do to ameliorate the negative effects of chlorine gas and ozone on crops and natural forest resources? Obviously, increasing our understanding of how air pollutants affect plants, and the mechanisms by which they act, is one step in the right direction. But, the scientist can only tell us why the crop or forest is suffering. In a general sense, the solutions require actions by people, companies and the government, and require that we focus our efforts on the source of the pollutants.

In the case of chlorine gas, we need to reduce the risk of accidental releases. Good substitutes for the use of chlorine gas exist for the production of PVC plastics, pesticides, refrigerants, solvents, and pulp and paper bleaching. These substitutes could decrease the chlorine use in Canada and the United States by as much as 75 percent![36] No good substitutes are yet available for chlorine use as a disinfectant without creating an unacceptable risk of biological infections.[37] However, chlorine-based disinfectants account only for 4 percent of the total chlorine use.[37] In concert with finding alternatives to chlorine, implementation of emergency response planning also can play an important role in minimizing the negative impact of accidental chemical releases.[38]

In order to mitigate adverse effects of tropospheric ozone on the health of people and vegetation, the most effective solution is to reduce ambient ozone concentrations. This will require a drastic decrease in emissions of both nitrogen oxides and VOC's, the chemical precursors of ozone. Ozone chemistry in the troposphere is quite complex, and unfortunately a decrease in only one of these precursors will not necessarily lead to lower

ozone concentrations.[39] Moreover, our experiments show that ozone has adverse effects on vegetation at relatively low concentrations. Thus, in practice it may prove very difficult to reach ozone concentrations low enough to avert all the negative impacts on forests and agricultural crops in the short term. However, there are steps we can take right away to start dealing with the ozone problem.

What can we, as individuals, do to reduce the risk of adverse effects of these air pollutants on health of people and vegetation? As consumers we certainly have a say in what types of and how we produce goods. For example, we can help decrease the use of chlorine gas by using paper that has not been bleached with chlorine, decrease our use of household bleach, buy refrigerators that do not require chlorine gas in their production process, and use non-PVC plastic. As mentioned earlier, there are many good alternatives to these uses of chlorine gas, and as consumers we do make a difference. If we decide to purchase products that do not need chlorine in their production process, the suppliers will respond to this demand. To reduce ozone concentrations, there is a definite role of individuals. Two important sources of the chemicals that lead to tropospheric ozone are exhausts from motor vehicles and power generation. We, as individuals, can limit how much we use our vehicles; and if we replace them, we can buy vehicles that are fuel efficient and low in emissions of air pollutants. Moreover, we can also limit our use of energy. This has two benefits: it would not only help to reduce ozone concentrations in the troposhere, but also emissions of carbon dioxide, a pollutant responsible for the "greenhouse effect".[40] In all of these measures, the government has a role to implement policies that encourage society to change to more environmentally friendly ways of

living. In this challenge we could look at other countries, with less polluting life styles, as examples.

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CHAPTER 7
Executive summary

Air pollution has long been recognized to cause adverse effects on human health, crops and natural vegetation. Adverse effects of air pollution have been observed on different scales, from the local scale to the regional and global scales. Many types of air pollution result from anthropogenic sources related to industrial activities and combustion of fossil fuels. Although additional research is needed, a number of key points emerge from this dissertation project, which are outlined below.

An example of air pollution that occurs on a on a local, short-time scale is chlorine gas. Typically, this gas is present in the atmosphere due to accidental releases. Chlorine gas has many applications in our society, such as in industrial processes, manufacturing of medicines and water treatment. The production volume of chlorine gas is projected to increase over the next decades. This may increase the risk of adverse effects from accidental chlorine gas releases, since chlorine gas is highly toxic to both public health and vegetation. Public health effects of chlorine gas are well known, and vary from irritation of eyes, nose and throat, to severe lung damage and even death. However, relatively little is known about the effects of chlorine gas on crops and natural ecosystems. The focus of the first part of this dissertation is on the long-term effects of acute chlorine gas exposure on a coniferous forest in the Rocky Mountains, USA. Although a few studies have reported on the visible injury symptoms after acute exposure to chlorine gas, this is the first study to report on long-term impacts of chlorine gas on morphology, physiology, and growth of coniferous trees.

