

Chlorophyll Fluorescence and Needle Chlorophyll Concentration of Fir (*Abies* sp.) Seedlings in Response to pH

B.M. Cregg

Department of Horticulture, Michigan State University, East Lansing, MI 48824, and Department of Forestry, Michigan State University, East Lansing, MI 48824

M.W. Duck, C.M. Rios, and D.B. Rowe

Department of Horticulture, Michigan State University, East Lansing, MI 48824

M.R. Koelling

Department of Forestry, Michigan State University, East Lansing, MI 48824

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Abstract. We assessed variable chlorophyll fluorescence (F/F_m) and needle chlorophyll concentration of seedlings of Macedonian fir (*Abies borisii regis* Mattfeld), subalpine fir [*A. lasiocarpa* (Hooker) Nuttall], Sakhalin fir [*A. sachalinensis* (Schmidt) Mast.], Siberian fir [*A. sibirica* (Ledeb.)], and Veitch fir (*A. veitchii* Lindl.) grown under varying soil media pH. Soil media pH was modified using liquid flowable dolomitic limestone, resulting in five pH levels (3.4, 4.0, 5.4, 6.0, and 6.8). Increasing media pH significantly reduced F/F_m and needle chlorophyll concentration in all of the species tested. The effect of pH on photochemistry was due to depressed nutrient uptake of P, Mn, B, and Cu. Because photosynthetic quantum yield may be related to deficiencies of several elements affected by pH, F/F_m may serve as a criterion to select for improved pH tolerance. Among the species examined, *A. veitchii* and *A. lasiocarpa* were most tolerant of increased pH based on F/F_m and needle chlorophyll concentration.

Evergreen conifers are an important component of landscapes in the upper Midwest. Conifers fulfill an important design function by providing year-round color, form, and texture in landscapes as well as providing cover for wildlife. However, horticulturists are recognizing that many of the principal conifers planted in the central United States are becoming overplanted and suffer from an increasing number of pest problems. For example, Scots pine (*Pinus sylvestris* L.), a mainstay of landscape and Christmas tree industries, is affected by at least 40 different insects and diseases (McCullough, personal communication). Eastern white pine (*Pinus strobus* L.), Austrian pine (*Pinus nigra* Arnold), and Douglas-fir [*Pseudotsugamenziesii* (Mirbel) Franco] likewise are often overplanted and are hosts to a variety of pests (Dix et al., 1986; Riffle and Peterson, 1986).

True firs (*Abies* sp. Miller) include a number of species that may add diversity to the conifers available for landscape planting. The genus *Abies* includes 50 to 60 species of trees native to North and Central America, Europe, Asia, and North Africa (Farjon and Rushforth, 1989; Liu, 1971). Most species of true firs occur in cool, moist habitats in northern latitudes or at high elevation in lower latitudes. Several firs such as grand fir [*Abies grandis* (Douglas ex D. Don) Lindley], pacific silver fir (*Abies amabilis* Douglas ex J. Forbes), and noble fir (*Abies procera* Rehder) are large trees

that are important commercial forest species in the Pacific Northwest. However, true firs have a number of characteristics that make them excellent ornamentals and Christmas trees. Firs grow straight and have a highly symmetrical growth habit. Firs also have a wide range of needle colors and lengths that can add year-round interest and variety to the landscape. While many firs are fast growers in their native environment, most are slower growing off-site.

In the upper Midwest, the use of true firs in landscapes is limited by their fairly exacting site requirements. In general, most *Abies* grow best on sites with good drainage, adequate moisture, and low soil pH. For example, Fraser fir [*Abies fraseri* (Pursh) Poir.] is native to mountainous sites with extremely acid soils; the A horizon pH is ≈ 3.5 , and the B horizon pH 3.8 to 4.2 (Beck 1990). Concolor fir [*A. concolor* (Gord. & Glen.) Hildebr.] and Canaan fir, in contrast, may grow on sites with considerably higher pH

(Brown, 2000; Laacke, 1990), indicating that *Abies* sp. vary in their pH tolerance. However, most information on the pH tolerance of firs is based on observational studies of soils in forest stands. Little direct comparative data on the relative pH tolerance of firs have been reported.

