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Effects of Simulated Acid Rain on Growth and Physiological Characteristics of *Ginkgo biloba* L. Seedlings and on Chemical Properties of the Tested Soil.[1]

I. Seed Germination and Growth

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人工酸性雨가 銀杏나무(*Ginkgo biloba* L.) 幼苗의 生長, 生理的 特性 및 土壤의 化學的 性質에 미치는 影響[1]

I. 種子發芽率과 生長

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ABSTRACT

Half-sib seeds and one-year-old seedlings of *Ginkgo biloba* were treated with various simulated acid rains (pH 2.0, pH 3.0, pH 4.0 and pH 5.0) to examine the effects of acid rain on seed germination and seedling growth. The seeds were sown in a pot (4500 cm^3) containing one of three different soils (nursery soil, mixed soil and sandy soil) and the seedlings were grown in the same pots as the seeds. Simulated acid rain was made by diluting sulfuric and nitric acid solution (H_2SO_4 : HNO_3 = 3:1, V/V) with tap water and tap water (pH6.4), and treated by 5mm each time for three minutes during the growing seasons (April to October 1985 and April to August 1986). Acid rain treatments were done three times per week to potted seeds and seedlings by spraying the solutions. The seed germination, seedling growth and physiological characteristics of potted seedlings were compared among three soil types as well as among the various pH levels. The results obtained in this study were as follows:

1. Seed germination of *Ginkgo biloba* decreased significantly at pH 2.0 level in the field test, and also at the levels of both pH 2.0 and pH 3.0 in the laboratory test, compared to that at control.
2. For two-year-old seedlings, total, top and root dry weights per seedling were significantly different among the three soil types and among the levels of pH, and shoot growth was different only among the levels of pH.
3. For one-year-old seedlings, height and total and stem-branch dry weights per seedling were significantly different among the levels of pH.

Key words: Simulated acid rain, Ginkgo biloba, *seedling growth, seed germination.*

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要 約

人工酸性雨가 銀杏나무의 種子發芽率과 幼苗의 生長에 미치는 影響을 究明하기 위하여, 天然降雨를 遮斷하고 苗圃土壤, 混合土壤 및 砂質土壤에 각각 盆植된 銀杏나무 種子와 幼苗(1-0, half-sib)에 人工酸性雨(黃酸과 窒酸을 3:1, v/v로 混合하여 수도물로 희석한 pH 2.0, 3.0, 4.0 및 5.0)와 수도물(pH 6.4)을 生育期間中(1985年 4月 28日～10月 19日 및 1986年 4月 12日～8月 19日)에 週 3回, 每回 5 mm씩 處理하여 얻어진 結果는 다음과 같다.

1. 銀杏나무 種子의 發芽는 野外實驗에서는 pH 2.0 處理區에서 發芽率이 減少했으며, 室內實驗에서는 pH 2.0 및 3.0 處理區에서의 發芽率이 對照區에 비하여 현저히 낮아졌다.
2. 2年生(1-1) 苗木의 경우, 個體當 乾重量, 地上部 및 地下部 乾重量은 土壤間에 그리고 pH 間에 有意差가 있었으며, 新梢生長은 pH 間에만 有意差가 認定되었다.
3. 1年生(1-0) 苗木의 경우, 苗高, 個體當 總乾重量, 樹幹-枝條 乾重量이 pH 間에 각각 有意性이 認定되었다.

Introduction

Air pollution is problematic not only in densely polluted, heavily industrialized areas, but also in larger regions geographically far from the major sources of pollution. Acid precipitation caused mainly by man-made emission of sulfur dioxide and nitrogen oxides occur regionally, with the impacted areas of further distance from the original sources of emission. The effects of acid precipitation on aquatic and terrestrial ecosystems are enormous and complicated. This involves the elimination of fish populations inhabiting acidified waters and the decline of aquatic invertebrate populations, and terrestrial problems and so forth. Among them, the effects on vegetations and soils are still uncertain. Some experiments with simulated acid rain, however, showed that nutrient leaching from plant tissues and soils increased, certain plant disease were either inhibited or stimulated, and foliar injury or growth effects in plants appeared. The construction structures and monuments made of stone or metal were deteriorated by acid rain. Recently wide-spread acid precipitation has become the major issue among environmental problems.

