



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

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Received: 3 November 2000 Returned for revision: 23 January 2001 Accepted: 3 April 2001 Published electronically: 25 June 2001

Following a single acute exposure to chlorine gas, persistent effects on epicuticular waxes, cuticular transpiration, tree growth and mortality were studied in foliage of *Pinus ponderosa* and *Pseudotsuga menziesii* for three growing seasons. Chlorine gas exposure caused foliar injury to both exposed foliage and foliage that flushed after exposure ($P < 0.05$). The tendency to form films of water rather than droplets was greater in directly exposed foliage ($P < 0.001$). Rates of cuticular transpiration were higher for directly and indirectly exposed foliage of *Pinus ponderosa* up to 1 year after exposure and up to 6 months after exposure for directly exposed *Pseudotsuga menziesii* ($P < 0.001$), after which *P. menziesii* needles defoliated. Total water content (TWC) and relative water content were significantly correlated with foliar injury ($P < 0.05$). TWC was lower for directly exposed foliage up to 1 year after exposure ($P < 0.001$). There was no persistent negative effect on F_v/F_m ratios after 1 year. Exposure to chlorine gas did not affect needle length or annual shoot increment growth, but exposure was correlated with increased bud production. Needle longevity of foliage that flushed 2 months after exposure was reduced significantly ($P < 0.001$). Annual stem increment growth for both species decreased over at least three growing seasons following chlorine gas exposure ($P < 0.001$), and depended on distance from the spill site. Cone production was lower for exposed *Pinus ponderosa* trees compared to controls ($P < 0.05$), and tree mortality was higher within approx. 50 m of the release site for *Pseudotsuga menziesii*. Growth responses for both conifers agreed well with predicted patterns of carbon allocation after defoliation caused by chlorine gas exposure. © 2001 Annals of Botany Company

Key words: *Pinus ponderosa*, *Pseudotsuga menziesii*, conifers, chlorine gas, leaf wettability, cuticular transpiration, water relations, growth, mortality.

INTRODUCTION

Accidents involving release of chlorine gas are not uncommon (Hall *et al.*, 1996), and can result in foliar injury to vegetation (Brennan *et al.*, 1965; Heck *et al.*, 1970; Temple *et al.*, 1998; Schreuder and Brewer, 2001). While acute effects have been reported for some species, persistent influences on physiological functions (e.g. tree water relations, photosynthesis and growth) following acute exposure have not been widely reported.

The effects of exposure to chlorine gas are similar to those of acid rain and acid mist because chlorine gas forms highly acidic solutions in contact with water (Morris, 1946; Schreuder and Brewer, 2001). It is well known that acid rain and acid mist affect the composition and structure of plant cuticles and their production of waxes (e.g. Garrec and Kerfourn, 1989; Percy *et al.*, 1992). Cuticular damage may lead to increased rates of cuticular transpiration rendering plants more susceptible to drought stress, both in summer (Mengel *et al.*, 1989) and winter conditions (Hadley and Smith, 1989). In addition to decreased photosynthesis (Velikova *et al.*, 1997; Momen *et al.*, 1999) and photosynthetic efficiency (Velikova *et al.*, 1997), exposure to acid mist has been reported to increase stomatal conductance (e.g. Flagler *et al.*, 1994).

In conifers, foliar damage from acid mist and rain consists of chlorosis, necrotic mottling, and necrosis (e.g. Heck *et al.*, 1970; Temple *et al.*, 1998; Schreuder and Brewer, 2001). Necrosis generally begins as tipburn, an orange-brown colouration extending from the tip to the base of the needle; this eventually kills the whole needle (Brennan *et al.*, 1966), which is then abscised prematurely (Heck *et al.*, 1970; Schreuder and Brewer, 2001). Defoliation can lead to decreases in vertical growth (Carlson *et al.*, 1988; Krause and Raffa, 1996), stem increment growth and total tree biomass (Christiansen and Fjone, 1993; Krause and Raffa, 1996). Furthermore, the combination of defoliation and increased water stress may increase susceptibility to insect injury in conifers (Christiansen and Fjone, 1993).

In this study we report the persistent physiological and growth effects on two conifer species after a single acute exposure to chlorine gas in a montane coniferous forest. Earlier studies indicated significant foliar injury and defoliation of exposed *Pinus ponderosa* and *Pseudotsuga menziesii* trees (Schreuder and Brewer, 2001) in the growing season immediately after exposure. Moreover, the foliage showed increased cuticular water loss and decreased photosynthetic efficiency following exposure to chlorine gas (Schreuder and Brewer, 2001). In this study, we examined the extent to which acute exposure to chlorine gas influenced cuticular transpiration, foliar water content, and susceptibility of trees to drought stress and insect damage. We predicted that the combination of these

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influences would lead to persistent negative effects on tree growth and mortality.

MATERIALS AND METHODS

Study site

The study sites were located in a narrow valley approx. 2 km west of Alberton, Montana, USA, in the Rocky Mountains (47°00'N, 114°30'W). On 11 April 1996 at approx. 0400 h, a train derailment released approx. 55 metric tons of chlorine gas into the atmosphere and the surrounding forest. Over the following week, measured chlorine concentrations at the site of 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 (Schreuder and Brewer, 2001). Since the chlorine gas reacts rapidly with the aqueous phase forming HCl and HOCl, the gas cloud could have been highly acidic (pH < 1; Schreuder, unpubl. res.). Our investigation focused on the two most common conifer species in the area, *Pseudotsuga menziesii* (Douglas fir) and *Pinus ponderosa* (Ponderosa pine).