Increasing soil pH may induce chlorosis in plants due to reduced uptake of one or more nutrients, particularly phosphorus, manganese, boron, and copper (Lucas and Davis, 1961). All of these nutrients are involved, either directly or indirectly, in photosynthetic processes. Therefore, understanding the effect of increasing soil pH on photosynthetic function may provide an opportunity for identifying species or genotypes that are adapted to relatively alkaline conditions. The efficiency with which photosystem II captures light energy may be rapidly and nondestructively estimated as the ratio of variable to maximal chlorophyll fluorescence (F/F_m) (Bjorkman and Demming, 1987). Because the function of the photosynthetic system is related to foliar nutrition, variable chlorophyll fluorescence may provide a rapid means to identify physiological response of plants to nutrient imbalances (Laing et al., 2000; Val et al., 1995).

The objectives of the present study were to 1) compare the response of five diverse species of true firs (*Abies* sp.) to varying soil pH, and 2) determine the utility of chlorophyll fluorescence as a tool to quantify this response.

Materials and Methods

The study was conducted in a glass greenhouse at the Michigan State Univ. Plant Science greenhouse complex. We planted plug +2 or 2–2 seedlings of five *Abies* sp. (Table 1) in 8-L containers filled with a mixture of 3 sphagnum moss : 1 perlite (by volume). The number of transplants per species varied from 20 to 35, depending on availability. Seedlings were irrigated as needed with a nutrient solution of well water (EC = 0.65 mS·cm⁻¹ and 105, 35, and 23 mg·L⁻¹ Ca, Mg, and S, respectively) acidified with H₂SO₄ to a titratable alkalinity 130 mg·L⁻¹ CaSO₃ and water-soluble fertilizer providing 125N–12P–125K–13Ca mg·L⁻¹ plus 1.0Fe–0.5Mn–0.5Zn–0.5Cu–0.1B–0.1Mo mg·L⁻¹ (MSU Special, Greencare Fertilizers, Chicago). Greenhouse photoperiod was extended to 16 h using high-pressure sodium lamps. Greenhouse temperature was maintained at 20 °C.

Seedlings from each species were assigned

Table 1. *Abies* species studied in this trial.

Scientific name	Common names	Geographic origin
<i>Abies lasiocarpa</i> (Hooker) Nuttall	Subalpine fir	W. North America
<i>Abies veitchii</i> Lindl.	Veitch fir, Veitch silver fir	Japan: Honshu
<i>Abies sachalinensis</i> (Schmidt) Mast.	Sakhalin fir	Russia (Sakhalin, S. Kuril Islands), Japan
<i>Abies sibirica</i> Ledeb.	Siberian fir	Russia E. from Volga River and S of 67°40' latitude; Turkistan; Mongolia; China
<i>Abies borisii regis</i> Mattfeld	King Boris fir, Bulgarian fir, Macedonian fir	Bulgaria, N. Greece, Albania, and the former Yugoslavia

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at random to one of five pH treatments. Treatments were applied in a completely randomized design. Media pH was modified by addition of liquid flowable dolomitic limestone (28% CaCO₃, 24% MgCO₃) (Limestone F, Cleary Chemical Corp., Dayton, N.J.) to the irrigation program. Based on a preliminary trial, four concentrations of flowable lime were applied to produce a range of soil pH conditions with at least 0.5 pH difference between each level of treatment. A fifth group of seedlings did not receive dolomitic lime. Media pH was determined using saturated media extract weekly or bi-weekly on a subset of plants from each treatment. Flowable lime was applied as needed to maintain the seedlings at the desired pH level.

