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Effects of Short-term, High Exposure to Chlorine Gas on Morphology and Physiology of *Pinus ponderosa* and *Pseudotsuga menziesii*

MAARTEN D. J. SCHREUDER* and CAROL A. BREWER
Division of Biological Sciences, The University of Montana, Missoula, MT 59812, USA

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Following an accidental spill, acute morphological and physiological effects of chlorine gas exposure were evaluated on two conifer species, *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus ponderosa* (L.), growing in a montane, coniferous forest in the Rocky Mountains, USA. Foliar injury, consisting of chlorosis, necrotic mottling and necrosis was observed only on foliage that was directly exposed to chlorine gas. Necrotic needles of both species defoliated during the months immediately following exposure. Buds of both species within 50 m of the gas release were killed; this gave rise to secondary shoot growth for *Pseudotsuga menziesii*. Cuticular injury was assessed by measuring droplet contact and retention angles on 1-year-old foliage (directly exposed) and current-year foliage (which flushed after the gas cloud had subsided). Chlorine gas exposure led to smaller droplet contact angles on needles in both age classes of *Pseudotsuga menziesii* ($P < 0{\cdot}0001$), but not on *Pinus ponderosa*. Moreover, exposure led to increased cuticular water loss and decreased total water content of needles in both age classes of *Pseudotsuga menziesii*, and for 1-year-old needles of *Pinus ponderosa* ($P < 0{\cdot}0001$). On exposed trees, needles in both age classes had lower F_v/F_m ratios ($P < 0{\cdot}0001$), suggesting reductions in photosynthetic efficiency. Thus, exposure of needles to chlorine gas may lead to increased drought susceptibility and damage to chloroplast membranes in conifers, and may have a negative influence on tree growth. Importantly, plant responses to chlorine gas are species-specific and are influenced by variation between sites and the stochastic movement of chlorine gas clouds.

Key words: *Pseudotsuga menziesii*, *Pinus ponderosa*, chlorine gas, foliar injury, defoliation, droplet contact angles, cuticular transpiration, chlorophyll fluorescence.

INTRODUCTION

Although foliar injury has been reported following exposure to chlorine gas, few data on the effects of acute exposure on physiological functions such as tree water relations, photosynthesis, and growth are reported in the literature. Chlorine, a yellowish-green gas, is about 2·5-times more dense than air, and moderately soluble in water. Chlorine gas has many applications in the chemical, pharmaceutical and paper industries (Yosie, 1996), and is also used to disinfect water (Richardson *et al*., 1996). Accidents involving release of chlorine gas are not uncommon. For example, the Hazardous Substances Emergency Events Surveillance System reported 138 accidental releases involving chlorine gas in nine states in the USA over the period 1990–1992 (Hall *et al*., 1996). About 25 % these accidents involved human injury and about 30 % led to evacuation of people (Hall *et al*., 1996).

Studies evaluating the influence of chlorine gas exposure on vegetation have followed either accidental chlorine releases into the environment (Brennan *et al*., 1969), or have been carried out under controlled conditions (e.g. Thornton and Setterstrom, 1940; Brennan *et al*., 1965; Griffiths and Smith, 1990). The most common foliar injury symptoms after exposure to chlorine gas include chlorosis (bleaching of tissues), necrotic mottling (red and black dark spots on the leaf surface), and necrosis (death of cells and cell tissue). These effects can be observed on foliage of both deciduous and coniferous species (Heck *et al*., 1970; Temple *et al*., 1998). On conifer needles, chlorine gas exposure causes tip-burn, an orange-brown colouration extending from the tip to the base of a needle, which eventually kills the whole needle (Brennan *et al*., 1966). Completely necrotic foliage generally abscises prematurely from the plant, which can significantly reduce the photosynthetic leaf area (Heck *et al*., 1970). Chlorosis and necrosis have been reported after exposure to chlorine concentrations as low as 0·5–3·0 ppm (Thornton and Setterstrom, 1940; Brennan *et al*., 1965). However, toxicity thresholds depend on several factors including 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 caused by acid rain and acid mist (Vijayan and Bedi, 1989; Whitney and Ip, 1991). This is expected because chlorine gas can form highly acidic solutions in the aqueous phase, consisting of hydrochloric acid (HCl) and hypochlorous acid (or bleach, HOCl; $pKa = 7{\cdot}58$ at 20 °C). The effect of acid mist on plant cuticles (e.g. Percy *et al*., 1992) may lead to changes in the way epicuticular waxes interact with liquid water, increased cuticular water loss, and susceptibility to drought stress (Hadley and Smith, 1990; Esch and Mengel, 1998). Furthermore, lower rates of photosynthetic gas exchange and photosynthetic efficiency have also been reported following exposure to acid mist (Momen *et al*., 1997; Velikova *et al*., 1997).