Two control sites were established, one approx. 65 km downwind (CD; 46°70'N, 114°00'W) and another approx. 4 km upwind (CU) from the site of gas release. Exposed study sites were selected based on foliar injury symptoms observed within 2 months after exposure (Schreuder and Brewer, 2001). These 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), and approx. 0.8 km and approx. 1.5 km downwind (foliage not visibly injured). Estimated peak chlorine concentrations at the study sites varied from approx. 1400 ppm 50 m downwind to 260 ppm 1.5 km downwind (Schreuder and Brewer, 2001). For some parameters, measurements were extended up to approx. 10 km downwind, the approximate distance over which symptoms of chlorine gas exposure could be documented visually (Olympus Environmental, 1996). All sites were similar in elevation, vicinity to the river, soil type and vegetation (Schreuder and Brewer, 2001). Tree health (based on visible symptoms of foliar injury) was judged to be uniform within each study site, but varied between sites.

Physiological and growth measurements were made over three growing seasons following the gas release. Samples were collected from five to 15 randomly selected trees with two to 15 replicates per tree (depending on the measurement). Samples for all measurements were collected from the lower canopy (1.5 to 2 m above the ground). In this study, the growing seasons will be referred to by calendar year or years since the chlorine gas release, and 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 insect damage was assessed in March 1997 and 1998, 1 and 2 years after exposure. Two branches with needles in the age classes -1, 0 and +1 yts were collected from five randomly selected trees at each site. Ten to 15 randomly chosen needles from each branch (50 to 75 in total) were scored in the laboratory according to the following five foliar injury categories: (1) 100 % green; (2) 5–25 % chlorotic; (3) >25 % chlorotic; (4) 5–25 % necrotic; and (5) >25 % necrotic. Beginning in March 1998, we checked for evidence of insect injury in the field during each sampling period.

Cuticular injury and transpiration

Effects of chlorine gas exposure on needle surfaces were assessed using droplet contact angles (CA, a measure of leaf wettability) and droplet retention angles (RT) according to Brewer (1996). CA and RT were determined for foliage from ten randomly selected trees at each site with two replicates per tree. Cuticular water loss was determined for exposed foliage from 1995 (-1 yts), and non-exposed foliage from 1996, 1997 and 1998 (0, +1 and +2 yts, respectively). One branch was collected from five randomly selected trees at each site. From this sample, two to four branch segments were selected at random for further analysis. Procedures used to measure cuticular water loss, minimal conductance to water vapour, G_{\min, H_2O_v} , total water content (TWC), and relative water content (RWC) are described in more detail by Schreuder (2000). TWC was expressed as g H₂O g⁻¹ d. wt. RWC was expressed as the ratio of fresh weight-dry weight to saturated weight-dry weight. Cuticular transpiration and foliar water content were measured quarterly from September 1998 for a total of three growing seasons following exposure to chlorine gas.

Growth measurements

Needle length was measured in March 1997 and 1998 for 15 randomly selected trees at each site (four replicates per tree). Annual shoot increment growth was assessed in the field in winter, spring and autumn 1997 by measuring the distance between nodes on leading branches in the lower canopy (five–15 randomly selected trees, two replicate branches per tree). The number of buds was counted each autumn (1996, 1997 and 1998) for ten–27 trees of each species, depending on availability at each site (two replicate branches per tree).

Retention of needle age classes was measured in the field in March 1998 at both control sites and at nine sites exposed to chlorine gas (up to approx. 10 km downwind of the site of release) for ten randomly selected trees per site (two replicates per tree). In April 1999, this survey was repeated at the two original control sites, an additional upwind control site, and eight exposed sites (≤1.5 km downwind of the site of release).

Tree cores were collected in May 1997 and November 1998 at both control sites and three exposed sites (≤ 0.8 km downwind from the release site). Sampling procedures at each site were the same as those used to estimate retention of living needle age classes. Annual core increment growth was calculated as the percentage change compared to an 18 year average (excluding the year of gas release). Relationships between foliar biomass and stem increment growth were described using a linear model (Nichols, 1988). In May 1998, large mature trees at all field sites were examined for the presence of cones, expressed as the percentage of trees that produced cones.

Tree mortality

Tree mortality was assessed in May 1999, 3 years after the gas release, at two upwind control sites and eight exposed sites (≤ 1.5 km downwind of the site of release). At each site, a 100 m² plot was established at random. All trees over 2 m tall were identified to species. We measured DBH (diameter at breast height, 1.37 m), and whether trees were alive (living foliage present on branches) or dead (no living foliage present). This was a conservative estimate of tree mortality because a conifer can be effectively dead while there is still active foliage present on the branches (P. Alaback, pers. comm.). Finally, tree mortality was simulated at the stand level using the FOREST-BGC model (Running and Gower, 1991).