Growth reduction of trees in New York was due probably to acid rain and low-level air pollution. The added stress could possibly become great enough to significantly reduce annual growth rates of some tree speces. Eventually, continuation of acid rain and air pollution could lead to the replacement of sensitive species by more tolerant species.[33] Similar forest decline and tree growth reduction were explained by Binns and Redfern, Cowling and Dochinger,[11] McLaughlin *et al.*[30] and Cho *et al.*[43]

Simulated acid rain caused early leaf-fall and yield reduction in several crops.[15,16,23,25] Of eleven species tested, eight species were affected by simulated acid rain at pH 4.0 or lower. Lee and Weber[28] suggest that acid rain, at concentrations typical of the northeastern United States, was strong enough to affect the germination rate or seedling growth rate of a large fraction of forest woody species. Lee *et al.*[27] reported that the direction and magnitude of effects on 28 crop species by artificial acid rain varied with species and with treatments. Similar results were reported in five species.[37] Soybean growth, pad yield and foliar elemental content were not affected by simulated acid rain.[19,22] However, Cates *et al.*,[9] Cole and Johnson[10] and Jones *et al.*[26] reported that atmospheric sulfur, wet or dry, was an effective amendment for improving soil nutrient availability, dry-matter yield, and nutrient up-take. Evans[12] explained that nutrients in rain could be absorbed by foliage and rain falls could wash off some bases from faliage. Abrahamsen *et al.*[2,3] investigated the effect of short-term acid rain treatment on height growth of some tree species and it was not significantly different between treatments and only differed from one species to another. Similar results were observed by others,[4,17,24,34,35] but Ogner[31] and Abrahamsen[1] reported that tree growth increased due to increased nutrient supply by acid rain. Anderson and Howells[5] insisted on our keeping the interatctions of vegetations and soils in mind, for the experiment of the effects of acid precipitation on vegetation, and also that significant time was required for feedback mechanisms, involving litter fall and decomposition in the field test. A considerable number of studies on the vegetative effects of acid precipitation have been published in recent days. However, because of limitation in research design, few of these differences in methodologies mke intercomparisons difficult, and the results appear to be inconsistant. More complex experimental designs and analysis may be necessary in order to examine and describe the possible subtle response of terrestrial vegetation to acid precipitation.[13,21]

In Korea, precipitation acidity was measured by National Environment Institute in Seoul. Park, *et al.*[39] reported that 63% of the individual rain, measured from May to September in 1982 in Seoul was classified as acid rain because of pH values ranging between 2.5 and 7.9. Kim[38] reported that 88% of 5-year rain data measured on the main campus of Seoul National University were acidic. The data reported by Son and Yang[40] indicate that acid precipitation was noticed mostly during the period between January and April and its average pH values ranged between 4.0 and 5.0. First acid rain treatment on five kinds of plants in Korea was done in 1982.[37] Then a few seminars were held on the conservation measures of environments and it was suggested to establish the cooperative network for research on precipitation chemistry and forestry and agriculture affected by acid precipitation.[38,41]

Although much research concerning acid rain phenomenon and its impact on plant growth, growth-related characters or soil characteristics have been conducted it would be difficult to assess the effects of acid precipitation on terrestrial ecosystems. Probably, it would not be possible to observe or measure the changes in natural ecosystems due to acid rain. However, such changes have been observed under controlled conditions. Recently, a few studies on rain chemistry and artificial acid rain effects on plant growth were conducted in Korea, but there were rare reports on tree growth and forest ecosystems affected by acid rain. Therefore, it is suggested to further study various, ecological impacts acid rain on both plant and soils, and soil microbes.

The objectives of this study were to determine the effects of different pH levels of simulated acid rain on the seed germination and seedling growth of *Ginkgo biloba* L. under controlled conditions.

Materials and Methods

Materials and Treatments

Plant Materials

Seeds and one-year-old half-sib seedlings of *Ginkgo biloba* L. were provided as plant materials. Seeds were collected from a 60-year-old tree growing on Seoul National University campus in Suwon, Korea. The seeds were sowed at the forestry nursery in Suwon in April 1984. Three seedlings were transplanted in each plastic pot (4500 cm^3 in approximate volume) on April 6, 1985 and April 12, 1986. Twelve seeds were sowed on April 12, 1986 in each plastic pot containing three kinds of soils (Table 1) and ten seeds were sowed in each plastic tray filled with sterilized sand on July 9, 1986. A total of 655 seedlings and 630 seeds were used for this study.