* For correspondence. Fax 001 406 243 4184, e-mail mschreuder@airsci.com

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TABLE 1. *Physical description of study sites*

Site	Elevation (m asl)	Soil type	Habitat type (after Pfister *et al.*, 1977)	Peak Cl_2 (ppm)	Average Cl_2 (ppm)
Upwind control (approx. 4 km)	876	Coarse loam	*Pseudotsuga menziesii*/ *Arctostaphylos uva-ursi*		
50 m downwind	897	Fine loam	*Pseudotsuga menziesii*/ *Physocarpus malvaceus*	14090	9580 ± 52 %
0·2 km downwind	898	Fine loam	*Pseudotsuga menziesii*/ *Physocarpus malvaceus*	2930	1990 ± 46 %
0·8 km downwind	900	Fine loam	*Pseudotsuga menziesii*/ *Physocarpus malvaceus*	560	380 ± 46 %
1·5 km downwind	902	Fine loam	*Pseudotsuga menziesii*/ *Symphoricarpos albus*	260	175 ± 46 %
Downwind control (approx. 65 km)	976	Fine loam	*Pseudotsuga menziesii*/ *Physocarpus malvaceus*		

Pinus ponderosa was the second most abundant tree species at all study sites. All sites were on level benches in close vicinity to the Clark Fork River, western Montana, USA. Estimated concentrations of chlorine gas are based on a dispersion model for chlorine gas with the following input variables (ATSDR, 1997): duration of the release 48 min; mass released approx. 55 tons; windspeed approx. 1·0 m s^{-1}; relative humidity 20 %. Values are the predicted peak concentrations (10 min averages), and average and relative standard deviation of concentrations over the whole cloud width from ground level to 1·5 m above the ground (10 min averages).

This study was initiated after an accidental release of chlorine gas into a montane, coniferous forest ecosystem. We investigated: (1) morphological symptoms related to acute chlorine gas exposure on conifers downwind from the site of gas release; (2) effects of chlorine gas exposure on cuticular waxes, cuticular transpiration and water content of conifer needles; and (3) the influence of acute chlorine gas exposure on photosynthetic capacity of conifer needles.

In an accompanying paper (Schreuder and Brewer, 2001) we report the effects of this chlorine, gas release on the physiology and growth of the two conifer species over several years following exposure.

MATERIALS AND METHODS

Study site

This study was conducted in a narrow (approx. 0·5 km wide) valley in the Rocky Mountains, approx. 2 km west of Alberton, Montana, USA (47°00′N, 114°30′W). On 11 Apr. 1996, at 0400 h, a 72-car train derailment released approx. 55 metric tons of chlorine gas into the soil and atmosphere (atmospheric conditions: light rain and windspeed < 1 ms^{-1}). Studies suggested that trees up to approx. 14 km downwind from the derailment site were exposed to chlorine gas (Olympus Environmental, 1996). Over the following week, measured chlorine concentrations at the site of the gas release varied from 12 to 50 ppm (1 h average), with peak concentrations reaching approx. 1400 ppm (Olympus Environmental, 1996). An atmospheric dispersion model predicted peak chlorine gas concentrations of approx. 260 ppm 1·5 km downwind from the release site, decreasing to approx. 5 ppm 9 km downwind (ATSDR, 1997). However, chlorine concentrations could have varied considerably across the width of the cloud and with distance from the ground (Table 1). Since the chlorine gas was released in conditions of high atmospheric humidity, it is likely that the cloud was highly acidic. Chemical modelling results show that the pH in the gas cloud could have been as low as 1, due to the rapid formation of HCl and HOCl when chlorine gas is in contact with the aqueous phase (Schreuder, unpubl. res.). Finally, several chlorophenol compounds were formed due to a ruptured railroad car containing potassium cresylate. Data on atmospheric concentrations of these organic pollutants were not available, but concentrations in the soil were reported to be well below levels expected to adversely affect public health (Olympus Environmental, 1996).

Four sites that had been exposed to chlorine gas were selected for study (Table 1, Fig. 1). Whole-tree conditions (based on relative presence of green, chlorotic and necrotic foliage) of *Pseudotsuga menziesii* and *Pinus ponderosa* were uniform within each site, but varied between sites. The sites were located as follows: within a 50 m radius of the site of the spill (foliage completely necrotic except for current-year needles); 200 m from the spill (foliage mainly chlorotic); and at 0·8 km and 1·5 km downwind (trees not visibly injured 2 months after gas exposure). Two control sites were established approx. 65 km downwind from the site of the gas release (CD; 46°70′N, 114°00′W; Table 1) and approx. 4 km upwind (CU; Table 1). There were no indications that chlorine gas had reached the control sites based on modelling studies and the absence of foliar injury characteristic of chlorine gas exposure. All field sites were comprised of mixed coniferous forests dominated by *Pseudotsuga menziesii* Mirb. Franco (Douglas fir) and *Pinus ponderosa* Laws (Ponderosa pine), and located on the bottom of the valley. Understorey vegetation and soils were similar at all sites (Table 1).