Statistical analysis

Data were analysed using SigmaStat (SPSS, 1997). Data that met the requirements for normal distribution were analysed using analysis of variance (reported as *F*, *P*-value), with a Bonferroni *post-hoc* test. The experimental design was a nested analysis of variance. Repeated measures (RM) techniques were used to analyse cuticular water loss data. In

the absence of significant differences between subsamples, they were pooled to increase the power of the statistical analysis ($P > 0.05$; Sokal and Rohlf, 1997). Data that did not meet the normality requirements were analysed using a Kruskal-Wallis ANOVA on ranks (reported as *H*, *P*-value). Frequency data, such as visual injury, cone production and tree mortality, were examined using a Chi-square analysis, extended Kruskal-Wallis test, or Fisher's Exact test.

RESULTS

Visual drought injury

In March 1997, 1 year after the spill, foliar injury for -1 and 0 yts needles of *Pinus ponderosa* was significantly higher within 0.2 km of the release site compared to control trees and trees 0.8 km downwind (Table 1). After 1 year, foliage that was chlorotic had progressed to necrotic. Even though new foliage (0 yts) had a healthy appearance after it flushed in 1996, 35–75 % of needles were necrotic by March 1997 on trees 50 m and 0.2 km downwind of the spill. There was a trend towards increased foliar injury in *Pseudotsuga menziesii* for the -1 and 0 yts age classes within 0.2 km of the site of release in March 1997 (+1 yts) and 1998 (+2 yts; Table 1). However, this trend was not statistically significant.

The extent of visual injury after 1 year (March 1997) was significantly correlated with visual injury in March 1998 for both species for 0 yts foliage ($r^2 = 0.90$, $P = 0.002$). However, this pattern was not reflected for -1 yts foliage ($r^2 = 0.58$, $P = 0.23$). Foliar injury of *Pinus ponderosa* and *Pseudotsuga menziesii* was negatively correlated with TWC and RWC up to 1 year after exposure ($r^2 \leq -0.70$, $P < 0.05$), and positively correlated with relative water loss and G_{\min, H_2O_v} for *P. menziesii* ($r^2 \geq 0.70$, $P < 0.05$). Thus, the index of foliar injury was a good indicator of the effects of chlorine gas exposure on cuticular water loss and needle water content up to 1 year after gas exposure.

TABLE 1. Foliar injury in March 1997 ($n = 5$ trees; 15 replicates per tree) and March 1998 ($n = 5$ trees; ten replicates per tree) for *Pinus ponderosa* and *Pseudotsuga menziesii* (years to spill in parentheses)

Site and date	<i>Pinus ponderosa</i> , needle age class			<i>Pseudotsuga menziesii</i> 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

Values shown are mean foliar injury scores. Categories are: (1) 100 % green; (2) 5–25 % chlorotic; (3) >25 % chlorotic; (4) 5–25 % necrotic; and (5) >25 % necrotic. The two control sites are indicated as CU (upwind control) and CD (downwind control). Needle age classes that were no longer present on the tree are indicated as 'Defoliated'. Sites with higher visual injury compared to all other sites are indicated by an asterisk, and apply within each needle age class, species and sampling dates (Chi-square test, $P < 0.05$).

Chlorine exposure increased susceptibility to insect damage on a very limited scale. By April 1998 (+3 yrs), only two mature *P. menziesii* trees, weakened by defoliation after chlorine gas exposure, had been killed by Douglas fir beetle (*Dendroctonus pseudotsugae* Hopkins). Several *P. menziesii* trees that had been killed directly by chlorine gas were infested by ambrosia beetles (*Trypodendron* spp.). There was no evidence of insect infestations on *Pinus ponderosa*.

Leaf wettability and cuticular water loss

Exposure to chlorine gas had persistent effects on leaf wettability for directly exposed *Pinus ponderosa* foliage (–1 yrs) up to 0.8 km downwind from the release site (Table 2), and caused defoliation of this needle age class in *Pseudotsuga menziesii*. Differences in CA for the other needle age classes (Table 2) could not be attributed fully to chlorine gas exposure due to variation between sites. CA decreased significantly with increasing needle age (Table 2) for both for *Pinus ponderosa* and *Pseudotsuga menziesii* ($F_{3,317} = 123.66$ and $F_{2,218} = 35.69$ respectively, $P < 0.0001$). Droplet retention angles (RT) did not differ significantly following exposure to different levels of chlorine for either species (data not shown).

Directly exposed *Pinus ponderosa* and *Pseudotsuga menziesii* needles (–1 yrs) up to 0.2 km downwind from the release site had a higher relative water loss compared to controls in October 1997 ($F_{2,56} = 9.04$, $P < 0.001$ and $F_{3,45} = 5.50$, $P = 0.007$, respectively; Figs 1A and 2A). New 0 yrs needles on trees up to 0.2 km downwind lost more water than those on trees at other sites (Figs 1B and 2B). Moreover, G_{\min, H_2O_v} of 0 yrs foliage for both *Pinus ponderosa* and *Pseudotsuga menziesii* was higher within 0.2 km of the release site compared to control sites ($F_{2,65} = 3.57$, $P = 0.034$ and $F_{2,73} = 4.24$, $P = 0.019$ respectively). Chlorine gas exposure did not affect relative

water loss and G_{\min, H_2O_v} of *Pinus ponderosa* needles that flushed in 1997 (+1 yrs). However, +1 yrs needles of *Pseudotsuga menziesii* had a higher relative water loss and G_{\min, H_2O_v} up to 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). Although relative water loss and G_{\min, H_2O_v} were correlated (mean $r^2 = 0.69 \pm 0.11$ s.e., $n = 19$; 75 % of r^2 values > 0.87), significant increases in relative water loss were not always reflected in significantly higher G_{\min, H_2O_v} values, and vice versa.