An example of air pollution on a regional scale is ozone. Ozone is the main component of photochemical smog, and is formed from nitrogen oxides and volatile organic carbons, VOC's, under the influence of sunlight. The main sources of the chemical precursors of ozone are road traffic, industrial processes and solvent use. Ozone concentrations considered harmful to vegetation are exceeded in urban and agricultural areas in the world, as well as at subalpine and alpine altitudes. Ozone is considered one of the factors contributing to forest decline, a phenomenon that has been observed in Europe and the western United States. One of the proposed mechanisms by which ozone may affect tree health is by increasing their susceptibility to drought stress. The second half of this dissertation addresses a possible mechanism of increased drought susceptibility due to ozone exposure, increased water loss via leaf cuticles, and the implications for tree water balance and tree growth. The interactions between leaf surfaces and atmospheric moisture, such as dew, rain and mist, were also studied, because leaf surface wetness can have a large impact on photosynthetic gas exchange and the uptake air pollutants by plants.

CHLORINE GAS EFFECTS ON VEGETATION

- Acute morphological injury symptoms of chlorine gas exposure on plants consisted of chlorosis (yellowing of tissues), necrosis (death of tissues), and necrotic mottling (black stipling of leaves). These symptoms occurred in conifers, broadleaved shrubs, herbaceous species and monocots such as grasses. In monocots the symptom was a streaky pattern, following the veins. In conifers, necrosis started at the tip of the needle gradually

extended to the base (also called tipburn). Completely necrotic conifer needles defoliated. Foliage in buds at the time of chlorine gas exposure was not visibly affected.

- Chlorine gas exposure increased leaf wettability of cuticles of needles of *P. menziesii* but not *P. ponderosa*, suggesting that chlorine gas exposure had changed plant cuticles of one species. Directly exposed foliage of both species also had higher cuticular water loss, and lower total foliar water content. Moreover, both foliage that was directly exposed to chlorine gas and foliage that flushed after exposure were affected similarly, suggesting that even indirect exposure weakened leaves. Thus, chlorine gas may have increased the susceptibility of exposed trees to drought stress, over at least three growing seasons.
- Chlorine gas exposure caused severe defoliation of both of *P. menziesii* and *P. ponderosa*. Not only were directly exposed needles dropped from the trees, foliage that flushed after chlorine gas exposure had decreased longevity, causing a significant decrease of photosynthetic biomass for exposed trees of both species.
- The combination of increased susceptibility to drought stress and decreased photosynthetic biomass led to reduced annual stem increment growth over at least three growing seasons for *P. menziesii*, up to 0.8 km downwind from the release, and, to a lesser extent, for *P. ponderosa*, up to 0.2 km downwind. Moreover, chlorine gas decreased reproductive output, as fewer exposed trees produced cones compared to unexposed trees. Increased tree mortality was only observed for *P. menziesii*, most of which occurred within months after chlorine gas exposure.
- Long-term responses of tree growth were species dependent. Factors that need to be considered when using data from this study to effects of future chlorine release need to

consider climatic and site characteristics factors, such as moisture availability, and differences between different conifers, and conifers versus deciduous tree species.

OZONE EFFECTS ON VEGETATION

- Leaf wetness of poplar leaves increases over time. Exposure to urban ozone concentrations delayed this increase by 2 to 4 weeks, and there were not differences in leaf wettability after 6 weeks of ozone exposure.
- Exposure of poplars to ozone concentrations characteristic for urban regions increased water loss via the cuticle for *P. Euramericana*, but not for *P. nigra*, suggesting that the way ozone exposure increases plant susceptibility to drought stress is species specific. Moreover, ozone exposure decreased photosynthetic biomass of both poplar species via production of fewer new leaves and premature abscission of foliage with symptoms of ozone injury. This was the case for ozone exposure regimes characteristic for urban as well as high elevation areas. Higher susceptibility to drought stress and decreased foliar biomass may have adverse effects on tree health and growth. However, plant responses to ozone exposure were species dependent within these experiments and may be also different for plants grown in field conditions.
- Although saplings of *P. menziesii* were exposed to ozone about four times longer than poplar saplings, negative effects were less pronounced for *P. menziesii*. While urban ozone exposure led to significant increases in leaf wettability of *P. menziesii*. This species did not show foliar injury symptoms, and cuticular water loss and tree growth parameters were not affected.

• These results suggest that coniferous trees were more tolerant to ozone exposure than deciduous trees. However, effects of ozone exposure may accumulate over several growing seasons, and the exposure period in our study may not have been long enough to find significant effects for *P. menziesii.*

LEAF SURFACE WETNESS AND AIR POLLUTION

• Exposure of conifers to chlorine gas and ozone increased leaf wettability of needle surfaces. This may lead to increased formation and duration of water layers on leaf surfaces, which can influence gas exchange over these leaf surfaces.