Visible morphological injury

Morphological injury to vegetation was assessed visually immediately after it was safe to access study sites on 15 May

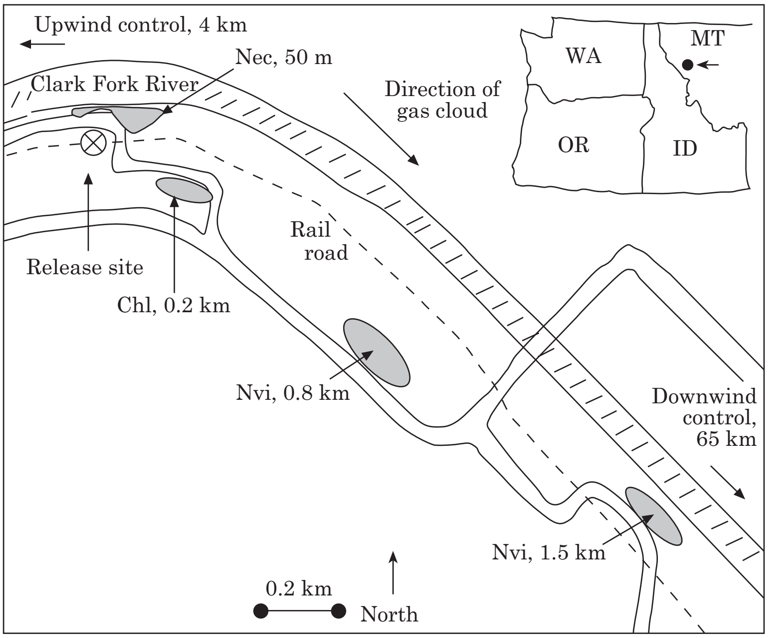

FIG. 1. Schematic map of the proximity of study sites to the site of gas release. Site descriptions are based on foliar injury 2 months after exposure, and are characterized as mainly necrotic (Nec), chlorotic (Chl), and no visible injury present (Nvi). More detailed description of the study sites and the estimated chlorine concentrations are shown in Table 1.

1996 (1 month post spill) and again on 10 June 1996. At each study site, ten trees of each species (representative for the site) were chosen at random for examination (two branches per tree; 1·5–2·0 m above the ground surface). Light microscopy techniques were used to examine foliage for damage. Foliage injury was scored using the following criteria: no visible injuries to foliage (0); necrotic mottling (1); chlorotic (2); tipburn (3); and completely necrotic (4). Comparisons from the two control sites were based on observations in March 1997 and 1998 (1–3-year-old foliage).

Secondary shoot growth

Survival rates of exposed buds (based on whether or not they opened) on *Pinus ponderosa* and *Pseudotsuga menziesii* were recorded at the end of May 1996. Data on secondary shoot growth were collected in the summer of 1999, to assess secondary shoot growth in the 1996 growing season. At each site, five randomly chosen trees of each species were evaluated, with two replicate branches per tree (approx. 1·5 to 2 m off the ground). The presence or absence of secondary shoots was recorded. When secondary shoots were present, the number per branch was recorded.

Cuticular injury

To assess cuticular injury, branches of *Pinus ponderosa* and *Pseudotsuga menziesii* were collected from sites 50 m, and 0·2 and 0·8 km downwind from the spill site as well as from an unexposed downwind control site (CD, approx. 65 km downwind). One-year-old needles (flushed in 1995) that had been exposed to chlorine and non-exposed current-year needles (flushed in 1996) were evaluated. Effects of chlorine gas exposure on the cuticle were assessed (four–ten needles per tree, $n = 10$ trees) by measuring contact angles of water droplets (CA; droplet size 5 µl) and droplet retention angles (RA; water droplet size 10 µl; Brewer *et al*., 1991; Brewer, 1996). RT is the angle at which a droplet on the surface starts to roll, with low angles representing poor retention (Brewer *et al*., 1991). CA and RT indicate changes in the outer layer of the cuticle, i.e. the epicuticular waxes, rather than in the whole cuticle.

Cuticular transpiration was determined for subsamples from five randomly selected trees at each site ($n = 2$–4 trees) using the methods of Hadley and Smith (1990). Samples were periodically weighed over the course of 3 d. Two stems per species from each site (representing each needle age class) were weighed, water-saturated overnight, and weighed again. Stems were then sealed with paraffin wax to avoid water loss via stem edges, and subsequently dried at approx. 30 °C in a drying oven. Minimal conductance to water vapour of the needles (Kerstiens, 1996) was derived using data on temperature and relative humidity in the drying oven, and specific needle area (SLA). Leaf area was determined using the glass bead method (Thompson and Leyton, 1971). Total water content (TWC, expressed as g H_2O g^{-1} d. wt) and relative water content of foliage (RWC, expressed as the ratio of fresh-dry weight to saturated-dry weight) were calculated.

Photosynthetic efficiency

Chlorophyll fluorescence measurements were made on needles of *Pseudotsuga menziesii* and *Pinus ponderosa* with an Opti-Sciences Modulated Fluorometer (Model OS-100, PP Systems, Haverhill, MA, USA). Measurements were made between 1000 and 1400 h (saturated light pulse = 0·8 s; F_0 = 100–125). Needle samples (n = 6 trees per species, with two replicates per tree) were dark-adapted for 15 min prior to measurements. F_0 (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 were analysed using the SPSS statistical package (SPSS, 1995). Data that met requirements for normal distribution were analysed using a *t*-test (reported as *T*, *P*-value) or 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 either two (F_v/F_m) or five replicates (CA and RT) per tree. Subsamples were tested for significant differences, and were pooled if within-subsample differences were not significant ($P > 0{\cdot}05$; Sokal and Rohlf, 1997). Repeated measures (RM) techniques were used to analyse foliar water loss data with two repeated factors, time of drying and sampling date. Droplet retention data were analysed using the Kolmogorov-Smirnov test (reported as ksz, *P*-value).

