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Responses of Maternal Siblings of *Pinus densiflora* to Simulated Acid Rain

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Because most research on acid rain does not consider the genetic composition of seeds and the inherent variations in seed mass and germination date, the potentially diverse patterns of plant response are neglected within a population. Therefore, we examined the effects of both family identity and simulated acid rain on *Pinus densiflora* seedlings over a 34-week period, beginning at germination. We were able to evaluate the importance of family and pH treatment independent of seed mass and germination date by considering the latter two variables as covariates. Seeds were collected from six randomly chosen trees that were assumed to represent different genotypes. After germination in a controlled environment, the seedlings were moved outdoors. Each was then subjected twice a week to 40 mL of simulated acid rain, at a pH of either 3.0 or 5.6. We examined variations in germination date, seedling height, needle numbers and lengths, and root and shoot masses. The maternal family effect was significant for all characters over the entire test period. Strong effects of pH treatment were detected only at the later growth stages. Artificial rain with a pH of 3.0 enhanced growth rates, particularly aboveground, compared with seedlings treated with pH 5.6 rain. However, regardless of pH, certain groups of maternal siblings consistently performed better in almost all parameters. The exception was for mean germination date, which differed among families only at a pH of 3.0. Therefore, although we demonstrated that acid rain affected both the date of germination as well as seedling growth rates in *P. densiflora*, the responses differed among maternal families, and were also associated with seed mass and germination date.

Keywords: Maternal family, *Pinus densiflora*, Seed mass, Seedling growth, Simulated acid rain

Decline in the forests of Europe and parts of eastern North America since the 1960s is largely attributed to air pollution, especially acid rain (Bormann, 1985; Falkengren-Grerup, 1986; Pitelka and Raynal, 1989; Schulze, 1989). Acid rain decreases a tree's survival and growth rate by shortening its foliar life span, lowering the photosynthetic rate, and weakening winter hardiness (Raynal et al., 1982a; Taylor et al., 1994; Finzi et al., 1998). It also adversely affects seed germination and seedling growth, the most critical life stages for both trees and agronomic crops (Houston and Dochinger, 1977; Lee and Weber, 1979; Raynal et al., 1982b; Kim, 1987; Fan and Wang, 2000). Reports often conflict about the negative effects of this acid deposition. For example, conifer seedlings may, in fact, perform better when subjected to acid rain and acidic air pollutants such as SO_2 and NO_x (Reich et al., 1987; Lee et al., 1990; Keller, 1991; Flagler et al., 1992; Leith et al., 1995).

Most research on acid deposition is conducted at the species or community level, and neglects the potentially diverse pattern of response within populations. However, recent studies have described genotype-specific responses to either ozone (*Pinus strobus*; Karnosky et al., 1993) or CO_2 (*Raphanus* and *Populus* species; Curtis et al., 1994; Mansfield et al., 1999). If genotypes react differently to air pollutants, the mean effect across genotypes is not as useful for predicting population or species changes in nature. Variations in seed mass and germination date among genotypes also may influence offspring performance (Mazer, 1987; Toon et al., 1991; Kang et al., 1992; Vaughton and Ramsey, 1998). Thus, plant response to acid rain can be confounded by associations between the genetic composition of seeds or seedlings, seed mass, and germination date. Unfortunately, most acid rain studies of conifers and other tree species begin with one- or two-year-old seedlings, rather than with seeds of known mass and family identity, and are usually conducted in a controlled environment for only a relatively short period (e.g., Lee and Weber, 1979; Raynal et al., 1982a, 1982b; Leith et al., 1995; Lee et al., 1998).

Acid rain has been a critical ecological and environmental issue in Korea since the 1980s. In contrast to

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the USA and Europe, where the levels and effects of acid precipitation have decreased over the past one or two decades (Likens et al., 1996), the acidification of Korean rainfall is expected to intensify (Chung, 1989). For example, 11 years of data (1989 through 1999) have shown that the overall pH of rain in 11 major cities has tended to decrease (Environmental Statistics Yearbook of Korea, 1989-1999), with the pH of 72% of the rainfall in Seoul in 1997 measuring $<$ 5.6 (Lee et al., 1997). Rural areas also experience acid precipitation (Kang et al., 1995; Lee et al., 1999). As the occurrence of acid rain has increased, a decline in *Pinus densiflora* populations has been observed, especially in cities and suburbs. Song et al. (1996) and Lee et al. (1998) have attributed this decline to air pollution such as acid rain. However, these studies, as in previous conifer/acid rain experiments, have neglected potentially confounding factors, e.g., family membership among seeds or seedlings, seed mass, and germination date. In this paper, therefore, we describe longer-term (34-week) responses of *P. densiflora* to simulated acid rain, from germination through early seedling development. Our sources of variation included maternal family identity, seed mass, date of germination, and germination rates.