After 30 weeks exposure to the pH treatments, we measured dark-adapted variable chlorophyll fluorescence (F_v) and maximum chlorophyll fluorescence (F_m) on newly formed needles using a portable chlorophyll fluorescence system (Plant Efficiency Analyzer, Hansatech Instruments Ltd., Norfolk, England). For each measurement, a randomly selected needle was dark-adapted for 15 min using the manufacturer's plastic/foam clips. Fluorescence illumination was provided by an array of six high-intensity light-emitting diodes (LED), which were focused onto the sample surface to provide even illumination over the exposed leaf surface. Red actinic light of a peak wavelength of 650 nm was provided. A fluorometer actinic light level of 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was determined sufficient to saturate PSII, according to our preliminary tests per the manufacturer's instructions (Hansatech Instruments, 1997). An algorithm was used to determine the line of best fit through the initial 8–24 data points at the onset of illumination. This line of best fit was then extrapolated from time zero to determine F_0 (initial or minimal fluorescence), and F_m (maximum fluorescence) was obtained at the same light intensity when the primary electron acceptor from PSII (QA) became fully reduced. Variable fluorescence (F_v) was calculated by subtracting F_0 from F_m , and F_v/F_m was calculated from the Plant Efficiency Analyzer (PEA) fluorometer (Hansatech Instruments, 1997).

After measuring F_v/F_m , we collected foliage from each seedling to determine chlorophyll content (Moran 1982; Moran and Porath, 1980). Ten 1.3-cm needle segments from each seedling were placed in glass vials with 5 mL *N-N'*-dimethylformaldehyde. Samples were incubated for 24 h at 5 °C and absorbance was read on a UV-Vis spectrophotometer (Model U-3110, Hitachi Ltd, Japan). Chlorophyll content was expressed on a leaf area basis. Total surface area of the needle segments was estimated as cross-sectional perimeter \times 1.3 cm \times 10 needles. Needle cross-section perimeter was measured on digital photomicrographs using image analysis software (SigmaScan Pro, SPSS Inc., Chicago). Cross-sectional perimeter varied significantly by species but not pH treatment (data not shown).

Statistical analyses. The effect of dolomitic lime treatment on soil media pH was determined by repeated measures analysis of

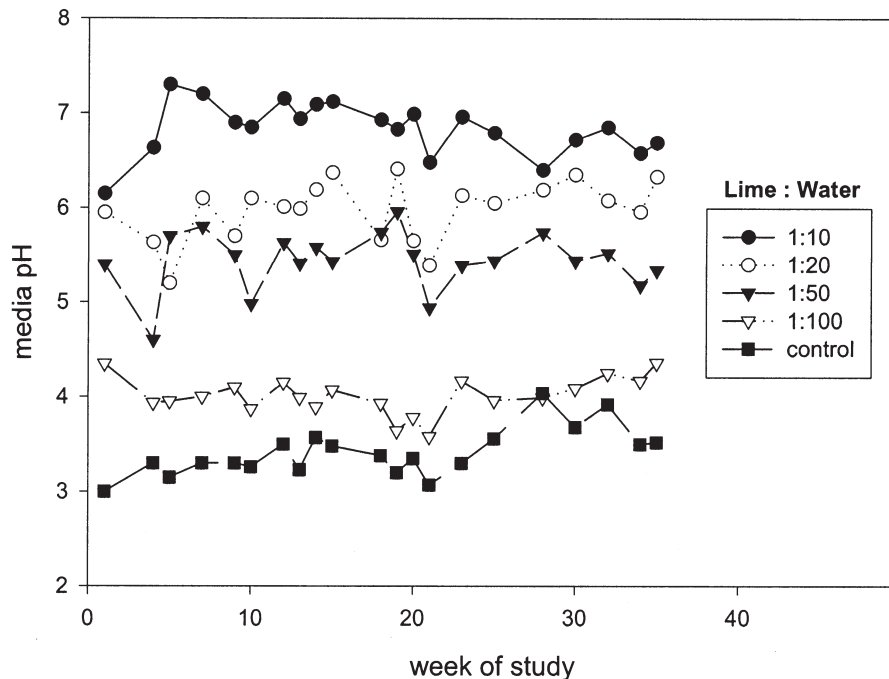


Fig. 1. Soil media pH of fir seedlings treated with various ratios of flowable liquid lime (Limestone F™) and water.

variance based on 26 periodic measurements during the study. Species and pH treatment effects on F_v/F_m and needle chlorophyll concentration were determined by analysis of variance using a fixed effects model:

$$y_{ijk} = \mu + \alpha_i + \gamma_j + \alpha\gamma_{ij} + \epsilon_{ijk}$$

where y_{ijk} = response of the seedling k ; α_i is the effect of species i ; γ_j is the effect of pH level j ; and ϵ_{ijk} is the error term. Foliar nutrition samples were pooled within species and pH treatment to insure adequate plant material for analysis. Main effects (species and pH treatment) were tested for foliar nutrition using the same model as above without species \times pH interaction.