Water content

Five months after exposure, TWC of directly exposed foliage (–1 yrs) was lower for *Pinus ponderosa* within 50 m of the release and for *Pseudotsuga menziesii* up to 0.8 km downwind (Table 3). Moreover, 0 yrs *P. menziesii* foliage also had lower TWC up to 0.8 km downwind (Table 3). RWC was lower for directly exposed foliage within 50 m of the release in September 1996 (Table 3), but for neither species nor any of the needle age classes was RWC affected after this date (data not shown).

Growth measurements

Shoot growth was higher at the upwind control site compared to the other sites for both *Pinus ponderosa* and *Pseudotsuga menziesii* (Table 4). However, the differences in shoot and needle growth were either present before exposure to chlorine gas, or there was no consistent pattern that appeared to be related directly to chlorine gas exposure (Table 4). Thus, differences in needle length and annual shoot growth were site-specific. At sites 0.2 and 0.8 km downwind, *Pinus ponderosa* had more buds in 1996 (0 yrs) and 1997 (+1 yrs), the two growing seasons following chlorine gas exposure (Table 4). A similar, but less pronounced, trend was observed for *Pseudotsuga menziesii*,

TABLE 2. Droplet contact angle (degrees, $n = 10$ trees, two replicates per tree) for *Pinus ponderosa* and *Pseudotsuga menziesii* for needle age classes 1995 (–1 yrs) to 1998 (+2 yrs), measured in August 1998

Species and site	1995 (–1)	1996 (0)	1997 (+1)	1998 (+2)
<i>P. ponderosa</i>				
Control, upwind	46 (3) ^a	44 (4)	57 (4)	75 (4) ^a
Control, downwind	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	$F_{2,58} = 11.66^{***}$	$F_{3,77} = 2.85^*$	$F_{3,77} = 0.47$, n.s.	$F_{3,77} = 3.15^*$
<i>P. menziesii</i>				
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 sites combined				
<i>Pinus ponderosa</i>	43 (2) ^a	47 (2) ^a	57 (2) ^b	77 (2) ^c
<i>Pseudotsuga menziesii</i>		61 (2) ^a	66 (2) ^a	77 (2) ^b

*** $P < 0.001$; * $P < 0.05$, n.s., not significant.

Superscripts 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 at the bottom of the table. Values in parentheses indicate 1 s.e.

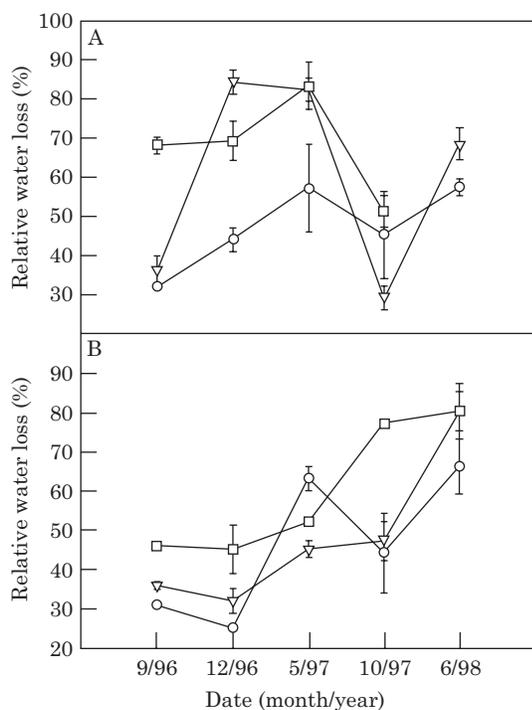


FIG. 1. Relative water loss of *Pinus ponderosa* foliage over time for 1995 foliage (–1 yrs, A) and 1996 foliage (0 yrs, B). Sites shown are the control sites (pooled; ○), 0.8 km downwind (▽), and 0.2 km downwind (□). Sites 0.2 km downwind were significantly different from the control sites (two-way ANOVA, $P < 0.001$; repeated factor: date, $n = 5$). Error bars represent 1 s.e.