MATERIALS AND METHODS

As a gymnosperm native to Korea, *P. densiflora* Sieb et. Zucc is an ecologically, economically, and culturally important species. In October 1997, we randomly chose six *P. densiflora* trees within a Forest Research Institute forest at Hongneung (37° 35'N, 127° 03'E), Seoul. Varying numbers of cones were collected from each tree, then labeled according to their maternal identity. We were able to obtain 11 to 34 seeds from each tree after their cones were dried in the laboratory for about two months. Seeds from each maternal family were individually weighed to the nearest 0.0001 g, and were refrigerated until sowing.

Simulated acid rain, with a pH of either 3.0 (1000 μ eq H + /L) or 5.6 (2.5 μ eq H + /L, control) was concocted according to Skelly et al. (1982), and contained major anions and cations whose mean concentrations approximated those found in rain from southwestern Virginia, USA. To fill the plug trays, soil collected from the A layer at the Hongneung forest was sifted through a 0.5 cm mesh. It was then mixed in equal parts with sand, perlite, and potting soil (TKS2, Floragard).

In April 1998, seeds representing each maternal family and identity were randomized to 134 positions in plug trays, and planted in four randomized blocks. Two trays each for pH 3.0 and 5.6 rain were prepared, and pH 5.6 rain was used to simulate the normal precipitation. The trays were placed in a growth chamber until germination began. Growing conditions included temperatures of 18/13°C (day/night), with 70% RH, and a 13-h photoperiod. Every day the trays were sprayed to saturation with our simulated acid rain, and evidence of germination was checked twice a day. Approximately one week after germination, the seedlings were transplanted to 11-cm-diameter pots, then relocated to an open field at Sungshin Women's University, Seoul, Korea. Twice a week, 40 mL of simulated acid rain (pH 3.0 or 5.6) was sprayed over each seedling. Tap water additionally was applied when the pot soil dried. The seedlings were harvested 34 weeks after germination, in January 1999. Thus, the plants were exposed to the seasonal climatic conditions typical of Seoul during the experimental period.

Seedling characters, i.e., plant height, number of needles, and maximum needle length, were measured at Weeks 2, 10, 18, 26, and 34 after germination. Individual primary needles were attached to the stems early in seedling growth, but needles bundled two to a fascicle (secondary needles) became evident about four months after seedling emergence. Therefore, leaf measurements were recorded for cotyledons at Week 2, for primary needles beginning after Week 2, and for secondary needles after Week 10. After the seedlings were harvested, the roots and shoots were separated and dried at 70°C for 3 d. These tissues were then weighed to determine the ratio of root dry mass to shoot dry mass (root/shoot ratio). Some pre-harvest seedling mortality, from an unknown cause, resulted in a small reduction in sample size.

We used a one-way ANOVA to examine differences in mean seed mass among maternal trees. To evaluate the influence of family identity and pH treatment on germination date (i.e., length of time to germination), a two-way ANCOVA was employed, with seed mass as a covariate. The effects of two class variables (family and pH) and two covariates (seed mass and germination date) on seedling performance were periodically assessed (at Weeks 2, 10, 18, 26, and 34 post-germination), using MANCOVAs. Their effects on each seedling character also were examined for each week with two-way ANCOVAs. At Weeks 26 and 34 (when both family and pH treatment effects were significant), the differences in overall seedling performance among families were tested based on a new variable (OVPERF). This cluster variable combined standardized values of seedling characters for which the family effects were significant in two-way ANCOVAs. The type III sums of squares was used to test the significance of factors in all of the ANOVAs. We conducted our statistical analyses with SAS (1985).