RESULTS

Morphological injury symptoms

Macroscopic injury to existing needles on coniferous trees was visually apparent. Needles on both *Pseudotsuga menziesii* and *Pinus ponderosa* showed extensive necrosis and tipburn. Needles of most *Pseudotsuga menziesii* trees up to approx. 0·5 km from the spill were almost completely necrotic (>85 % of foliage up to 2 m above the ground was necrotic). After 2 months, no green needles were present and there were few chlorotic needles. Necrotic needles abscised during the summer. Most exposed *Pseudotsuga menziesii* trees developed new green foliage over the course of the growing season. Foliage of *Pinus ponderosa* up to 0·2 km downwind of the derailment site was mostly necrotic or chlorotic (90 % of foliage up to 2 m above the ground was necrotic and 75 % chlorotic), although newly flushed green needles were present. At distances beyond 0·2 km downwind, needles on *Pinus ponderosa* were mostly green, and only minimal visual injury was observed.

The degree of foliar damage generally decreased with increasing distance from the site of the gas release for both *Pseudotsuga menziesii* and *Pinus ponderosa* ($T_2 = 2{\cdot}016$, $P < 0{\cdot}05$; Fig. 2). However, foliar injury downwind of the release site was higher than at the two control sites, especially for *Pseudotsuga menziesii*. Variation in foliar injury, between patches of trees as well as within individual trees, was high. For example, the bottom half of a *Pinus ponderosa* tree approx. 0·8 km downwind from the derailment was completely necrotic, while the upper half showed no visual injury. Healthy green foliage, as well as chlorotic, necrotic, necrotic mottled, or tipburn foliage often occurred within the same tree.

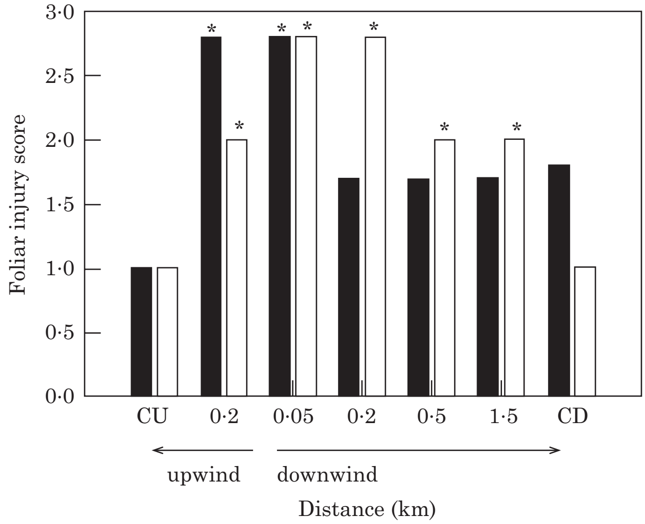

FIG. 2. Foliar injury on *Pinus ponderosa* (■) and *Pseudotsuga menziesii* (□), for all needle age classes measured in June 1996, 2 months after exposure to chlorine gas. The scores are means (n = 10 trees, two replicate branches per tree) for the following categories of visually determined injury: non-visibly injured (0); necrotic mottling (1); chlorotic (2); tipburn (3); and completely necrotic (4). Data for the upwind (CU, 4 km upwind) and downwind control (CD, 65 km downwind) sites are based on observations made in March 1997 and 1998 (1–3-year-old foliage). Exposed sites were generally on level terrain, except the site 0·2 km uphill of the gas release (0·2 up). Asterisks indicate sites that are statistically different from both upwind and downwind control sites within each species (*t*-test, $P < 0{\cdot}05$).

Secondary shoot growth

By the end of May 1996, 100 % of *Pseudotsuga menziesii* buds had flushed on trees at control sites and sites more than 0·2 km downwind from the chlorine release. In *Pseudotsuga menziesii*, 30 % of buds on trees within 50 m of the release were killed compared to control sites (Fisher's exact test, $P = 0{\cdot}005$). The absence of healthy apical buds gave rise to the growth of secondary shoots (mean = 9 ± 2 s.e. secondary shoots per major branch; n = 20). However, there was no obvious pattern in the distribution of the secondary shoots over shoot increments of different ages. Within 50 m of the gas release, 75 % of *Pinus ponderosa* buds were killed (Fisher's exact test, $P = 0{\cdot}031$). At distances >50 m from the spill, 100 % of *Pinus ponderosa* buds opened at all study sites. Although bud mortality within 50 m of the gas release was much higher for *Pinus ponderosa* than for *Pseudotsuga menziesii*, no secondary shoots were produced on *Pinus ponderosa* in 1996.

Wettability of needle surfaces

Exposure to chlorine gas altered the interaction between needle surfaces and water droplets. The droplet contact angle (CA) of exposed 1-year-old *Pseudotsuga menziesii* needles decreased in comparison to control needles (Table 2), which may have led to increased leaf wettability and a greater tendency for water layers to form over the needle surface. However, on *Pinus ponderosa* needles, lower CAs were apparent only for 1-year-old needles of trees growing 0·8 km downwind from the release site (Table 2).