In order to develop a dose-response to pH, we modeled the response of F_v/F_m and chlorophyll content to varying pH using a linear model:

$$y = b_0 + b_1x_i$$

where b_1 and b_0 are coefficients; $y = F_v/F_m$ or $\ln(\text{chlorophyll concentration})$; and $x = e^{(\text{pH})}$. Differences in the model coefficients among species were compared at the 0.05 level following a Bonferroni adjustment (Neter et al., 1985). Correlations among pH, F_v/F_m , chlorophyll concentration, and nutrient concentrations were determined by Pearson's correlation coefficients (Steel and Torrie, 1980).

Results

Flowable lime application was effective in producing a consistent range of pH conditions among the treatments (Fig. 1). Averaged across the study period, the dolomitic lime application maintained at least a 0.58 unit difference between each pH level (Table 2). The total pH range achieved was \approx 3.5 pH units (3.38–6.80).

Photosynthetic quantum efficiency, as in-

dicated by variable chlorophyll fluorescence (F_v/F_m), declined with increasing pH, especially at the highest pH level (Fig. 2, Table 3). Analysis of covariance indicated a significant ($P = 0.05$) interaction between species and pH. At the highest pH level, F_v/F_m of *A. lasiocarpa* was significantly higher than *A. borisii regis*. A Bonferroni-adjusted comparison among coefficients indicated a significant difference in F_v/F_m response to pH between *A. veitchii* and *A. borisii regis*.

Needle chlorophyll concentration decreased significantly with increasing pH (Fig. 3, Table 3). This effect reflected visible chlorosis symptoms, which were evident in seedlings at 1 lime: 50 water ratio and higher pH treatments. Analysis of covariance indicated that the response of chlorophyll concentration to varying pH differed significantly among species. Chlorophyll concentration response of *A. veitchii* to increasing pH was the least sensitive among the species tested (Fig. 3).

Lime treatment and lime \times species interaction effects on seedling height growth were nonsignificant ($P > 0.1$). However, species varied significantly in height growth (Table 3). *Abies sachalinensis* grew more than all the other species except *A. sibirica*.

Foliar concentration of several nutrients

Table 2. Mean pH of saturated media extract from 8-L conifer containers treated with various concentrations of liquid lime.

Liquid lime to water ratio	Mean pH ^a (standard error)
Control	3.38 (0.05)
1:100	4.02 (0.04)
1:50	5.38 (0.07)
1:20	5.96 (0.08)
1:10	6.80 (0.06)

^aMean calculated from 26 periodic measurements throughout study.