Soils

Soils used in this study were nursery soil (4.73% in organic matter), river sand (0.94% in organic matter) and mixture of above two kinds of soils (nursery soil: river sand = 1:1, V/V). The physical and chemical properties of the soil used in this study were shown in Table 1.

Simulated Acid Rain Treatment

Simulated acid rain was prepared as follows:

Table 1. The physical and chemical properties of the soil used in this study.

Soil	Texture	pH H_2O 1:1	Loss on ignition (%)	C.E.C. (me/100g)	Exch. Cations (me/100g)				Bast sat. (%)	Exch. Al (me/100g)	Avail. P_2O_5 (ppm)	SO_4 -S (ppm)	NO_3 -N (ppm)
					Ca^{++}	Mg^{++}	K^+	Na^+					
Nursery soil	SiC	4.91	4.37	12.14	3.18	.53	.59	.35	38.30	1.38	41.29	54.25	4.50
Sandy soil	SL	5.75	0.94	3.19	1.51	.26	.06	.21	62.02	.22	8.09	14.61	3.50
Mixed above	SCL	5.04	1.64	6.26	2.10	.35	.25	.22	48.24	.67	23.31	32.78	3.90

Table 2. Major cations and anions concentration of simulated acid rain treated in this study.

Treatments		Electric conductivity (mS/cm)	Major cations (ppm)					Major anions (ppm)		
			Ca	Mg	K	Na	Al	P_2O_5	SO_4-S	NO_3-N
Control (pH 6.4)		.19	27.11	5.01	1.61	12.24	3.27	.23	21.15	4.10
Simulated	pH 5.0	.42	30.06	4.77	2.00	9.40	3.34	.58	33.64	9.20
acid rain	pH 4.0	.56	30.08	4.76	2.00	10.85	3.37	.64	46.90	11.91
	pH 3.0	.85	31.07	5.02	1.97	12.83	3.46	.14	117.53	16.36
	pH 2.0	24.50	39.98	6.28	2.15	8.76	4.05	.04	1135.66	327.00

sulfuric and nitric acids (H_2SO_4: HNO_3 = 3:1, V/V) were diluted with tap water and adjusted to pH 2.0, 3.0, 4.0 and 5.0. Tap water (pH 6.4) was treated as control. Major cation and anion concentrations of simulated acid rain treated in this study were shown in Table 2.

Simulated acid rain was sprayed mainly between 15:00 and 18:00 with plastic sprinkler (3 times/weeks, 5mm/time) for 3 minutes, from April 28, 1985 to October 19, 1985, and also from April 12, 1986 to August 20, 1986. respectively.

Experimental Design

Transplanted and seeded pots were arranged in an artificial bed made of Styrofoam to eliminate the effect of other soils. Semitransparent plastic slate was roofed at 1.5m in height to avoid natural precipitation, and then a light screening net was covered to prevent pot-soil from drying and to provide uniform light intensity.

A split-plot design was adopted; soil types as a main-plot factor and pH of treated solution as a sub-plot factor. During the period of second year experiments in 1986, the screening net was removed, and experimental design was similar to the first year design with slight modification.

Seed Germination and Growth Measurement

Germination test was conducted in both laboratory and field. In the Laboratory, ten seeds were sowed in each plastic tray filled with sterilized sand on July 9, 1986. Germination trays placed in an incubator (20-30°C) were moistened with simulated acid rain and tap water, 3 times a week from July 9 to August 19, 1986. In the field, germination rate was observed monthly from May to August 1986.

All the plants were harvested on October 20, 1985 and August 20, 1986, respectively. Total height and shoot growth were measured. Dry matters of leaf, stem and root were weighed to the 0.01g after drying at 85°C for 72 hours in an oven, and then top to root (T-R) ratio was claculated. The mean values of 6 seedlings per each sub-plot were used for statistical analysis.

Results and Discussion

Effects of Simulated Aid Rain on Seed Germination and Seedling Growth

Seed Germination

Seeds and seedlings are critical life stages for the woody plants, especially at infertile, dry sites. Acid rain gives seedlings and additional stress that may affect regeneration by impacting seedbed properties, germination nutrient status and growth.[29]

Table 3. Germination rate of *Ginkgo biloba* in the field by soil types and by pH levels.