TABLE 2. *Droplet contact angle (CA) and droplet retention angles (RT) for 1-year-old needles (1995; n = 10 trees), and current-year needles (1996; n = 10 trees), measured in July 1996*

Species and site	Contact angle (CA) degrees		Droplet retention (RT) degrees	
	1995	1996	1995	1996
Pinus ponderosa				
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)
Pseudotsuga menziesii				
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)

Letters indicate statistically significant differences within each species and needle age class (within columns; nested one-way ANOVA for CA, and Kolmogorov-Smirnov for RT; $P < 0{\cdot}0001$; five replicates per tree for the 1995 needle age class; two replicates per tree for the 1996 needle age class). Values are means (±s.e.).

Current-year *Pinus ponderosa* foliage (not directly exposed to chlorine gas) did not show changes in leaf wettability (Table 2). In contrast, current-year *Pseudotsuga menziesii* foliage had significantly lower CA up to 0·2 km from the release site compared to control needles (Table 2). Significant changes in droplet retention (RT) were found only for *Pseudotsuga menziesii* foliage that was directly exposed to chlorine gas (Table 2).

Cuticular water loss

Cuticular water loss and minimal conductance to water vapour were species dependent, and did not necessarily correspond with the severity of foliar injury or distance from the release site. Cuticular water loss from exposed, 1-year-old *Pinus ponderosa* foliage (flushed in 1995) was significantly higher at all exposed sites compared to control sites ($F_{3,96} = 66{\cdot}14$, $P < 0{\cdot}001$; Fig. 3A). However, minimal conductance to water vapour over cuticles (assuming closed stomata; $G_{min,H2Ov}$) was higher at only two of the exposed sites ($F_{3,96} = 33{\cdot}72$, $P < 0{\cdot}001$; Fig. 3B). In comparison, there was no consistent trend that could be attributed to chlorine gas exposure for current-year needles (flushed in 1996 after the spill), since cuticular water loss, expressed both as relative water loss ($F_{3,96} = 44{\cdot}19$, $P < 0{\cdot}001$; Fig. 3A) and $G_{min,H2Ov}$ ($F_{3,96} = 6{\cdot}73$, $P = 0{\cdot}006$; Fig. 3B), was higher only on trees growing 0·8 km downwind of the spill.

Effects on cuticular transpiration were also observed on foliage of *Pseudotsuga menziesii*. Relative water loss from 1-year-old and current-year foliage was greater at some sites ($F_{3,96} = 6{\cdot}60$, $P = 0{\cdot}007$ and $F_{3,96} = 4{\cdot}25$, $P = 0{\cdot}029$, respectively) compared to control sites (Fig. 4A). Necrotic 1-year-old foliage of *Pseudotsuga menziesii* (50 m from release site) had significantly lower $G_{min,H2Ov}$ ($F_{3,84} = 12{\cdot}75$,

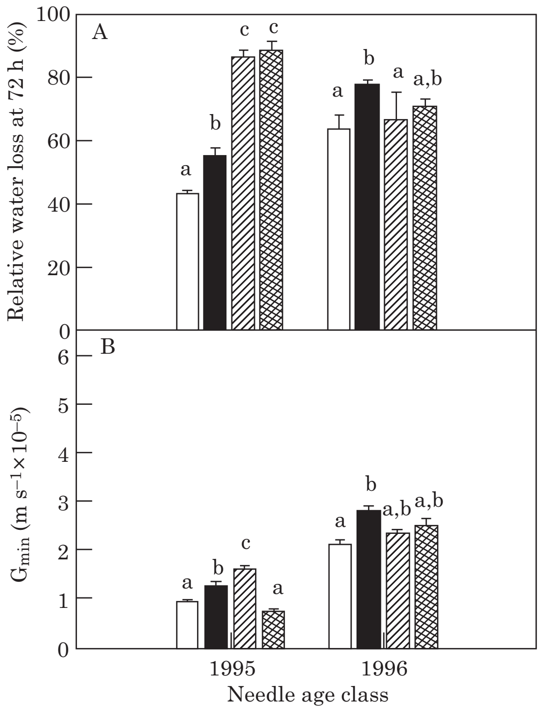

FIG. 3. Relative water loss (A) and minimal conductance $G_{min,H2Ov}$ (B) for 1-year-old (1995) and current-year (1996) foliage *Pinus ponderosa*. Although statistical analyses are based on data from 0 to 72 h, relative water loss is shown after 72 h ($n = 4$; nested two-way ANOVA, $P < 0{\cdot}001$; repeated factor: drying time, ten measurements over time). $G_{min,H2Ov}$ is shown as the average from 2 to 72 h ($n = 4$; nested two-way ANOVA, $P < 0{\cdot}001$; repeated factor: drying time, nine measurements over time). Bars represent the downwind control site (□), 0·8 km downwind (■), 0·2 km downwind (▨), and 50 m downwind (⊠). Error bars are ± 1 s.e.

$P < 0{\cdot}001$; Fig. 4B), which can be attributed to the low water content of the necrotic foliage (Table 3). Current-year foliage on exposed *Pseudotsuga menziesii* trees also had higher $G_{min,H2Ov}$ values when compared to control needles ($F_{3,84} = 7{\cdot}03$, $P = 0{\cdot}006$; Fig. 4B).