• Simulated leaf surface wetness events, such as dew, rain and mist, increased ozone deposition to poplar leaves by 1.5 to 5 times, especially in dark conditions. This was the result of two processes, lower ozone uptake due to decreased stomatal conductance, and higher ozone deposition to wet leaf surfaces.

• Photosynthetic gas exchange was 15-20 % lower for wet leaves. Moreover, CO_2 exchange over wet leaves in dark conditions was 60 to 100 % lower compared to dry conditions. In light conditions the effect was attributed to lower photosynthesis due to limited diffusion of CO_2 through the water present on the surface. However, more research is needed to assess whether the observed response in dark conditions was due to CO_2 gas dissolving in the aqueous phase or to an actual decrease in dark respiration.

• In dark conditions, leaf surface wetness effects on ozone uptake and respiration were strongly influenced by the pH and ionic composition of the solution on the leaf surface. This suggests that the chemistry of the aqueous phase is an important factor in mediating responses of ozone deposition and gas exchange to leaf surface wetness.

DIRECTION FOR FUTURE RESEARCH

These results have increased our understanding of the adverse effects of chlorine gas and ozone on plants, as well as our understanding of some of the mechanisms responsible for these effects. Moreover, the results point to areas where additional research is needed.

- This research pointed out that effects of acute chlorine gas exposure on forests are not limited to short-term foliar injury symptoms, but that long-term impacts on forest health and growth can be expected after severe defoliation. Moreover, even trees that were not visibly affected three months after exposure showed adverse effects. Thus, studies limited to descriptions of foliar damage a few weeks after exposure are not sufficient to appropriately assess the real damage of chlorine gas exposure on forest ecosystems. In order to understand the impact of accidental exposure to pollutants on physiological processes, growth, and survival, monitoring over several years after the exposure is necessary. Incorporating effects on soils and soil microbiology would further our understanding of accidental exposure to materials, such as chlorine gas effects on forests.
- Data presented in this work reported on effects of chlorine gas over three growing seasons following the exposure event. However, these effects may persist much longer than the time frame studied. Three years after chlorine gas exposure, exposed trees still had lower photosynthetic biomass and shorter needle longevities, which may have lingering effects on tree vigor and health. Reduced vigor of exposed trees was manifested as lower stem increment growth and reproduction. These effects may continue to persist for an unknown period of time. In order to address these effects, long-term study sites

have been established. These sites will be used to collect data on foliar injury, needle age classes present, insect injury, stem increment growth, cone production, and tree mortality, and will be visited on an annual basis.

- In case of future chlorine gas releases, managers need to take into account that species respond in different ways to chlorine gas exposure. These differences appear to be related to needle longevity, relative contribution of needle age classes to whole tree photosynthesis, and drought tolerance. Moreover, long-term responses will be mediated by climatic and site characteristics, such as moisture availability, and differences between different conifers, as well as coniferous versus deciduous tree species.
- Beyond the scientific arena, society needs to address the risks of the use of chlorine gas, because not only is it highly toxic to humans, but exposure also causes long-term negative effects on vegetation. It is crucial to continue to explore safer alternatives for chlorine gas use, implement these into production processes, and to work on regulations that would lead to safer transportation of hazardous materials.
- At this point there are no methods to distinguish between the stomatal and the cuticular components that contribute to minimal conductance to water vapor in dark conditions. The only methods that do so are methods that chemically isolate cuticles from intact leaves. However, these methods may alter cuticular characteristics, and cannot be used to study indirect effects of air pollution on the development of cuticles, such as those observed for poplar leaves presented in this dissertation research. Development of methods that can separate stomatal and cuticular components so we can understand minimal conductance to water vapor on intact leaves will be extremely valuable in increasing our understanding of air pollution effects on plant water relations.

• Studying the effects of ozone exposure on tree saplings under laboratory conditions helped identify some of the mechanisms by which ozone exposure may affect plants in field conditions. However, tree responses to air pollutants are influenced by many factors, including species (as was shown by my research), tree size, and environmental conditions encountered in the field. Thus, additional research is needed to assess the role of identified ozone injury mechanisms in field conditions, and for trees of different size.