RESULTS

Family Effect on Seed Mass

Seed mass ranged from 7.1 to 20.6 mg, with a mean of 13.4 ± 1.3 mg (N = 134) (Fig. 1). The mean family seed mass varied greatly among our six maternal trees ($F_{5,128} = 95.93$, $P < 0.0001$). For example, seeds produced from Family 5 were twice as heavy as those from Family 6.

Family and pH Effects on Germination Rate and Germination Date

The average germination rate was 92.5%. Our two-way contingency table analyses (i.e., the number of germinated seeds by family and by pH) showed that the proportion of germinated seeds was associated with neither family ($X^2 = 0.77$, $P \gg 0.05$, N = 134) nor pH ($X^2 = 0.04$, $P \gg 0.05$, N = 134). When seed masses within families were compared, the germinated and non-germinated seeds did not differ significantly in their mean masses (paired t-test T = −0.38, $P > 0.05$; X ± 1 SD = 13.1 ± 3.3 mg and 12.9 ± 4.1 mg, respectively).

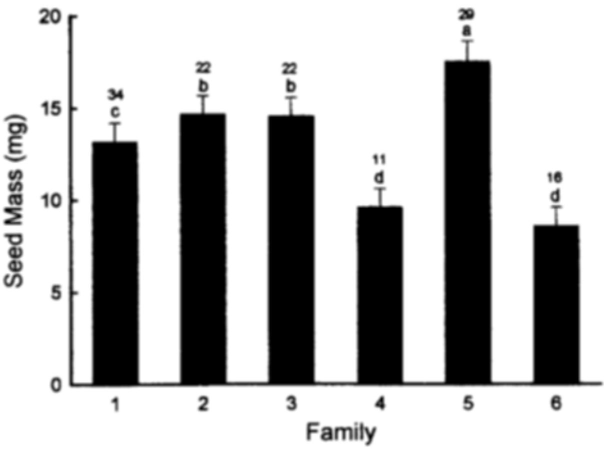

Figure 1. Mean seed mass (X ± 1 SE) among six maternal trees of *P. densiflora* sampled in Hongneung. Different letters on bars represent significant differences at α = 0.05 level. Numbers indicate the number of seeds within each family.

Table 1. Effects of maternal family and pH on germination date in *P. densiflora*.

Source of variation	df	F	P
Family	5	2.13	ns
pH	1	0.11	ns
Family * pH	5	2.01	(*)[a]
Cov Seed mass	1	0.19	ns
Model	12,111	1.81	(*)[b]

Cov refers to covariate used in the model. (*)[a] P = 0.0830; (*)[b] P = 0.0541.

Seeds germinated an average of 18.1 ± 3.8 d after sowing. The family x pH effect on germination date was marginally significant, whereas seed mass had no significant effect (Table 1). A subsequent one-way ANOVA for each pH treatment showed that the mean germination date differed significantly among families only for the pH 3.0 treatment (pH 3.0 $F_{6,53} = 2.71$, $P < 0.05$ vs. pH 5.6 $F_{6,57} = 1.56$, $P > 0.05$), with seeds from Family 3 germinating an average of five days earlier than those from Families 4 and 6 (Fig. 2; $P < 0.05$ by Tukey's test). Because the variables of seed mass and germination date were independent of each other (r = −0.03, $P > 0.05$), both were used as covariates in subsequent MANCOVAs.

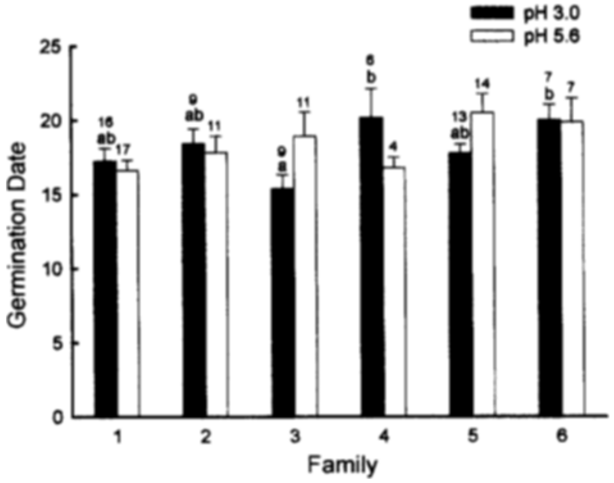

Figure 2. Mean germination date (X ± 1 SE) of *P. densiflora* seeds from six maternal trees, by pH treatment. Mean family germination dates differed significantly only in the pH 3.0 treatment: different letters on filled bars represent significant differences at α = 0.05 level. Numbers indicate the number of germinated seeds within each family.