Water content

Total water content (TWC) of exposed 1-year-old needles decreased with increasing levels of visual injury (Table 3). Foliage of both conifer species within 0·2 km of the release site had a lower TWC than foliage at control sites or from trees further downwind. However, current-year needles on trees within 50 m of the release site had a significantly higher TWC than at the other sites (Table 3). Differences in RWC were less pronounced than for TWC (Table 3). Only 1-year-old *Pseudotsuga menziesii* needles within 50 m of the release site had a significantly lower RWC compared to control needles (Table 3).

Chlorophyll fluorescence

Exposed 1-year-old and current-year needles of both *Pinus ponderosa* and *Pseudotsuga menziesii* had significantly lower F_v/F_m ratios compared to controls (Table 4). The

TABLE 3. *Total water content (n = 4 trees) and relative water content (n = 2 trees) of 1-year-old (1995) and current-year needles (1996)*

	Total water content (g H_2O g^{-1} d. wt)		Relative water content (%)	
Species and site	1995	1996	1995	1996
Pinus ponderosa				
Control, downwind	1·21 (0·04)[a]	1·92 (0·06)[a]	92 (1)	94 (1)[a]
0·8 km downwind	1·30 (0·01)[a]	2·03 (0·01)[a]	94 (0)	100 (1)[b]
0·2 km downwind	1·01 (0·03)[b]	2·03 (0·01)[a]	95 (2)	99 (1)[a,b]
50 m downwind	0·36 (0·04)[b]	2·14 (0·04)[b]	88 (1)	98 (0)[a,b]
ANOVA	$F_{3,13} = 279{\cdot}30$, $P < 0{\cdot}0001$	$F_{3,5} = 8{\cdot}55$, $P = 0{\cdot}026$	$F_{3,13} = 5{\cdot}38$, $P = 0{\cdot}069$	$F_{3,5} = 11{\cdot}07$, $P = 0{\cdot}021$
Pseudotsuga menziesii				
Control, downwind	1·26 (0·03)[a]	1·63 (0·09)[a]	95 (3)[a]	94 (2)
0·8 km downwind	1·12 (0·02)[b]	1·75 (0·03)[a]	93 (1)[a]	96 (1)
0·2 km downwind	0·87 (0·02)[c]	1·70 (0·07)[a]	89 (1)[a]	99 (1)
50 m downwind	0·12 (0·01)[d]	2·57 (0·34)[b]	67 (1)[b]	91 (4)
ANOVA	$F_{3,13} = 644{\cdot}07$, $P < 0{\cdot}0001$	$F_{3,5} = 7{\cdot}86$, $P = 0{\cdot}004$	$F_{3,13} = 65{\cdot}05$, $P = 0{\cdot}0008$	$F_{3,5} = 2{\cdot}36$, $P = 0{\cdot}213$

Data were collected in July 1996. Letters indicate statistically significant differences within each species and age class (one-way ANOVA). Values are means (±s.e.).

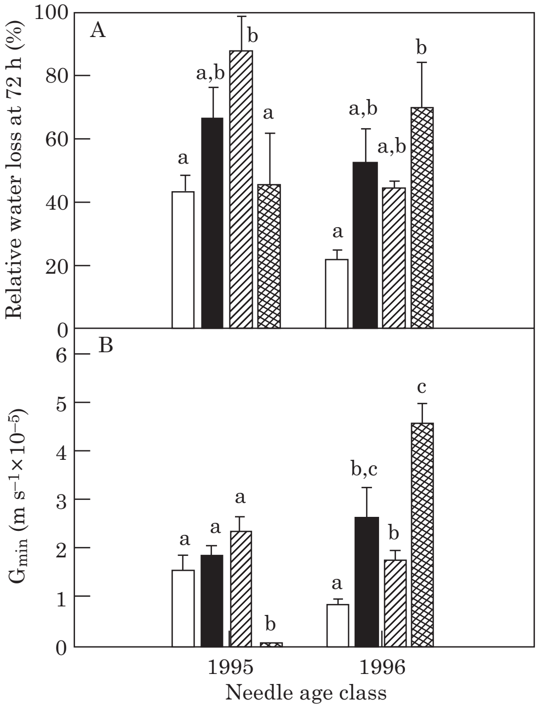

FIG. 4. Relative water loss (A) and minimal conductance $G_{min,H2Ov}$ (B) for 1-year-old (1995) and current-year (1996) foliage of *Pseudotsuga menziesii*. Relative water loss is shown after 72 h ($n = 4$, nested two-way ANOVA, $P < 0{\cdot}001$; repeated factor: drying time, ten measurements over time). $G_{min,H2Ov}$ is shown as the average from 2 to 72 h ($n = 4$; nested two-way ANOVA, $P < 0{\cdot}001$; repeated factor: drying time, nine measurements over time). Bars represent the downwind control site (□), and sites 0·8 km downwind (■), 0·2 km downwind (▨), and 50 m downwind (⊠). Error bars are ± 1 s.e.

reduction in the F_v/F_m ratio for 1-year-old foliage was more pronounced in *Pinus ponderosa* that in *Pseudotsuga menziesii* needles, suggesting that foliage in these age classes on exposed trees had decreased photosynthetic efficiency.