• Leaf surface wetness experiments identified the influence of leaf surface wetness on ozone deposition in laboratory conditions, as well as the underlying mechanisms. Leaf surface wetness is a common phenomenon in nature, and has been shown to significantly increase ozone deposition rates. The next step is to study the role of these processes and the relative contribution of leaf wetness events to the total ozone uptake by plants in field conditions. Moreover, ozone uptake by plant canopies often is assessed using models. In order to improve ozone uptake models, influences of leaf wetness events should be incorporated.

• Ozone deposition to wet leaves was influenced by pH and water chemistry due to reactions of ozone in the aqueous phase. Additional research is needed to increase our understanding of the chemical interactions of ozone with the aqueous phase, especially with solutions similar to dew, rain, and mist. Moreover, the chemical fate of reaction products of ozone in the aqueous phase needs to be studied. Key questions include identifying the reaction products, if these compounds take up by the plant, and the extent to which these products have adverse effects on cuticles or other plant processes.

• Ozone deposition was significantly higher on wet leaf surfaces in dark conditions. Since stomata are closed during the night, increased ozone deposition to the cuticle may

lead to considerably higher ozone uptake, especially at high elevations where nighttime ozone concentrations remain relatively high. Additional research is needed to assess the importance of nighttime ozone uptake for plants, both in light of the total ozone dose and its contribution to adverse effects on plants.

- Finally, leaf surface wetness decreased the exchange of CO_2 over leaf surfaces, causing lower rate of photosynthesis. In dark conditions, leaf surface wetness had a large impact on CO_2 exchange over leaves. The observed decrease in CO_2 emission could potentially be attributed to lower rates of dark respiration. These results are the first to report such an effect of leaf surface wetness on CO_2 emission, and many uncertain factors remain. Additional study is needed to understand whether CO_2 gas was adsorbed by the aqueous phase, or whether dark respiration was limited by O_2 diffusion. If the latter was the case, to what extend was dark respiration reduced by leaf surface wetness and what are the physiological consequences for plants?

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Appendix 2.1: Acute foliar injury of chlorine gas exposure on conifers, shrubs, forbs and grasses

INTRODUCTION

Only a few studies have reported on the effects of chlorine gas exposure on vegetation. Foliar injury symptoms of chlorine gas exposure on vegetation consist of chlorosis, (bleaching and yellowing of leaf tissues) and necrosis (death of cells and cell tissue). These effects can be observed on foliage of both coniferous and deciduous plant species (e.g. Heck, Daines and Hindawi, 1970), although the patterns in which these symptoms occur differs between conifers, broadleaves shrubs and forbs (dicots), and grasses (monocots). On conifer needles, chlorine gas exposure causes tipburn, an orange-brown coloration extending from the tip to the base of a needle, which eventually kills the whole needle (Brennan et al., 1966). In broad-leaved species necrosis may be confined to leaf margins, extending from the edge to the center and base of a leaf, and be interveinal (Brennan, et al. 1965; Heck et al. 1970). In monocots, such as corn, onion, and grass species, necrosis occurs in a streaky pattern following the course of veins. Foliar injury symptoms have been found at concentrations as low as 0.5 – 3.0 ppm Cl_2 at exposures times varying from 4 to 24 hours (e.g.Thornton and Setterstrom, 1940; Brennan et al., 1965). Threshold chlorine concentrations that cause visible injury depend on plant species and duration of exposure, as well as environmental conditions (Brennan et al., 1965; Griffiths and Smith, 1990). This appendix describes the acute foliar injury effects on natural vegetation after an accidental chlorine gas release in western Montana, USA.

METHODS

Description of the chlorine gas exposure

The study sites were located in a narrow valley in the Rocky Mountains, ~2 km west of Alberton, Montana, USA. On April 11, 1996, at 0400 hr, a 72-car train derailment at the site released ~55 metric tons of chlorine gas into the atmosphere and the surrounding forest. Forests up to ~14 km downwind from the derailment site were exposed to chlorine gas (Olympus Environmental, 1996). Chlorine gas concentrations at the site of the gas release varied from 12-20 ppm to ~50 ppm (1-hr average), with peak concentrations reaching ~1400 ppm (Olympus Environmental, 1996). Atmospheric dispersion models reported peak chlorine gas concentrations ranging from ~165 ppm at about 1.2 km, to ~5 ppm at 9 km downwind from the point of release (ATSDR, 1997). In addition to chlorine gas, unknown concentrations of chlorophenols were present in the gas cloud. However, levels in the soil were well below levels reported to adversely affect public health, and residues of toxic chlorinated organic compounds were removed from the site by excavation of the polluted soil layers (Olympus Environmental, 1996).