Family and pH Effects on Seedling Characters

The MANCOVA for very young plants (e.g., at Week 2 after germination) indicated that overall seedling performance differed significantly among families, but not between pH treatments (Table 2). In separate two-way ANCOVAs for each character, the significant effect of family identity was detected only for height (Table 2). The mean family height ranged from 9.5 to 15.4 mm, with an increasing order for Families 6 < 4 < 3 < 5 < 2 < 1. Based on the adjusted means for both covariates, seedlings from Family 1 were significantly taller than those from Family 5 ($P = 0.0185$). Data pooled over families and pH treatments showed that seedlings produced from larger seeds were taller ($r = 0.28$, $P < 0.01$), and bore more and longer cotyledons than those from smaller seeds ($r = 0.74$ and 0.44, respectively; $P < 0.0001$ for both). Later-germinating seedlings were taller than those emerging earlier ($r = 0.23$, $P < 0.05$), but their cotyledons were shorter than in the latter ($r = -0.27$, $P < 0.01$).

An overall model showed that seedling performance at Week 2 also was significantly associated with both covariates, i.e., seed mass and germination date. In subsequent analyses for each character, germination date was associated with both seedling height and cotyledon length, while seed mass was correlated with only the latter (Table 2). Unlike the other characters measured at Week 2, the number of cotyledons per seedling was independent of all other factors. Thus, Week-2 performance was related to family identity and both covariates, although the pattern of association was not entirely coincident among characters.

During the intermediate growth stage, Weeks 10 to 18, the overall pattern of MANCOVA matched that of

Table 2. Two-way ANCOVA of the effects of maternal family and pH on *P. densiflora* seedling characters at Week 2 after germination.

Source of variation	df	Seedling height		Primary needle length		No. needles/plant		Overall MANOVA	
		F	P	F	P	F	P	F	P
Family	5	2.58	*	2.07	ns	2.00	ns	2.18	**
pH	1	0.13	ns	0.00	ns	1.67	ns	0.62	ns
Family * pH	5	1.16	ns	0.46	ns	0.80	ns	0.82	ns
Cov Seed mass	1	2.86	ns	51.76	****	0.72	ns	18.50	****
Germination date	1	9.63	**	16.88	****	0.00	ns	13.36	****

Cov refers to covariate used in the model. MANOVA criterion is based on Wilks' Lambda. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

Table 3. Two-way ANCOVA of the effects of maternal family and pH on *P. densiflora* seedling characters at Week 10 after germination.

Source of variation	df	Seedling height		Primary needle length		No. needles/plant		Overall MANOVA	
		F	P	F	P	F	P	F	P
Family	5	2.20	ns	3.51	**	1.95	ns	2.27	**
pH	1	0.80	ns	0.35	ns	2.79	ns	1.14	ns
Family * pH	5	1.82	ns	1.29	ns	1.02	ns	1.05	ns
Cov Seed mass	1	4.60	*	55.03	****	8.12	**	19.81	****
Germination date	1	19.84	****	4.90	*	0.34	ns	10.57	****

Cov refers to covariate used in the model. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

Table 4. Two-way ANCOVA of the effects of maternal family and pH on *P. densiflora* seedling characters at Week 18 after germination.

Source of variation	df	Seedling height		Primary needle length		Secondary needle length		Overall MANOVA	
		F	P	F	P	F	P	F	P
Family	5	1.80	ns	1.72	ns	4.46	**	2.64	***
pH	1	2.95	ns	0.73	ns	0.44	ns	1.85	ns
Family * pH	5	2.00	ns	0.85	ns	0.13	ns	1.10	ns
Cov Seed mass	1	0.63	ns	16.56	****	0.20	ns	5.79	**
Germination date	1	29.65	****	15.97	****	4.74	*	22.19	****

Cov refers to covariate used in the model. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