TABLE 4. F_v/F_m *ratios (n = 6 trees) for 1-year-old (1995) and current-year needles (1996)*

	F_v/F_m ratio	
Species, site	1995	1996
Pinus ponderosa		
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	$F_{3,9} = 25{\cdot}87$, $P < 0{\cdot}0001$	$F_{3,9} = 3{\cdot}94$, $P = 0{\cdot}015$
Pseudotsuga menziesii		
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	$F_{3,9} = 16{\cdot}00$, $P < 0{\cdot}0001$	$F_{3,9} = 14{\cdot}29$, $P < 0{\cdot}001$

Data were collected in July 1996. Letters indicate statistically significant differences within each species and age class (nested one-way ANOVA; two replicates per tree). Values are means (±s.e.). Needle age classes that were no longer present at the time of measurement are indicated as 'n.a.'.

DISCUSSION

This is the first study to report physiological responses of conifers growing under natural conditions to chlorine gas exposure. Acute exposure to chlorine gas has immediate negative effects on exposed native vegetation and these effects can be found up to 1·5 km downwind from the immediate spill area. Acute foliar injury symptoms, consisting of chlorosis, necrotic mottling, tipburn and necrosis occurred in both *Pinus ponderosa* and *Pseudotsuga menziesii*. Necrotic needles defoliated during the months following exposure. While foliage that was still in bud at the time of the gas release was generally not visibly affected, there were negative influences on water loss and photosynthetic efficiency. The results of this study suggest that chlorine exposure leads not only to visual foliar injury, but may also influence longer-term tree growth through defoliation, increased susceptibility to drought stress, and decreased photosynthetic efficiency. Importantly, these effects were species-specific and were spatially variable, probably due to the patchy movement of chlorine gas clouds across a complex landscape.

Morphological injury symptoms

Foliar injury symptoms were most severe within a radius of about 100 m from the site of gas release. The most prevalent foliar injury symptoms observed on *Pseudotsuga menziesii* and *Pinus ponderosa* were chlorosis, necrosis and tipburn, and many trees partially-to-fully defoliated over the growing season. These symptoms are similar to the foliar injury reported for other conifer species after exposure to chlorine gas (Brennan *et al*., 1966; Heck *et al*., 1970; Temple *et al*., 1998). Bud mortality was observed in the present study but was not observed by Brennan *et al*. (1969). However, trees in our study sites were exposed to considerably more chlorine gas (up to 1400 ppm; Olympus Environmental, 1996) than trees in the Brennan study (10 ppm; Brennan *et al*., 1969). Formation of secondary shoots has been reported for other conifers, e.g. *Picea abies*, as a result of defoliation (Salemaa and Jukola-Sulonen, 1990). *Pseudotsuga menziesii* responded to defoliation by producing secondary shoots but *Pinus ponderosa* did not. Thus, new *Pinus ponderosa* foliage arose only from surviving buds. Interestingly, many deciduous tree species in the vicinity of the spill were not visibly injured by chlorine exposure, possibly because these broad-leaved species had not yet experienced bud break at the time of gas exposure. However, chlorosis, necrotic mottling and necrosis were observed on directly exposed foliage of the shrubs and herbaceous species present (Schreuder, 2000).

Two mechanisms may explain foliar injury: chloride accumulation in plant tissues (e.g. Führer and Erisman, 1980; 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 average natural background levels reported for native plants in boreal forests in Canada (approx. 1100 ppm Cl^-; range 15–5000 ppm; Sheppard *et al*., 1999), and 30- to 300-times greater than concentrations reported for foliage of crop species (range 100–1000 ppm Cl^-; Führer and Erisman, 1980). Although accumulation of chloride in plant tissues has been observed after exposure to chlorine gas (Vijayan and Bedi, 1989), this is not necessarily the only cause of tissue damage (Brennan *et al*., 1965). It is likely that plants in our study 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 7·0–8·5 in plant tissues (Smith and Raven, 1979). Based on a chemical modelling approach, the pH of the chlorine gas cloud in the vicinity of the spill site could have been as low as pH 1 (Schreuder, unpubl. res.). Under strongly acidic conditions, plasmolysis may occur in plant cells due to the accumulation of acid in the apoplast (Thornton and Setterstrom, 1940; Heath, 1980). Moreover, competition between H^+ and Mg^{2+} ions may occur in chlorophyll molecules. This is a potential causal mechanism for the chlorosis observed after chlorine gas exposure. Although either damage mechanism described above is plausible, the rapid appearance of foliar injury suggests that tissue acidity following exposure may be the most likely immediate cause of foliar injury. However, some of the longer-term effects we observed could be related to chloride accumulation in the exposed foliage.

In general, heavy gasses tend to remain close to the ground (e.g. Griffiths and Fryer, 1988), especially at wind speeds as low as those reported at the time of the gas release. Hence, variation in the extent of foliar injury within trees and between sites can be explained, in part, by rapid changes in chlorine gas concentrations in the gas clouds that lingered in the area for several days. For example, branches of a *Pinus ponderosa* tree approx. 1 km downwind had completely necrotic needles on the lower 2 m and there was no recovery over the period of this study. Surprisingly, needles on branches in the upper part of the tree showed no visual injury. This same pattern of spatially partitioned injury within a tree also was observed in *Juniperus scopulerum*, further implicating highly variable movement of chlorine gas clouds through the forest.