Study sites

Study sites that had been exposed to chlorine gas were established within 50 m of the site of the gas release, 0.2 km above the release, and 0.2, 0.5, 1.0 and 1.5 km downwind from the release. A control site was established at ~65 km downwind from the site of the gas release. All field sites were mixed coniferous forests and had similar vegetation and soils (fine or coarse, mixed loam). More information on the study sites is given in Table 2.1

Visible morphological injury

Morphological injury to vegetation was assessed immediately after it was safe to access study sites on May 15 (1 month post spill) and again June 10, 1996 (2 months post spill). Visible injury to foliage was assessed in the field for several species of trees (*Pseudotsuga menziesii* (Mirb.) Franco, *Pinus ponderosa*, and *Abies lasiocarpa*), shrubs (*Prunus virginia, Amelanchier alnifolia, Juniperus communis*, and *Juniperus scopulerum*), herbaceous species *(Mahonia repens, Arctostaphylos uva-ursi* L., *Arabis rectissima)* and grasses (*Digitaria sanguinalis, Elymus sp.*). Samples were collected up to 1.5 km downwind from the spill site based on availability and public access (see previous section), and were representative of common vegetation present at each study site. The number of plant samples varied based on availability at the study sites, ranging from n=1 to 10, depending on the species. Light microscopy techniques were used to examine foliar damage for different species.

RESULTS

Morphological injury symptoms

Deciduous tree species were not visually injured by chlorine exposure. Since new leaves were still in buds when they were exposed to chlorine gas, they were not directly exposed to the chlorine gas cloud. However, visual injury to existing needles on coniferous trees was apparent. Needles on both Douglas fir and Ponderosa pine showed extensive necrosis and tipburn (Figure 2.2C and 2.2D). Needles on Douglas fir trees at the spill site (n=4) and up to ~500 m (n=5) from the spill were almost completely necrotic, with a few chlorotic needles and no green needles present 2 mo after exposure.

Necrotic needles dropped over the course of the summer. Douglas fir trees 1 and 1.5 km downwind from the spill (n=3) had chlorotic, necrotic and green needles. Most exposed Douglas fir trees developed new green foliage. Ponderosa pine foliage was less visibly affected than Douglas fir. Ponderosa pine trees at the derailment site (n=3) and ~200 above the derailment site (n=1) had mainly necrotic and chlorotic needles, as well as newly flushed green needles present. However, needles on Ponderosa pine trees ~0.2 to 1.5 km downwind (n=3) were mostly green, with only minimal visual injury observed.

The degree of foliar damage generally decreased with increasing distance from the site of the gas release for both Douglas fir and Ponderosa pine (t_7=2.016, $p<0.05$; Figure 2.3). However, foliar injury downwind from the release site was higher than at the two control sites, especially for Douglas fir. Variation in foliar injury, between patches of trees as well as within individual trees, was high. Healthy green foliage, as well as chlorotic and necrotic foliage, necrotic mottling, and tipburn often occurred within the same tree. For example, a Ponderosa pine tree ~0.8 m downwind from the derailment was completely necrotic on the bottom half of the tree, while the upper half showed no visually injury.

Four different shrub species were examined. *Juniperus communis* (Rocky mountain juniper; n=3) growing within a 50 m radius of the gas release had been killed. Further downwind the juniper species showed chlorosis, necrotic mottling, tipburn , and necrosis, although most of the foliage was necrotic. One *Juniperus scopulerum* (common juniper) tree growing about 1.5 km downwind showed extended necrosis in the bottom half of the tree, whereas the top half of the tree was visibly unaffected. Foliage on the bottom half of the tree was shed later due to the chlorine gas exposure and a subsequent

disease. This was evidence that the chlorine gas cloud tended to stay near the surface, which is common for heavy gas clouds. Another broad-leaved shrub, *Amelanchier alnifolia* (western service berry; n=1), located ~0.5 km downwind had mainly green foliage, but also chlorotic and necrotic foliage, and necrotic mottling. Necrosis in this species varied from a few spots to whole leaves. *Prunus virginiana* L. (common choke cherry, n=1), located about 1.5 km downwind, was mainly unaffected but showed some bifacial necrosis, extending from the tip to the base of the leaves.