Week 2 (Tables 3 and 4); seedling performances differed among families when the effects of the covariates were factored out. At Week 10, the mean primary needle lengths among the six families were significantly different, ranging from 26.4 to 38.5 mm (Table 3). At Week 18, a significant family effect was detected only for secondary needle lengths (Table 4). Here, mean lengths ranged from 42.9 to 66.2 mm, with an increasing order of Family 6 < 1 < 2 < 5 < 4 < 3. Based on adjusted means, the secondary needles of seedlings from Family 3 were significantly longer than those from Families 1 and 2 (P = 0.0014 and 0.0093, respectively). Likewise, seedlings from Families 3 and 4 had longer secondary needles than those from Family 6 (P = 0.0068 and 0.0027, respectively).

The correlation between both seed mass and germination date and the other seedling characters appeared to vary with growth stage (Table 5). Larger seeds produced taller seedlings with more and longer primary needles at Week 10; only the length of primary needles was correlated with seed mass at Week 18. When germination occurred earlier, the seedlings were shorter at both Weeks 10 and 18, but tended to have longer than average primary and secondary needles by Week 18. The MANCOVAs confirmed these significant patterns of association between covariates and seedling characters (Tables 3, 4, and 5).

The overall pattern of MANOVA changed somewhat in the later growth stages, 26 and 34 weeks after germination (Tables 6 and 7). Family identity still exerted a strongly significant effect on seedling performance, but an important overall pH treatment effect was detected only after Week 26. The OVPERF results between Weeks 26 and 34 were consistent with family rankings (Fig. 3). For example, seedlings from Families 1 and 3 performed best, those from Families 2, 4, and 5 were intermediate, while those from Family 6 had the worst overall growth at both measuring points.

Table 5. Pearson correlation coefficients between both seed mass and germination date and seedling characters of *P. densiflora* at Weeks 10 and 18 after germination. Data were pooled over families and pH treatments. N ranges from 110 to 116.

		Seedling height	Primary needle length	No. needles /plant	Secondary needle length
Seed mass	Week 10	0.26**	0.75****	0.56****	---
	Week 18	0.05	0.52****	---	0.08
Germination date	Week 10	0.34***	–0.16	–0.01	---
	Week 18	0.46****	–0.34***	---	–0.23 *

* P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

Table 6. Two-way ANCOVA of the effects of maternal family and pH on *P. densiflora* seedling characters at Week 26 after germination.

Source of variation	df	Seedling height		Primary needle length		No. needles/plant		Overall MANOVA	
		F	P	F	P	F	P	F	P
Family	5	2.40	*	4.27	*	5.85	****	3.49	****
pH	1	9.09	**	1.70	ns	18.54	****	8.29	****
Family * pH	5	0.76	ns	0.67	ns	1.19	ns	0.90	Ns
Cov Seed mass	1	0.00	ns	0.38	ns	0.67	ns	0.30	Ns
Germination date	1	12.96	***	1.84	ns	4.61	*	7.08	***

Cov refers to covariate used in the model. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

Table 7. Two-way ANCOVA of the effects of maternal family and pH on *P. densiflora* seedling characters at Week 34 after germination.

Source of variation	df	Seedling height		Primary needle length		Secondary needle length		Root dry mass		Shoot dry mass		Overall MANOVA	
		F	P	F	P	F	P	F	P	F	P	F	P
Family	5	3.01	*	4.53	***	6.01	****	0.96	ns	0.60	ns	2.82	****
pH	1	8.29	**	5.34	*	19.49	****	1.40	ns	0.06	ns	7.95	****
Family * pH	5	1.11	ns	1.78	ns	1.19	ns	2.26	ns	0.63	ns	1.43	Ns
Cov Seed mass	1	0.47	ns	1.16	ns	0.81	ns	11.20	**	2.02	ns	2.53	*
Germination date	1	18.72	****	0.12	ns	5.46	*	60.76	****	19.23	****	17.00	****

Cov refers to covariate used in the model. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