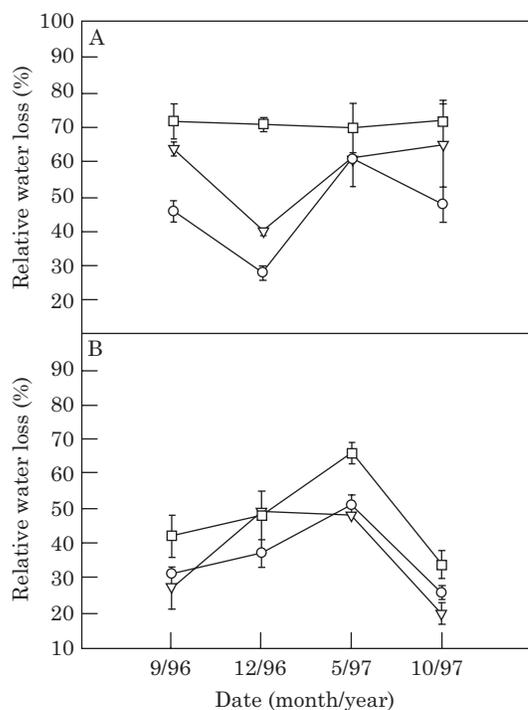


FIG. 2. Relative water loss of *Pseudotsuga menziesii* foliage over time for 1995 foliage (–1 yrs, A) and 1996 foliage (0 yrs, B). Sites shown are the control sites (pooled; ○), 0.8 km downwind (▽), and 0.2 km downwind (□). Sites 0.2 km downwind were significantly different from the control sites (two-way ANOVA, $P < 0.001$; repeated factor: date, $n = 5$). Error bars represent 1 s.e.

TABLE 3. Total water content and relative water content ($n = 4$ trees) of 1995 (–1 yrs) and 1996 (0 yrs) needles, measured in September 1996

Species and site	Total water content (g g^{-1})		Relative water content (%)	
	1995 (–1)	1996 (0)	1995 (–1)	1996 (0)
<i>Pinus ponderosa</i>				
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	$F_{3,13} = 49.23^{***}$	$F_{3,13} = 26.62^{***}$	$F_{3,13} = 12.70^{***}$	$F_{3,13} = 11.68^{***}$
<i>Pseudotsuga menziesii</i>				
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.

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

Superscripts indicate statistically significant differences within each species and needle age class (one-way ANOVA, compare within columns). Values in parentheses indicate 1 s.e.

with increased numbers of buds on trees 0.8 km downwind in 1996 (0 yrs) and 0.2 km downwind in 1997 (+1 yrs; Table 4). In 1998, there were no differences in numbers of buds for either species.

By spring 1998 (+2 yrs), only 40–75 % of needle age classes were present on *Pinus ponderosa* trees within 0.5 km of the site of gas release compared to control trees and trees

further downwind (Fig. 3A). Moreover, in 1999, needle retention for *P. ponderosa* was lower up to 1.5 km downwind ($H_{10} = 56.8$, $P < 0.001$; compared to 0.5 km in 1998), and exposed *P. ponderosa* had even fewer needle age classes present than in 1998. Reduced needle retention was observed for *Pseudotsuga menziesii* up to 10 km downwind of the release site, and was especially pronounced within

TABLE 4. Summary of growth measurements over three growing seasons following chlorine gas exposure (values ± 1 s.e.; in parentheses years to spill)

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

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. Superscripts 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 analysed 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.'

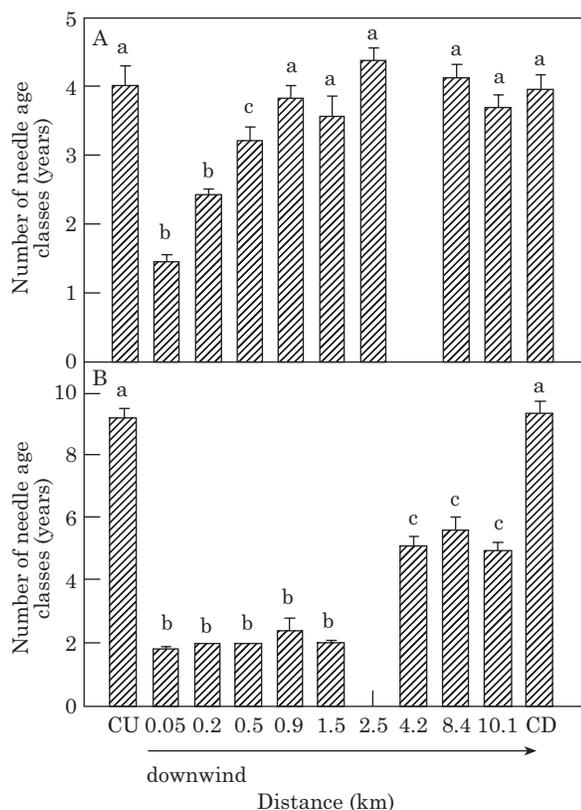


FIG. 3. Needle age classes present on *Pinus ponderosa* (A, $n = 10$ trees) and *Pseudotsuga menziesii* (B, $n = 10$) over a 10 km gradient downwind from the site of gas release, measured in spring 1998 (+2 yts). The two control sites are the upwind control (CU) and the downwind control (CD). Letters indicate statistically significant differences within each species and needle age class (Kruskal-Wallis with Tukey post-hoc test, $P < 0.001$).

1.5 km of the site (Fig. 3B). These differences were still present in 1999 ($H_{10} = 123.1$, $P < 0.001$).

There were no differences in annual core increment growth between study sites in 1994 and 1995, the two growing seasons before chlorine exposure ($P > 0.05$). At control sites, stem growth increased in *Pinus ponderosa* and *Pseudotsuga menziesii* from 1994 to 1998 ($F_{4,120} = 2.86$ and $F_{4,120} = 5.61$ respectively, $P < 0.001$; Fig. 4). In contrast, both species had a significantly lower core increment growth in the 3 years following chlorine gas exposure compared to the years prior to exposure at sites 0.2 and 0.8 km downwind of the spill [$F_{4,120} = 2.86$ ($P < 0.05$), $F_{4,120} = 5.61$ ($P < 0.001$), respectively; Fig. 4]. In 1996, reductions in stem growth for *Pseudotsuga menziesii* corresponded to the severity of observed foliar injury: -26% ($\pm 6\%$) for chlorotic trees and -56% ($\pm 7\%$) for necrotic trees ($T_{3,3}$, $P = 0.009$).