Mahonia repens (n=5) showed chlorosis and interveinal necrosis (Figure 2.2A and 2.2B). The severity of the injury symptoms did not differ between sites located about ~0.2 km above point of gas release and sites ~1.5 km downwind. Leaf tips commonly were more affected than the base and center of leaves. The extent of the symptoms varied between plants and within plants. Chlorine gas killed the buds of *Mahonia repens*, followed by the formation of new buds during the next growing season. *Arctostaphylos ura-ursi* (n=1) located ~0.2 km above gas release also showed interveinal necrosis. Necrosis on this species was bifacial, and varied from a light degree of mottling to complete necrosis. Exposed foliage of *Arabis rectissima* (Recter's rock cress, n=2,) ~1 km downwind showed chlorosis and necrosis, occurring predominantly on the older leaves. Moreover, both buds and flowers of this species had a "bleached" appearance, resembling chlorosis.

Two grass species, located ~1 km downwind from the gas release also were examined for visual damage. Both an *Elymus* sp. and *Digitaria sanguinalis* (crab grass) showed bifacial chlorosis, mottling (black and yellow spots scattered over the leaf surface), and necrosis. Necrotic injury extended from the tip to the base of the leaves. On

Digitaria sanguinalis, chlorosis occurred predominantly on the abaxial surface of the leaves, whereas necrosis was most common on the adaxial surface. Chlorosis and necrosis on these two grass species occurred in the direction of the veins, resulting in a streaky pattern of damage. *Digitaria sanguinalis* had not put out new green foliage one month after the gas release, whereas the other two grass species did. New foliage and flowers that came out after the exposure to chlorine gas were visibly unaffected.

CONCLUSIONS

This study confirmed that chlorine gas exposure led to chlorosis and necrosis in natural vegetation. On conifers, necrosis occurred as tipburn, i.e. necorsis extending from the tip to the base of the needle. Although many conifer needles became completely necrotic over time, followed by defoliation, conifer shoots and buds were generally not affected (i.e., no visible injury, alive). Moreover, deciduous foliage that was exposed as buds was not visibly affected by chlorine gas exposure. Visual injury symptoms of chlorine gas exposure shrubs, forbs and grasses also consisted of chlorosis and necrosis. In grasses (monocots) these foliar injury symptoms occurred in a streaky pattern, following the veins. This research confirmed foliar injury symptoms of chlorine gas exposure reported in the literature. These injury symptoms can be used to describe the geographic extent and severity of effects of chlorine gas exposure after accidental releases in natural vegetation as well as crops.

Appendix 3.1: Summary of water loss and water content for Ponderosa pine. Data are shown for downwind- and upwind control sites (CD and CU), and sites 1.5, 0.8, 0.2 km and 50 m downwind. "*" indicate statistically significant differences compared to the control sites for all dates within each needle age class (Repeated Measures with Tukey post-hoc test; $p<0.05$). Values in parentheses indicate one SE. Needle age classes that were no longer alive are indicated as "Absent" and sites that were not measured as "n.m.".

Date and site	Relative water loss, %		$G_{min,H2O}$, m $s^{-1}*10^{-3}$		TWC, g H_2O /g dry weight		RWC, %	
	1995	1996	1995	1996	1995	1996	1995	1996
December '96	n=2	n=2	n=2	n=2	n=2	n=2		
CD	44±3	37±4	1.0±0.3	0.9±0.3	1.04±0.22	1.35±0.07	n.m.	n.m.
0.8 km	84±3	49±1	1.9±0.3	1.2±0.3	1.09±0.04 *	1.35±0.04	n.m.	n.m.
0.2 km	69±5 *	48±5	1.7±0.3	1.2±0.3	1.09±0.02 *	1.38±0.06	n.m.	n.m.
50 m	Absent	48±9	Absent	1.4±0.3*	Absent	1.57±0.07	n.m.	n.m.
March '97					n=3	n=3	n=3	N=3
CU	n.m.	n.m.	n.m.	n.m.	1.05±0	1.15±0.03	91±0.03	94±0.1
CD	n.m.	n.m.	n.m.	n.m.	1.11±0.01	1.30±0.01	96±0.3	98±0.2
0.8 km	n.m.	n.m.	n.m.	n.m.	1.21±0.02 *	1.31±0.01	93±0.2	94±0.3
0.2 km	n.m.	n.m.	n.m.	n.m.	0.81±0.01 *	1.10±0.04	93±0.2	93±0.2
50 m	n.m.	n.m.	n.m.	n.m.	Absent	1.10±0.02	Absent	91±3
May '97	n=2	n=2	n=2	n=2	n=2	n=5	n=2	N=5
CU	n.m.	51±5	n.m.	1.0±0.3	n.m.	1.04±0.15	n.m.	93±1
CD	57±11	51±2	1.3±0.6	1.0±0.3	0.82±0.01	1.01±0.01	92±1	94±1
0.8 km	82±3	48±1	1.6±0.6	1.1±0.3	0.63±0.01 *	0.92±0.04	80±3	92±1
0.2 km	83±9	58±3	1.8±0.8	1.9±0.6	0.63±0.02 *	1.41±0.09	76±3	84±1
50 m	Absent	73±3	Absent	2.8±0.8 *	Absent	1.46±0.10	Absent	85±2