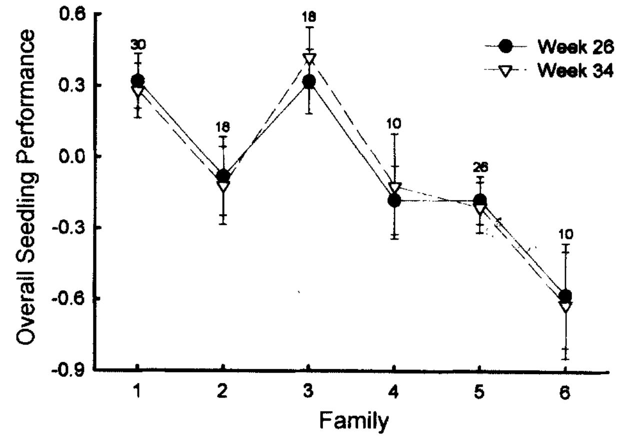

Figure 3. Mean overall seedling performance (OVPERF) of *P. densiflora* seedlings of six families measured at Weeks 26 and 34 after germination. Overall seedling performance differed significantly among families at both times, and the OVPERF ranking of families was quite consistent between weeks. Numbers indicate the number of seedlings within each family.

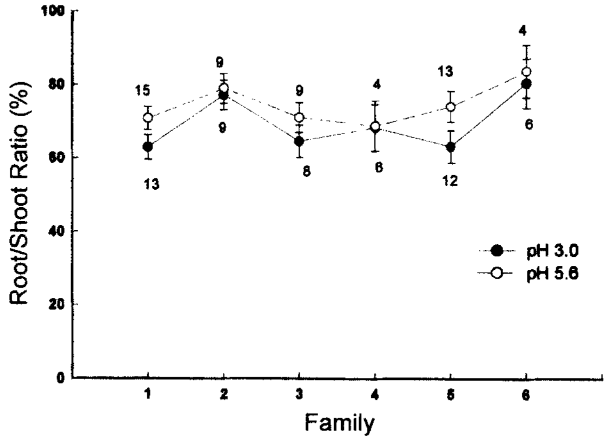

Figure 4. Adjusted mean root/shoot ratio of *P. densiflora* seedlings on covariate seed mass and germination date, by pH treatment. Numbers indicate the number of seedlings within each family and per pH treatment.

No significant interaction was found between the main factors; regardless of family identity, seedlings treated with pH 3.0 rain were larger than those under pH 5.6. At Week 26, seedlings treated at pH 3.0 were taller, and had longer primary and secondary needles than those grown under the pH 5.6 rain (seedling height X_{adj} ± 1 SE = 2.97 ± 1.03 cm and 2.58 ± 1.03 cm, respectively, $P < 0.01$; primary needle length X_{adj} = 32.9 ± 0.5 mm and 32.0 ± 0.5 mm, respectively, $P > 0.05$; secondary needle length X_{adj} = 94.2 ± 1.9 mm and 83.1 ± 2.0 mm, respectively, $P < 0.001$). At Week 34, pH 3.0 seedlings were still significantly taller, with longer primary and secondary needles, but pH did not similarly affect root and shoot dry masses (Table 7; seedling height X_{adj} = 3.10 ± 1.02 cm and 2.76 ± 1.02 cm, respectively, $P < 0.05$; primary needle length X_{adj} = 35.1 ± 0.5 mm and 33.5 ± 0.5 mm, respectively, $P < 0.05$; secondary needle length X_{adj} = 96.6 ± 2.0 mm and 85.1 ± 2.0 mm, respectively, $P < 0.001$).

During later development, germination date was more strongly associated with seedling characters than was seed mass (Tables 6 and 7). For example, seed mass was significantly correlated only with root dry mass at Week 34, whereas germination date continued to exert strong, persistent effects on seedling growth up to Week 34. When data were pooled over families and pH treatments, seedlings that germinated earlier were shorter at both Weeks 26 and 34 (r = 0.27 and 0.33, $P < 0.01$ and $P < 0.001$, respectively), but had longer secondary needles (r = –0.33 and –0.34, $P < 0.01$ and $P < 0.001$, respectively). By Week 34, the earlier seedlings also had larger roots (r = –0.25, $P < 0.01$).