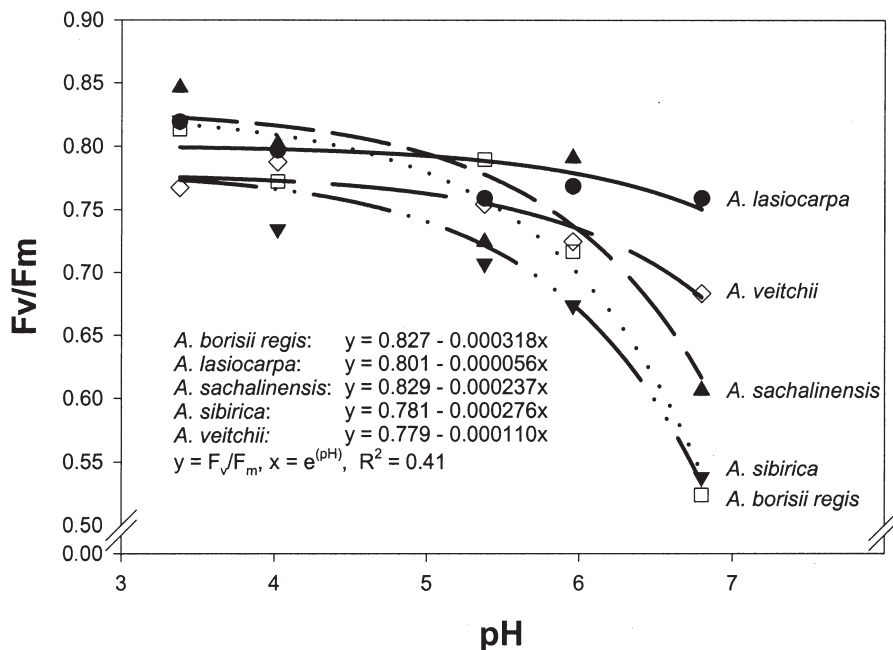


Fig. 2. Relationship between variable chlorophyll fluorescence and soil media pH of seedlings from five *Abies* species.

differed significantly among treatments and species (Table 3). Nutrient concentrations were generally lower in *A. borisii regis* relative to the other species. Foliar concentrations of N, P, Mn, B, and Cu declined in response to increasing pH. Foliar Mg and Ca increased with pH, due to increased availability of these ions with the dolomitic limestone treatment. Foliar Fe, S, Zn, and K were not significantly affected by lime treatment.

Chlorophyll concentration and F_v/F_m were highly correlated ($r \geq 0.71$, $P < 0.001$). Correlation analyses suggested that photochemical response of *Abies* to increased pH is related to depression of P, Mn, and Cu uptake (Table 4). Correlations between Ca and Mg and other cations, such as K, Fe, and Mn, were nonsignificant. This indicated that increased uptake of Ca and Mg from the dolomitic limestone treatment did not cause an antagonistic depression of uptake of other cations. Increased

uptake of cations such as K and Ca can result in an antagonistic suppression of other cations (Sun and Payn, 1999).

Discussion

The results of this study indicate that the photochemistry of seedlings of *A. lasiocarpa* and *A. veitchii* is more tolerant of increasing soil alkalinity than the other species tested. Since these species have evolved in distant regions of the world, they likely have adapted to varying edaphic conditions. *Abies* species are known to vary widely in their response to various biotic and abiotic stresses, including resistance to fungal diseases and drought and cold tolerance (Eiga and Sakai, 1987; Guehl et al., 1991; Hinesley et al., 2000; Sakai, 1982). Although we have no specific information on the soils from which these species were selected, other *Abies* species occur on diverse soils and differ

in pH tolerance. Among North American *Abies* species, optimum pH varies widely. Fraser fir and Pacific silver fir grow on strongly acid soils (pH 3.3–4.2) (Beck, 1990; Crawford and Oliver, 1990). Balsam fir grows under a wide range of soil pH conditions and achieves its best growth on soils with pH between 6.5 and 7.0 (Frank, 1990). *Abies lasiocarpa*, which was relatively insensitive to increasing pH in this study, has an extensive native range and occurs across a range of soil pH levels from 4.5 to 5.9 (Alexander et al., 1990).

The general decline in chlorophyll concentration and photosystem II efficiency with increasing pH observed here is consistent with the response reported for other firs. Bryan et al. (1989) found that seedlings of Fraser fir grew best at a soil pH between 4.2 and 4.5. Moreover, they noted that the seedlings became visibly chlorotic at soil pH greater than 5.0. Chlorosis of declining *A. alba* trees in forest stands in Switzerland was attributed to a shift in pH from 3.7–4.4 to 7.0–7.1 (Hiltbrunner and Flückinger, 1996).

Based on the constant-feed program in our greenhouse, all essential macro- and micro-nutrients were present in adequate amounts. Foliar nutrient levels in the control seedlings were in excess of published sufficiency values for related fir species (Hockman et al., 1989; Kopp and Burger, 1990; Timmer and Stone, 1978). Photosystem II efficiency of the control seedlings approached the theoretical optimum of 0.832 suggested by Bjorkman and Deming (1987).