At the control sites, 75% of *Pinus ponderosa* trees produced cones in 1998 (+2 yts), compared to 30–40% ($\pm 27\%$) at exposed sites up to 10 km downwind (Fisher's Exact test, $P = 0.05$ to 0.001; range 10 to 70%). One exception was a heavily managed *Pinus ponderosa* stand approx. 2.5 km downwind, with 70% of the trees bearing cones. Cone production for *Pseudotsuga menziesii* at sites 50 m and 0.5 km downwind was significantly lower (10% of trees with cones) than at control sites (50% of trees with cones; Fisher's Exact test, $P = 0.02$).

Mortality

In spring 1999, 3 years after gas exposure, there were no effects of chlorine gas exposure on mortality in *Pinus ponderosa*. The average mortality was 10% ($\pm 5\%$) over

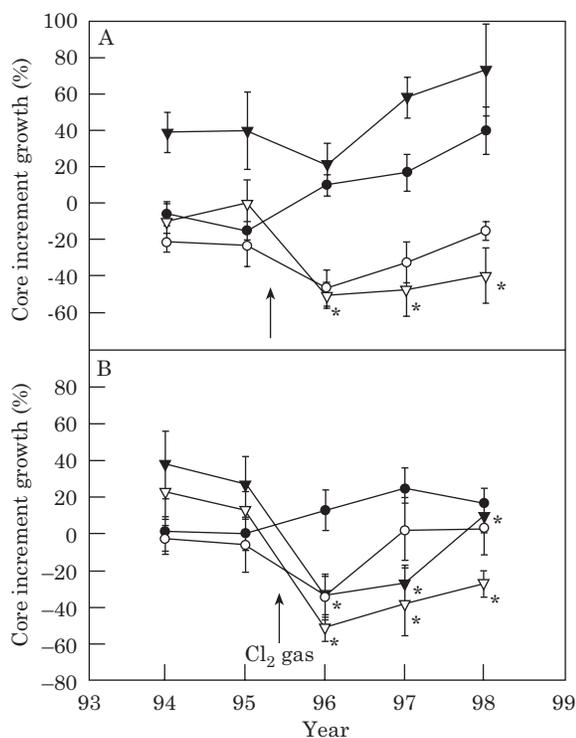


FIG. 4. Core increment growth of *Pinus ponderosa* (A) and *Pseudotsuga menziesii* (B). Shown are the control sites (pooled; ●), and sites 1.5 km downwind (○), 0.8 km downwind (▼), and 50 m downwind (▽). * indicates years within a site that are statistically significantly different compared to before chlorine gas exposure ($n = 10$ for 1995 and 1996; $n = 5$ for 1997 and 1998; nested two-way ANOVA, $P < 0.001$; repeated factor: year, $n = 5$).

nine sites within 1.8 km of the site of gas release (range 0 to 30%). Mortality in *Pseudotsuga menziesii* was significantly higher at the two sites within 50 m of the site of gas release

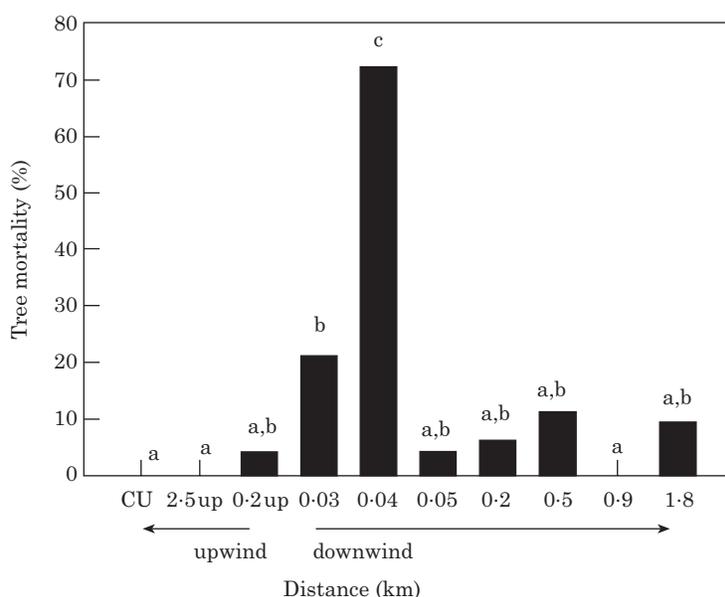


FIG. 5. Tree mortality for *Pseudotsuga menziesii* ($n = 25$) over 1.8 km downwind of the site of gas release and at the upwind control site (CU), measured in May 1999 (+3 yts). Letters indicate statistically significant differences between sites (Fisher's Exact test, $P < 0.001$).

compared to control sites (Fig. 5). Although mortality of *Pseudotsuga menziesii* at distances beyond 50 m downwind of the release site was not significantly different from the upwind control sites, tree mortality at the exposed sites tended to be higher than at the control site (Fig. 5).