Root/shoot ratios differed significantly between families and pH treatment when the effect of germination date was controlled (model $F_{13,95} = 2.35$, $P < 0.01$; family $F_{5,95} = 3.52$, $P < 0.01$; pH $F_{1,95} = 4.23$, $P < 0.05$; family x pH $F_{5,95} = 0.46$, $P > 0.05$; seed mass $F_{1,95} = 1.30$, $P > 0.05$; germination date $F_{1,95} = 9.98$, $P < 0.01$). When the adjusted means of the root/shoot ratios among families were pairwisely tested (based on $\alpha = 0.01$), Family 2 allocated relatively more resources to shoot growth than to root development, compared with Family 1 (66.8% vs. 78.0%, respectively, $P = 0.0035$). Adjusted mean root/shoot ratios of 69.3% vs. 74.5% for seedlings treated with pH 3.0 and 5.6 rain, respectively, suggest that the pH 3.0 seedlings allocated relatively more resources to shoots than to roots. The effects of family and pH on root/shoot ratios are clearly demonstrated in Figure 4.

DISCUSSION

In many species, heavier seeds are more likely to germinate or to germinate earlier, and to produce larger seedlings than do smaller seeds (Mazer 1987; Toon et al., 1991; Kang et al., 1992). However, our results showed that the larger *P. densiflora* seeds had no advantage in germination probability and date. Although seeds with greater mass grew into larger seedlings, this effect tended to weaken over time, exerting little or no influence on seedling growth by Weeks 26 and 34. We caution that this seemingly transient effect of mass may or may not be a significant influence on seedling development because the test period was only 34 weeks long. Previous conifer studies have also reported that the seed mass effect disappeared at later stages of seedling growth (Kriebel et al., 1972; Snyder and Namkoong, 1978; but see Toon et al., 1991). If variation in seed size is to be considered an important factor affecting seedling performance, longer-term studies are required.

The two-fold difference in seed mass among our six maternal trees is not atypical of the intra-specific variations that naturally occur in *P. densiflora*. In fact, mass can vary 1.7-, 4.2-, and 2.3-fold among populations at Kwanak, Dobong, and Hongneung, respectively (Kang, 1999). The relatively weak effect of seed mass on offspring performance may be related to extremely low seed set. In *P. densiflora*, a high rate of early abortion means that only 7% of the ovules mature into seeds, far lower than the seed sets of other conifer species (Kang, 1999). However, if resources are adequate for seed maturation through these high abortion rates, their masses do not fluctuate so widely that they may not strongly influence seedling performance. Future studies should examine the relationship among seed set, seed mass variability, and the effect of seed mass on offspring among conifer species with differing seed sets.

Two characters, germination rate and length of time to germination, were not directly correlated with maternal identity, pH treatment, and seed mass variation. The approximately 93% rate was consistent with that typically found for *P. densiflora* germinated without pretreatment (Yoon and Lee, 1999). This high rate indicates that, regardless of maternal identity and seed mass, most seeds are germinative even when treated at the low pH of 3.0. Such acidic precipitation also did not affect germination in several other tree species (Raynal et al., 1982b; Fan and Wang, 2000). Acid rain may exert no negative effect because germination is usually accomplished only through imbibition of water and the subsequent physiological processes within the seed (Eldhuset et al., 1994).

Early germination often confers a size advantage that exploits the resources available to competing seedlings. Surprisingly, the early-germinated seedlings of *P. densiflora* were consistently larger in all dimensions except height over the 34-week test period. If leaf area is an important component in photosynthesis and subsequent competitive ability, early germination is critical to seedling success, as has also been demonstrated in a tropical tree, *Pithecellobium pedicellare* (Kang et al., 1992). Our data suggest that the timing of germination is a far more ecologically important trait than seed mass when evaluating the response of plants grown under environmental stress. Therefore, variations in germination date should be seriously considered in experiments initiated with seeds and young seedlings.

The family effect on all studied parameters persisted from seed germination through Week 34, with the exception of germination rate. Furthermore, maternal siblings showed similar responses to different pH treatments, except for date of germination. Genotype-specific responses to air pollutants or other stresses have also been observed in herbs and in other conifer species (Libby et al., 1981; Govindaraju and Dancik, 1987; Mansfield et al., 1999). This differential sensitivity among families to acid rain could potentially serve as a selective filter that induces changes in the gene pool of populations. A certain group of our maternal siblings germinated earlier than others when subjected to pH 3.0 rain, which, again, could have provided an advantage when competing for resources. However, siblings from Families 1 and 3 had consistently better seedling growth than those from Family 6, despite the variation in germination date among families treated with low pH. These results strongly suggest that the genetic composition of seeds or seedlings used in acid rain experiments can confound patterns of response. Therefore, the family effect must be controlled by using seeds or seedlings with known provenance, such as from a single clone (e.g., Momen et al., 1999).