Increasing chlorosis and reduced photosynthetic activity in firs in response to pH are attributed to reduced uptake of several key elements, particularly Mn, P, B, and Cu. This nutritional response closely follows the classic pattern for organic soils depicted by Lucas and Davis (1961). Their graphic representation of nutrient availability indicates that B, P, Zn, and Cu are severely reduced as pH increases above 6 and that Mn availability declines sharply at pH higher than 5.5. All of these nutrient elements affected by pH are involved with photosynthetic processes and may be associated with the decreases observed

Table 3. Variable chlorophyll fluorescence, chlorophyll content, height growth, and foliar nutrition of seedlings of five *Abies* species grown in peat–perlite under varying levels of flowable dolomitic limestone.

Species ¹	F_v/F_m	Ht												
		Chl a+b (mg·cm ⁻²)	growth (cm)	N	P	K	Mg	Ca	S	Mn	Fe	Zn	B	Cu
<i>A. borisii regis</i>	0.73 ab ²	0.95 a	1.56 a	28.9 a	2.3 a	19.9 a	1.9 a	3.9 a	2.0 a	224 a	74 a	43.0 a	18.6 a	5.2 a
<i>A. lasiocarpa</i>	0.77 a	1.76 b	0.90 a	32.5 ab	2.7 ab	17.7 a	2.2 ab	4.4 ab	2.5 ab	499 a	105 a	45.0 a	21.6 ab	4.8 a
<i>A. sachalinensis</i>	0.75 ab	0.93 a	3.82c	37.8b	3.0b	25.0b	2.8b	6.4b	3.0b	320 a	108 a	46.0 a	29.2bc	5.2 a
<i>A. sibirica</i>	0.70 b	1.16 a	3.14bc	36.5 ab	3.0b	24.4 b	2.2 ab	5.3 ab	3.0b	420 a	104 a	59.8 a	31.4 c	4.8 a
<i>A. veitchii</i>	0.74 ab	1.21 a	1.87 ab	34.5 ab	2.9b	21.3 ab	2.4 ab	4.4 ab	2.5 ab	390 a	88 a	41.2 a	20.4 ab	4.4 a
Treatment	F_v/F_m	Ht ³												
(lime–water)		Chl a+b (mg·cm ⁻²)	growth (cm)	N	P	K	Mg	Ca	S	Mn	Fe	Zn	B	Cu
Control	0.81 x	1.65 x	2.25 x	39.9 x	3.1 xy	20.5 x	1.8 x	3.6 x	2.8 x	449 xy	100 x	43.0 x	28.2 xy	5.6 xy
1:100	0.77 xy	1.39 xy	2.38 x	40.9 x	3.4 x	23.3 x	2.2 xy	4.1 x	2.9 x	528 x	104 x	50.2 x	32.2 x	6.8 x
1:50	0.74 xy	1.15y	2.07 x	30.7 y	2.6 xy	22.7 x	2.5 xy	5.2 xy	2.5 x	342 xy	98 x	51.4 x	21.6 yz	4.4 xy
1:20	0.73 y	1.10 y	2.27 x	28.0 y	2.5z	20.8 x	2.3 xy	4.6 x	2.3 xy	183 y	89 x	38.4 x	15.6z	4.2 xy
1:10	0.62z	0.62z	2.43 x	30.8 y	2.4z	21.1 x	2.7 y	6.9 y	2.5 x	351 xy	88 x	52.0 x	23.6 xyz	3.4 y

¹Mean separation within each variable by Tukey's studentized range test, $P \leq 0.05$.

²Species means averaged across treatments.

³Treatment means averaged across species.