DISCUSSION

This is the first report of persistent physiological and growth effects on a natural forest ecosystem after acute chlorine gas exposure. Effects were species-specific and might continue to have a negative influence on tree health and survival due to increased susceptibility to drought stress. Chlorine gas exposure led to increased leaf wettability of directly exposed needles of *Pinus ponderosa*, suggesting cuticular damage. Moreover, directly exposed foliage had a lower TWC, while relative water loss across cuticles tended to be higher for exposed trees within 0.2 km of the release. In general, chlorine exposure caused severe defoliation and led to decreased leaf longevity, stem increment growth and cone production in affected trees.

Leaf wettability and cuticular water loss

Two years after chlorine gas exposure, directly exposed needles had defoliated from *Pseudotsuga menziesii*. Foliage from *Pinus ponderosa* (-1 yts) 0.8 km downwind had lower CA than controls, suggesting that epicuticular waxes had yet not recovered to pre-spill levels (Table 2). There were no lasting effects on CAs of foliage that flushed after chlorine gas exposure (Table 2), even though +1 yts *P. menziesii* needles had lower CAs 5 months after gas exposure (Schreuder and Brewer, 2001). CA and RT decreased with increasing needle age (Table 2), which can be attributed to erosion and degradation of cuticular waxes over time (Staszewski *et al.*, 1998). The observed changes in CA of

–1 yts *P. ponderosa* foliage may have been caused, in part, by the acidity of the chlorine gas cloud (e.g. Paparozzi and Tukey, 1984; Percy *et al.*, 1992). These changes may have implications for cuticular transpiration (DeLucia and Berlyn, 1984; Hadley and Smith, 1989), and foliar water content (Mengel *et al.*, 1989).

It appears that chlorine gas exposure tended to increase cuticular water loss for conifer foliage within 0.2 km of the release. This effect persisted for up to 1 year after exposure for foliage of *Pinus ponderosa* and for up to 6 months for foliage of *Pseudotsuga menziesii* (Figs 1 and 2). After 1 year, directly exposed *P. menziesii* needles (–1 yts) were too sparse to sample. Interestingly, increased cuticular transpiration was statistically higher for directly exposed –1 yts foliage when expressed as relative water loss, and for the 0 yts needle age class when expressed as G_{\min, H_2Ov} (Figs 1 and 2; Schreuder, 2000). We also observed a reasonably strong correlation between relative water loss and G_{\min, H_2Ov} . It appears that cuticular damage to the –1 yts needle age class occurred through direct exposure to chlorine gas, while damage to the 0 and +1 yts needle age classes occurred via indirect mechanisms (e.g. wax synthesis; Percy *et al.*, 1992).

Increased cuticular transpiration for *Pinus ponderosa* and *Pseudotsuga menziesii* led to lower TWC for –1 yts foliage within 0.2 km of the release site up to 1 year after exposure (Table 3; Schreuder, 2000). Moreover, indirectly exposed foliage of *P. menziesii* had a lower TWC until October 1997, 1.5 years after chlorine exposure (Schreuder, 2000). RWC was generally not affected (Table 3), but in September 1996, RWC values of necrotic and chlorotic foliage within 50 m of the site of gas release were close to the lethal threshold reported for *P. menziesii* in Montana (approx. 55%; Pharis and Ferrell, 1966). Low RWC may have had several negative influences, including decreased stomatal conductance, and consequently, lowered photosynthetic rates (Pallardy *et al.*, 1991), as well as defoliation (Pharis and Ferrell, 1966). Further work is needed to determine the extent to which these patterns scale to whole tree water and carbon balance.

Growth

It has been reported that conifers allocate carbon to different tissues according to the following prioritization (e.g. Running and Gower, 1991; Dewar *et al.*, 1994): (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. Moreover, carbon tends to be allocated to acquisition of the most limiting resource, e.g. light, nutrients or water (Mooney and Winner, 1991). Using this carbon allocation scheme as a baseline, we predicted that chlorine gas exposure, and the subsequent defoliation, would have the most negative influence on tissues that tend to be lower priorities in conifers for carbon allocation. The results obtained from our study were consistent with this prediction. Allocation to high priority carbon sinks (i.e. needles, buds and branches) was generally not affected by chlorine gas exposure (Table 4) based on the parameters we measured. However, allocation to lower priority sinks (i.e.

reproduction and stem increment growth) was negatively impacted. Cone production can be a significant carbon sink in coniferous trees (Dewar *et al.*, 1994); thus the considerable reduction in cone production of exposed vs. non-exposed trees, especially in *Pinus ponderosa*, was not surprising. Both *Pinus ponderosa* and *Pseudotsuga menziesii* significantly reduced annual stem increment growth over at least three growing seasons following chlorine gas exposure (Fig. 4). Interestingly, stem increment growth of the deciduous western larch was not affected (Schreuder, unpubl. res.), supporting the interpretation that the growth reduction observed in evergreen conifers was related to direct exposure of foliage to chlorine gas and subsequent defoliation. Nichols (1988) reported a relationship between annual stem increment growth and the percentage of foliage retained in the previous growing season for *Pseudotsuga menziesii*. Using this model with data collected in our study, we found a strong relationship between annual stem increment growth and the percentage of foliage retained in the previous growing season for both *Pseudotsuga menziesii* ($r^2 = 0.86$, $P < 0.005$), and *Pinus ponderosa* ($r^2 = 0.80$, $P < 0.001$). Based on Nichol's model (1988), as well as the Forest BGC model (Running and Gower, 1991), stem increment growth could be expected to return to normal after 5–6 years for *Pseudotsuga menziesii* and approx. 4 years for *Pinus ponderosa*. This was consistent with observed recovery times for stem growth of defoliated conifer species in our study (up to 5 years depending on the severity of defoliation).