Appendix 3.1: Continued

Date and site	Relative water loss, %		$G_{min,H2Ov}$ m s^{-1}*10^{-5} *		TWC, g H_2O /g dry weight		RWC, %	
	1995	1996	1995	1996	1995	1996	1995	1996
October '97	n=2	n=2	n=2	n=2	n=2	n=2	n=2	n=2
CU	45±11	26±2	0.9±0.3	0.4±0.1	0.86±0.01	0.91±0.03	77±1	78±5
CD	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
0.8 km	29±3	20±3	0.5±0.3	0.3±0.1	0.74±0.04	0.94±0.02	84±2	87±1
0.2 km	51±4	34±4	1.0±0.3	0.6±0.3	0.83±0.01	0.87±0.03	80±2	87±1
50 m	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
March '98					n=5	n=5	n=5	n=5
CU	n.m.	n.m.	n.m.	n.m.	0.86±0.01	0.96±0.01	85±2	89±1
CD	n.m.	n.m.	n.m.	n.m.	0.85±0.03	0.86±0.08	79±1	74±6
1.6 km	n.m.	n.m.	n.m.	n.m.	0.90±0.02	0.96±0.01	83±2	84±1
0.8 km	n.m.	n.m.	n.m.	n.m.	0.89±0.01	0.97±0.01	83±1	84±1
50 m	n.m.	n.m.	n.m.	n.m.	Absent	0.89±0.02	Absent	84±1
June '98	n=4	n=4	n=4	n=4	n=4	n=4;	n=4	n=4
CU	52±1	46±3	1.2±0.3	1.2±0.3	0.84±0.02	0.92±0.02	83±1.	81±1
CD	62±2	55±2	1.3±0.3	1.2±0.6	0.82±0.01	0.88±0.01	80±1	80±1
0.8 km	68±4	54±1	1.8±0.6	1.4±0.6	0.88±0.01	0.99±0.02	80±1	79±1
50 m	Absent	65±2	Absent	1.5±0.3	Absent	1.02±0.02	Absent	81±2

Appendix 3.1: Continued

Date and site	Relative water loss, %		$G_{min,H2Ov}$ m $s^{-1}*10^{-5}$		TWC, g H_2O /g dry weight		RWC, %	
	1995	1996	1995	1996	1995	1996	1995	1996
September '98					n=4	n=4	n=4	n=4
CU	n.m.	n.m.	n.m.	n.m.	1.00±0.03	1.05±0.02	82±1	85±4
1.6 km	n.m.	n.m.	n.m.	n.m.	0.83±0.06	1.05±0.01	77±6	82±1
0.8 km	n.m.	n.m.	n.m.	n.m.	1.05±0.01	1.16±0.01	79±2	82±1
50 m	n.m.	n.m.	n.m.	n.m.	Absent	1.06±0.03	Absent	85±1

Appendix 3.2: Summary of water loss and water content for Douglas fir. Data are shown for downwind- and upwind control sites (CD and CU), and sites 1.5, 0.8, 0.2 km and 50 m downwind. . "*" indicate statistically significant differences compared to the control sites for all dates within each needle age class (Repeated Measures with Tukey post-hoc test; $p<0.05$). Values in parentheses indicate one SE. Needle age classes that were no longer alive are indicated as "Absent" and sites that were not measured as "n.m.".