Treatment	Soil type			Mean±S.E.
	Nursery	Mixed	Sandy	
Control (pH 6.4)	41.7	33.3	50.0	41.7± 4.82
pH 5.0	58.3	25.0	25.0	36.1±11.10
pH 4.0	41.7	-	41.7	41.7± 0.00
pH 3.0	30.0	33.3	41.7	35.0± 3.48
pH 2.0	16.7*	0.0*	0.0*	5.6± 5.57
Mean±S.E.	37.68±6.81	22.9±7.88	31.68±8.90	

S.E. indicates standard error
* indicates significance at 5% level.

Table 4. Germination rate in the laboratory by pH levels.

	Treatment				
	Control (pH 6.4)	pH 5.0	pH 4.0	pH 3.0	pH 2.0
Germination rate (%)	36.67	40.00	40.00	16.67*	10.00*

* indicates significance at 5% level.

Germination rate was determinated by the rate of seeds producing visible plants above soil surface during the experimental period. Table 3 gives the germination rate of *Ginkgo biloba* by the levels of pH for each of the soil types in the field, and Table 4 does the germination rate of *Ginkgo biloba* in the laboratory. Germination in all types of soil was reduced at pH 2.0 level, and its differences were significant between soil types and between the test methods of seeds in both laboratory and field. In the field, germination rate was not severely reduced at pH 3.0 or higher, but in the laboratory it was reduced at pH 3.0 and 2.0. No germination was observed in mixed and sandy soils at the levels of pH 2.0, but a few were germinated in nursery soil at pH 2.0. Mean values of germination rate in nursery soil were higher than those in mixed and sandy soils.

These results agreed with those reported by MacDonald *et al.*,[29] who found that slight reduction in germination of conifer seeds was observed on acidified A horizon soil, and also similar to those of *Magnolia kobus, Magnolia sieboldii* and *Diospyros lotus* seeds on acidified sandy soil.[42] However, there is no clear evidences of increased germination at low pH levels as reported by Lee and Weber[28] for eastern white-pine, eastern redcedar and yellow birch. From these results, germination of *Ginkgo biloba* seeds were not severely affected by short-term acid rain treatment in this study.

Two-Year-Old Seedling Growth

Mean values of two-year-old seedling height, shoot growth, and total, top and root dry weights per seedling are shown in Table 5.

Seedling height was not significantly different between soil types and between the levels of pH, but total, top and root dry weights per seedlings showed significant differences between soil types at 5% level, between the levels of pH at 1% level. Shoot growth and top to root ratio were different at 1% significance level only between the levels of pH. Shoot growth of the seedlings grown in all types of soil was reduced severely at pH 2.0 levels, compared with control. Shoot growth in mixed and sandy soils decreased with decreasing pH levels of acid rain whereas that in nursery soil increased with decreasing pH levels (Table 5).

Mean values of total dry weight per seedling showed significant differences between soil types, 3.61g, 3.19g and 2.87g in nursery, mixed and sandy soil, respectively. The differences seemed to be resulted from the differences in nutrient status of soils, nutrient uptake of root and photosynthetic efficiency. Total dry weight per seedling at pH 2.0

Table 5. Mean values of growth variables of two-year-old seedlings by soil types and by pH levels.