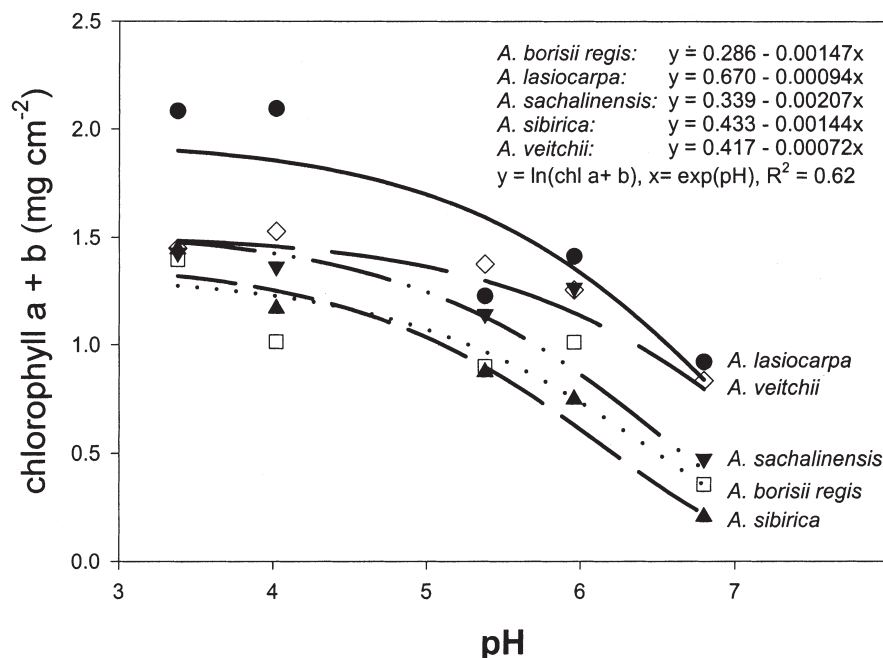


Fig. 3. Relationship between needle chlorophyll content and soil media pH of seedlings from five *Abies* species.

Table 4. Simple correlation coefficients for chlorophyll fluorescence, chlorophyll concentration, and foliar nutrients of *Abies* sp. seedlings under varying container media pH.

	F _v /F _m	Chl a	Chl b	Chl a+b	N	P	K	Mg	Ca	Mn	Fe	Cu
PH	-0.74***	-0.74***	-0.74***	-0.74***	-0.67**	-0.66**	-0.06	0.52**	0.59**	-0.42*	-0.25	-0.72***
F _v /F _m		0.71***	0.72***	0.72***	0.36	0.40*	-0.13	-0.42*	-0.57**	0.36	0.26	0.53**
Chla			0.99***	0.99***	0.40	0.47*	-0.26	-0.44*	-0.61**	0.47*	0.34	0.42*
Chlb				0.99***	0.38	0.45*	-0.26	-0.47*	-0.64**	0.48*	0.33	0.43*
Chl a+b					0.39	0.46*	-0.26	-0.45*	-0.62**	0.47*	0.34	0.40*
N						0.85***	0.51**	0.02	0.05	0.56**	0.48*	0.66**
P							0.44*	0.04	-0.09	0.48*	0.45*	0.65**
K								0.35	0.45	-0.06	0.31	0.31
Mg									0.84***	-0.07	0.35	-0.23
Ca										0.38	0.38	-0.27
Mn											0.55**	0.36
Fe												0.35

*, **, ***Significant at $P \leq 0.05$, 0.01, or 0.0001, respectively.

in F_v/F_m and/or chlorophyll content. Variable chlorophyll fluorescence appears to provide a rapid and effective mechanism to identify pH tolerance among *Abies* species. F_v/F_m was highly correlated with chlorophyll content and key elements, such as P, B, and Cu. Val et al. (1995) found that F_v/F_m was indicative of Fe- and Mn-induced chlorosis in pear trees. However, with respect to foliar nutrition, F_v/F_m is best regarded as a general indicator of nutrient dysfunction. Relationships between chlorophyll fluorescence and nutrient deficiency have been established for P (Loustou et al., 1999), S (Kastori et al., 2000), Fe (Morales et al., 2000), B (Kasotri et al., 1995), and Mg (Laing et al., 2000).

In summary, increasing media pH significantly reduced F_v/F_m and needle chlorophyll concentration of the five *Abies* species tested. The effect of pH on photochemistry was due to depressed nutrient uptake of P, Mn, B, and Cu. Because photosynthetic quantum yield may be related to deficiencies of several elements affected by pH, F_v/F_m may serve as a criterion to select for improved pH tolerance. Among the species examined, *A. veitchii* and *A. lasiocarpa* were most tolerant of increased pH based on

F_v/F_m and chlorophyll concentration. We should note however, that significant intraspecific variation has been identified in *Abies* for other abiotic stresses. Therefore, additional gains in pH tolerance may be realized from seed source testing as well as species comparisons.

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