Date and site	Relative water loss, %		$G_{min,H2Ov}$, m $s^{-1}*10^{-3}$		TWC, g /g		RWC, %	
	1995	1996	1995	1996	1995	1996	1995	1996
December '96	n=2	n=2	n=2	n=2	n=2	n=2		
CD	28±2	25±1	0.7±0.3	0.6±0.3	1.11±0.01	1.34±0.01	n.m.	n.m.
0.8 km	40±1	32±3	1.0±0.3	0.9±0.3	1.34±0.03	1.95±0.58	n.m.	n.m.
0.2 km	71±2 *	45±8	1.7±0.3	0.7±0.3	0.96±0.01 *	1.03±0.32	n.m.	n.m.
50 m	Absent	44±3	Absent	1.0±0.3 *	Absent	1.45±0.03	n.m.	n.m.
March '97					n=3	n=3	n=3	n=3
CU	n.m.	n.m.	n.m.	n.m.	0.99±0.01	1.25±0,08	95±2	98±0.2
CD	n.m.	n.m.	n.m.	n.m.	1.19±0.0	1.31±0.02	97±0.4	98±0.3
0.8 km	n.m.	n.m.	n.m.	n.m.	0.99±0.07	1.30±0.01	94±0.04	98±0.2
0.2 km	n.m.	n.m.	n.m.	n.m.	0.96±0.08	1.24±0.05	95±0.01	97±2
50 m	n.m.	n.m.	n.m.	n.m.	0.92±0.05 *	1.39±0.02	93±0.05	95±1
May '97	n=2	n=2	n=2	n=2	n=2	n=5	n=2	n=5
CU	n.m.	62±3	n.m.	1.0±0.3	n.m.	1.02±0.02	n.m.	93±1
CD	61±8	63±2	1.4±0.6	1.1±0.3	0.82±0.01	0.97±0.01	80±3	91±2
0.8 km	61±1	45±1	1.7±0.9	1.0±0.3	0.92±0.01	0.96±0.002	78±1	80±0.3
0.2 km	70±7 *	40±1	1.2±0.6	1.0±0.3	0.49±0.07 *	1.08±0.14	74±2	82±2
50 m	Absent	62±1	Absent	1.5±0.3 *	Absent	0.94±0.003	Absent	70±3

Appendix 3.2: Continued

Date and site	Relative water loss, %		$G_{min,H2Ov}$, m s^{-1}*10^{-5}		TWC, g /g		RWC, %	
	1995	1996	1995	1996	1995	1996	1995	1996
October '97	n=2	n=2	n=2	n=2	n=2	n=2	n=2	n=2
CU	48±5	44±10	1.2±0.3	1.0±0.3	0.84±0.07	0.96±0.04	81±0.03	85±5
CD	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
0.8 km	65±12	47±5	1.5±0.6	1.0±0.3	0.74±0.15	1.07±0.01	81±2	90±4
0.2 km	72±6 *	73±1	1.7±0.6	1.6±0.3	0.79±0.22	0.81±0.12	73±4	85±1
50 m	Absent	80±1	Absent	2.1±0.6 *	Absent	1.33±0.65	Absent	84±1
March '98					n=5	n=5	n=5	n=5
CU	n.m.	n.m.	n.m.	n.m.	0.74±0.04	0.84±0.05	78±5	80±1
CD	n.m.	n.m.	n.m.	n.m.	0.79±0.02	0.83±0.03	77±1	75±2
1.6 km	n.m.	n.m.	n.m.	n.m.	Absent	0.79±0.06	Absent	76±3
0.8 km	n.m.	n.m.	n.m.	n.m.	Absent	0.79±0.07	Absent	75±3
50 m	n.m.	n.m.	n.m.	n.m.	Absent	0.75±0.06	Absent	65±3
June '98		n=5		n=5		n=5		n=5
CU	n.m.	62±6	n.m.	1.3±0.3	n.m.	1.03±0.05	n.m.	87±1
CD	n.m.	69±8	n.m.	1.3±0.3	n.m.	0.97±0.03	n.m.	88±1
0.8 km	Absent	80±7	Absent	1.7±0.6	Absent	1.00±0.03	Absent	81±1
50 m	Absent	80±5	Absent	1.7±0.6	Absent	1.02±0.06	Absent	84±2

Appendix 3.2: Continued

Date and site	Relative water loss, %		$G_{min,H2Ov}$, m s^{-1}*10^{-5}		TWC, g /g		RWC, %	
	1995	1996	1995	1996	1995	1996	1995	1996
September '98						n=4		n=4
CU	n.m.	n.m.	n.m.	n.m.	n.m.	0.88±0.03	n.m.	76±4
1.6 km	n.m.	n.m.	n.m.	n.m.	Absent	0.94±0.01	Absent	78±2
0.8 km	n.m.	n.m.	n.m.	n.m.	Absent	0.94±0.03	Absent	78±3
50 m	n.m.	n.m.	n.m.	n.m.	Absent	0.90±0.03	Absent	74±1