Soil types (main-plot)	pH levels (sub-plot)	Seedling height (cm)	Shoot growth (cm)	Total dry wt. (g)	Top dry wt. (g)	Root dry wt. (g)	Top to root
Nursery soil	Control (pH 6.4)	18.87	6.57 ab	3.40 a	1.19 ab	2.21 a	.54 a
	pH 5.0	19.87	6.93 a	4.19 a	1.52 ab	2.68 a	.57 a
	pH 4.0	19.10	6.10 ab	3.71 a	1.24 ab	2.47 a	.50 a
	pH 3.0	20.37	7.00 a	4.46 a	1.67 a	2.79 a	.61 a
	pH 2.0	18.53	5.10 b	2.31 b	1.10 b	1.21 b	.92 b
Mixed soil	Control (pH 6.4)	21.07	7.13 a	3.55 a	1.21	2.34 a	.52 a
	pH 5.0	19.77	6.30 ab	3.54 a	1.23	2.31 a	.54 a
	pH 4.0	19.13	5.13 ab	3.38 a	1.11	2.27 a	.50 a
	pH 3.0	18.93	6.07 ab	3.24 a	1.09	2.15 a	.51 a
	pH 2.0	18.87	4.77 b	2.26 b	1.02	1.23 b	.84 b
Sandy soil	Control (pH 6.4)	20.30	6.53 a	3.25 a	1.06 a	2.19 a	.48 a
	pH 5.0	19.20	6.17 a	3.45 a	1.08 a	2.38 a	.45 a
	pH 4.0	19.47	5.80 ab	3.32 a	1.09 a	2.23 a	.49 a
	pH 3.0	17.80	5.43 ab	2.97 a	.99 a	1.97 a	.50 a
	pH 2.0	17.40	4.97 b	1.37 b	.64 b	.73 b	.88 b
F-values							
between main-plot		.657	1.016	13.597*	10.663*	10.380*	3.017
between sub-plot		2.099	4.978**	33.357**	8.023**	46.824**	38.558**
interaction		.853	.668	2.363*	2.139	1.437	.575

* and ** indicate significances at 5% and 1% levels, respectively

Differences in letters in vertical columns indicate significant difference at 5% level for Duncan test.

levels markedly decreased, which might be due to severe root and leaf damage and early leaf-fall in July by acid rain treatment. In nursery soil, total dry weight per seedling, 4.46g at pH 3.0 level was the largest among all of the pH levels, and those at pH 4.0 and 5.0 levels were larger than that at control. In mixed and sandy soils, total dry weight per seedling at pH 3.0 level was smaller than that control, which differed from the results in nursery soil (Fig. 1).

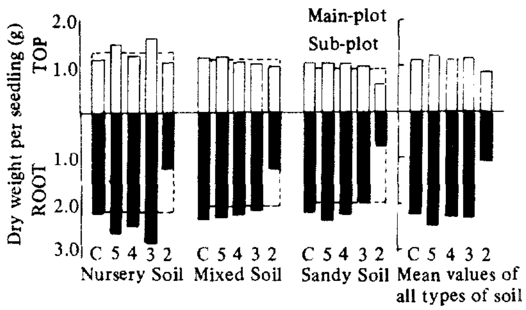

Fig. 1. Dry weight of two-year-old seedlings between soil types and between the levels of pH.

Means of total dry weight per seedling was 3.40g at control, 3.73g at pH 5.0 level, 3.47g at pH 4.0 level and 3.56g at pH 3.0 level.

Considering the means of total dry weight per seedling, pH 3.0 or higher of simulated acid rain might benefit the growth of two-year-old *Ginkgo biloba* seedlings. The T-R ratio was the highest at pH 2.0 levels among all the levels of pH, and differences in the ratios between the levels of pH were not constant at other levels of pH. This means that root growth inhibition is occurred only at pH 2.0 levels.

Increase in shoot growth in nursery soil with decreasing acid rain pH levles was similar to other reports that showed increase in shoot growth of conifers treated with simulated acid rain.[2,35] However shoot growth in mixed and sandy soils decreased as rain pH decreased. These differences between soil types might be due to such soil properties as buffering capacity, organic matter content and cation exchange capacity. Increase in total dry weight with decreasing acid rain pH levels in nursery soil was similar to the stimulation of seedling growth by simulated acid rain at pH 4.0 or higher for tree species,[28] which would be explained that acid rain might stimulate plant growth by the action of nutrient supply in high buffered or calcareous soil.[9,10,26,31] The results in mixed and sandy soils agreed with those reported by Binns and Redfern,[6] McLaughlin *et al.*,[30] Puckett,[33] Bisessar *et al.*,[7] Evans and Lewin,[15] Jacobson *et al.*,[23] who mentioned forest growth declined by air pollution and acid rain, in Germany and in North America, and growth reduction of some crops by simulated acid rain, respectively. Variations in seedling growth among soil types were probably caused by the differences in magnitude of nitrogen and sulfur,[1,32] soil buffering capacity, modification of nutrient phytoavailability by acid rain and by interacts between plant and soils.[5,34] Referring to the reports that alfalfa grown in acidic soil (pH 4.2) showed no injury to aluminum toxicity[20] and tree species were more resistant to aluminum toxicity than crop species, In this study, *Ginkgo biloba* seedlings at pH 3.0 or higher were not injured by aluminum toxicity, but those at pH 2.0 levles (soil pH and exchangeable aluminum are 2.78-3.37 and 1.57-2.80 me/100g, respectively) might have severe root injury by hydrogen and aluminum toxicity as reported by Foy *et al.*[18] and Hecht-Buchholz and Foy.[20]