Influences on cuticles and leaf moisture content

Increased wettability has been reported for many species after exposure to acid mist (e.g. *Picea rubens*: Percy *et al*., 1992; beech: Paoletti *et al*., 1998). In this study, chlorine gas exposure also led to a greater tendency for water films to collect on needle surfaces of exposed new needles of *Pseudotsuga menziesii* (Table 2). Interestingly, higher leaf wettability was not related to the severity of foliar injury observed. Because the tendency of water to form films instead of droplets on leaves can lead to reductions in rates of gas exchange (e.g. Brewer and Smith, 1994), increased leaf wettability can form an additional avenue by which chloride exposure could negatively influence photosynthesis (Brewer *et al*., 1991).

One direct effect of acid rain and mist on plant epicuticular waxes is the erosion of cuticular waxes (Paparozzi and Tukey, 1984). Indirect effects mediated through wax biosynthesis include decreased cuticular thickness and reduction in surface waxes (Garrec and Kerfourn, 1989; Percy *et al*., 1992). Ultrastructural changes (Percy *et al*., 1992) and changes in the chemical composition of the cuticle (Percy *et al*., 1992; Günthardt-Görg, 1994) have also been reported. Morphological changes such as wax erosion and decreased cuticle thickness (DeLucia and Berlyn, 1984; Hadley and Smith, 1990) have been correlated with increased cuticular water loss.

Water loss and $G_{min,H2Ov}$ of *Pinus ponderosa* and *Pseudotsuga menziesii* foliage were influenced at some of the study sites. Within 50 m of the release site, $G_{min,H2Ov}$ of

1-year-old necrotic needles was lower than that at other sites (Figs 3B and 4B), especially for *P. menziesii*. This can be explained by the low values of TWC in necrotic needles (Table 3; Hadley and Smith, 1990). Because current-year *Pseudotsuga menziesii* needles on exposed trees had increased cuticular water loss and $G_{min,H2Ov}$ (Fig. 4), it is likely that cuticles were influenced via indirect pathways. In *Pinus ponderosa* however, differences in cuticular transpiration of current-year needles between study sites could not be attributed solely to chlorine gas exposure (Fig. 3A).

Higher values of $G_{min,H2Ov}$ suggest increased susceptibility to drought and winter desiccation in affected trees. Directly exposed foliage of both species had a lower TWC (Table 3), and exposed chlorotic *Pinus ponderosa* needles were abscised during dry conditions in the summer of 1996, but not at control sites. Even though chlorine gas exposure was not generally correlated with TWC in needles that flushed after gas exposure, these needles did show substantial drought damage over the summer, especially in *Pinus ponderosa*. Due to the substantial decrease in photosynthetic biomass via abscised needles, negative influences on tree carbon balance would be expected in affected trees.

The stomatal response to acid rain is influenced by factors such as ambient temperature (Momen *et al*., 1999), chemical composition of the aqueous phase (Eamus and Murray, 1993), and tree age (Momen *et al*., 1997). However, acid rain does not necessarily affect stomatal conductance (e.g. Anderson *et al*., 1997). Although data on $G_{min,H2Ov}$ suggest stomata were closed on the needles we measured, a stomatal component to $G_{min,H2Ov}$ cannot be ruled out completely due to the possibility of incomplete closure, which has been reported in leaves exposed to acid rain (e.g. Barnes *et al*., 1990; Kerstiens, 1996).

We did not evaluate the extent to which whole tree water balance was affected by exposure to chlorine gas. Consequently, one should be cautious when extrapolating these patterns to the whole tree level because exposure was highly variable given the movement of the gas clouds and it is not known if foliage throughout the canopy was always affected to the same extent.

Influences on photosynthetic efficiency

Our data agree with those reported in other studies on the adverse influences of acid rain on photosynthetic efficiency in, for example, *Picea abies* (Šiffel *et al*., 1996). Exposure to chlorine gas led to decreased photosynthetic efficiency of exposed 1-year-old needles of *Pseudotsuga menziesii* and *Pinus ponderosa* (Table 4) compared to needles from control trees. Moreover, foliage that developed on exposed trees after the chlorine cloud dissipated also had lower photosynthetic efficiency (Table 4), suggesting that photosynthetic membranes of new needles were affected indirectly by the gas exposure. The combination of defoliation, increased drought susceptibility and decreased photosynthetic efficiency are known to adversely affect tree growth and mortality (Salemaa and Jukola-Sulonen, 1990; Christiansen and Fjone, 1993). However, additional studies are needed to fully document the longer-term effects of chlorine gas exposure on tree growth and mortality (Schreuder and Brewer, 2001).

CONCLUSIONS

Plant responses to chlorine gas exposure are species-specific. Moreover, variation in the extent of foliar injury is considerable, depending on site conditions, the unpredictable movement of chlorine gas clouds, and rapid changes in chlorine gas concentrations in the cloud. Results of this study suggest that acute chlorine gas exposure caused visible needle injury, and more subtle cuticular changes, as well as greater water loss during the growing season. Both conifer species in this study lost photosynthetic biomass due to defoliation, while influences on photosynthetic efficiency varied between species. Based on the negative effects reported here, we expect greater susceptibility to drought stress and lower growth in trees exposed to chlorine gas. Studies over a time period of at least several years are needed to address multi-year influences of chlorine gas exposure on growth and mortality of forest trees.

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