Acid rain may either exert inhibitory effects (Houston and Dochinger, 1977; Schulze, 1989; Likens et al., 1996; Finzi et al., 1998) or else stimulate plant growth (Reich et al., 1987; Lee et al., 1990; Keller, 1991; Flagler et al., 1992; Leith et al., 1995). In some cases, acid rain adversely affects conifers and hardwoods only when pH is ≤ 2.0 (e.g., Raynal et al., 1982a; Lee et al., 1998; Fan and Wang, 2000). In our study, differences in growth for seedlings treated with pH 3.0 or 5.6 rain became apparent 34 weeks after germination; those under more acidic conditions showed more vigor. For example, secondary needles on pH 3.0 seedlings were 12 mm longer. This beneficial effect might be attributed to the nitrogen and sulfur nutrients found in acid rain, or to the warmer foliage resulting from this precipitation (Lee and Weber, 1979; Keller, 1991; Flagler et al., 1992; Leith et al., 1995).

However, pH 3.0 rain also resulted in reduced root/shoot ratios. Plants treated with other pollutants, e.g., NO_2, SO_2, or ozone, have shown similar reductions (Tingey et al., 1976; Mooney et al., 1988; MacDonald and Ducsay, 1997). In *P. densiflora*, acid rain can increase chlorophyll content in the needles while decreasing net photosynthesis (Shan, 1998). In our previous experiments (unpublished data), photosynthetic rates for seedlings grown under pH 3.0 rain were much lower than for the controls. Thus, *P. densiflora* seedlings subjected to more acidic rain may allocate relatively more resources to shoots than to roots to compensate for reduced photosynthetic efficiency. Such a reduction in root/shoot ratios under pH 3.0 rain may ultimately be disadvantageous for *P. densiflora* because this species inhabits largely xeric sites.

Furthermore, the initial size advantage for seedlings under pH 3.0 rain would not enhance plant fitness in long-lived species like *P. densiflora*. Conifers such as *Abies firma* and *Picea sitchensis* have shown reduced growth and earlier needle-shed when treated with acid fog or acid mist for more than two years (Carreira et al., 1997; Igawa et al., 1997). Nevertheless, expression of such adverse effects may be delayed because of a soil's acid-buffering capacity (Wood and Bormann, 1977; Likens et al., 1996). For example, two-year-old *P. densiflora* seedlings exhibited a decrease in photochemical reactions after growing four months in soil pretreated with an H_2SO_4 solution (Lee, 1997). Exposure to acid rain may also confound the effects of other environmental stresses. For example, the initially rapid growth of plants under acid rain, acid fog, NO_2, or SO_2 was accompanied by increased sensitivities to frost and drought (Lee et al., 1990; Igawa et al., 1997; Mena-Petite et al., 1999; Caporn et al., 2000). Thus, the early size advantage for seedlings growing under acidic precipitation probably does not guarantee long-term success in the field.

In this study, acid rain affected germination date and seedling performance, but these responses differed among maternal families, and were associated with seed mass and germination date. This differential sensitivity among families could serve as a selective filter that alters the gene pool. However, such changes may require particularly strong selection pressure for trees because of their complex mode of inheritance and their naturally long life spans (Taylor et al., 1994). The acidity and frequency of polluted rain also varies seasonally and annually. Thus, the effect of acid rain on the natural gene pool could be more complex than can be experimentally evaluated.

We acknowledge that our results might not directly apply to *P. densiflora* in nature. A test period that spans less than a year may not be sufficient for understanding the general pattern of response to acid rain. We could not identify a relationship between resource allocation to roots vs. shoots and plant fitness because each seedling was amply supplied with water during the experimental period. In addition, we did not consider morphological and anatomical characters that might be related to seedling performance and, ultimately, to plant fitness. However, our results demonstrate that variations in seed mass and germination date, genetic composition of seeds, as well as our analytical methods all are important in understanding the pattern of responses to pollutants such as acid rain.

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