Potential causal factors for the negative effects on stem growth and reproduction include toxicity of chloride, drought stress, and defoliation (Sidhu and Stanforth, 1986). Defoliation has been reported to decrease stem increment growth of several conifer species (e.g. Nichols, 1988; Christiansen and Fjone, 1993). Defoliation of necrotic foliage from exposed trees was considerable during the first growing season after exposure. A conservative defoliation estimate, based on visual injury data 2 months after exposure, was approx. 75% for *Pinus ponderosa* and approx. 90% for *Pseudotsuga menziesii*. Moreover, reduced needle longevity was found both for directly exposed needles as well as for needle age classes that flushed during the two growing seasons following exposure (Fig. 3), suggesting increased susceptibility to environmental stress factors.

Tree mortality

While we saw no difference in mortality of *Pinus ponderosa* trees during the 3 years following chlorine gas exposure, mortality of *Pseudotsuga menziesii* was significantly higher at the two sites nearest to that of the chlorine release, and generally higher at the other exposed sites compared to control sites (Fig. 5). Our results were generally consistent with other studies (e.g. Alfaro *et al.*, 1982), in that mortality was greatest in younger trees (approx. 90% of the mortality in trees with DBH < 6 cm), which have lower carbon reserves than larger trees (Webb, 1981). Based on results from the Forest BGC model (Running and Gower, 1991), some mortality was expected when defoliation exceeds approx. 85% (due to a negative

carbon balance). This was the case for trees closest to the release site.

Species-specific responses

Pseudotsuga menziesii and *Pinus ponderosa* responded differently to chlorine gas exposure. In general, *Pinus ponderosa* is known to be more drought-tolerant than *Pseudotsuga menziesii*, and has a deeper root system. Moreover, *Pinus ponderosa* replaces its foliar biomass at a faster rate (approx. 4 years) compared to *Pseudotsuga menziesii* (approx. nine needle age classes on healthy trees). Current-year foliage of *Pinus ponderosa* tends to contribute proportionally more (approx. 70%) to whole tree photosynthesis compared to that of *Pseudotsuga menziesii* (approx. 36%; Chabot and Hicks, 1982). Thus, one would expect faster recovery for *Pinus ponderosa* compared to *Pseudotsuga menziesii*. This pattern was observed over the geographical area where chlorine gas exposure led to needle loss (Fig. 3), and this influenced replacement rates of lost needle age classes (Fig. 3) and stem growth (Fig. 4). Although 1-year-old needles of *Pseudotsuga menziesii* and *Pinus ponderosa* contribute about equally to whole tree photosynthesis (approx. 25%; e.g. Chabot and Hicks, 1982; Rundel and Yoder, 1998), this age class was generally retained on *Pinus ponderosa* but abscised on *Pseudotsuga menziesii*. This pattern may be explained by higher drought tolerance and higher relative photosynthetic gain from the youngest, current-year needle age class in *Pinus ponderosa* (Chabot and Hicks, 1982; Rundel and Yoder, 1998). The greater negative influences on reproduction in *Pinus ponderosa* may be attributed to repair of exposed tissues and a higher proportion of sapwood, leading to higher respiration costs (e.g. Margolis *et al.*, 1988).

CONCLUSIONS

These data suggest that acute exposure to chlorine gas not only leads to immediate visible injury symptoms, but also has persistent physiological and growth effects on the conifer species studied here. Depending on the severity of exposure, chlorine gas led to increased susceptibility to drought stress, decreased photosynthetic biomass, and decreased stem growth and reproduction. Moreover, increased tree mortality was observed close to the site of gas release. Although effects on drought susceptibility only lasted approx. 1 year, effects on foliar biomass and growth were expected to persist 4 to 7 years based on modelling results. Interestingly, effects of exposure to chlorine gas were highly species-specific: *Pseudotsuga menziesii* was more susceptible to defoliation than *Pinus ponderosa*, thus, the incidence of defoliation of *Pseudotsuga menziesii* was a useful indicator of chlorine gas exposure years after the gas release. Tree responses may differ between dry and moist sites, as well as between dry and moist climates and growing seasons, because exposure tended to increase drought susceptibility of directly exposed foliage. When extrapolating results from this study to other tree species, general data on species-specific responses to defoliation may be useful for predicting longer-term responses to chlorine gas

exposure. Factors such as site characteristics, drought tolerance, climate and timing of exposure need to be taken into account for such an assessment.

ACKNOWLEDGEMENTS

We thank A. Sala for the use of the Opti-Sciences Modulated Fluorometer and critical review of an earlier draft of this manuscript, and P. Alaback, T. Deluca and D. Six for their help with developing research methodology. We also thank the field assistants who helped out in this research. This study was funded in part by a grant from NSF OSR-955450 to J. Stanford.

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