Thus, growth response to acid rain varied with tree species[2,28] and with crop species,[14,37] and *Ginkgo biloba* was considered resistant to air pollutants.[36] Effects of acid rain on plant growth must be studied simultaneously with its effects on soil chemical properties.[13,21] Forest growth reduction by long-term acid rain treatment was reported by many researchers.[6,30,33] Growth of *Ginkgo biloba* seedling might be reduced in some years later if treated continuously with artificial acid rain of pH 5.0 or lower.

One-Year-Old Seedling Growth

Mean values of one-year-old seedling height, total, stem-branch, leaf and root dry weights, and T-R ratio are shown in Table 6. All the growth variables were not significantly different between soil types. However, seedling height was significant-

Table 6. Mean values of growth variables of one-year-old seedling.

Soil types (main-plot)	pH levels (sub-plot)	Seedling height (cm)	Total dry wt. (g)	Top dry wt. stem-branch (g)	Top dry wt. leaf (g)	Root dry wt. (g)	T-R ratio
Nursery soil	Control (pH 6.4)	13.2 a	1.79 a	.56 b	.47	.76	1.35
	pH 5.0	13.6 a	2.07 a	.59 a	.50	.78	1.73
	pH 4.0	13.9 a	1.91 a	.60 a	.56	.75	1.74
	pH 3.0	10.6 b	1.62 a	.48 a	.65	.49	2.39
	pH 2.0	3.0 c	0.22 b	.01 b	.06	.15	.44
mixed soil	control (pH 6.4)	11.5 a	1.35	.44	.34	.57	1.40
	pH 5.0	11.7 a	1.59	.60	.40	.59	1.70
	pH 4.0	–	–	–	–	–	–
	pH 3.0	9.6 b	1.34	.47	.45	.42	2.42
	pH 2.0	0.0	0.00	.00	.00	.00	–
Sandy soil	Control (pH 6.4)	13.5 a	1.44	.50	.44	.50	2.26
	pH 5.0	12.3 a	1.65	.54	.56	.55	2.48
	pH 4.0	12.5 a	1.74	.44	.64	.66	2.71
	pH 3.0	10.9 b	1.56	.52	.49	.55	2.02
	pH 2.0	0.0	0.00	.00	.00	.00	–
F-value							
between main-plot		.787	.446	.009	.772	.734	3.046
between sub-plot		9.458**	2.261*	3.316*	1.805	1.869	2.525

* and ** indicate significances at 5% and 1% levels, respectively.

Differences in letters in vertical columns indicate significant difference at 5% level for Duncan test.

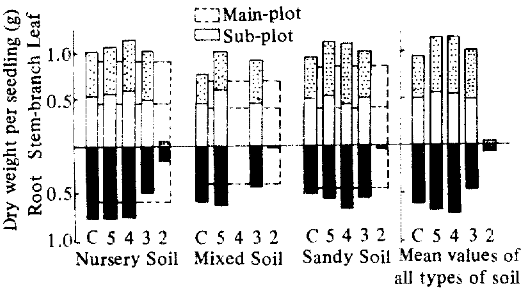

Fig. 2. Dry weight of one-Year-old seedlings between soil types and between the levels of pH.

ly different at 1% level, and total and stem-branch dry weights per seedling significantly different at 5% level between the levels of pH.

Seedling height grown in mixed and sandy soils was reduced as acid rain pH levels decreased, whereas increased in nurssery soil as rain pH levels decreased. Total and stembranch dry weights at pH 2.0 levels markedly decreased, but those at pH 4.0 or higher slightly increased, compared with those at control (Fig. 2).

Growth responses of one-year-old seedlings to acid rain treatment, in general, were similar to the results of two-year-old seedlings. However, more sensitive responses were observed on total dry weight and seedling height, compared to the growth variables of two-year-old seedlings. It is suggested that plants at earlier growth stages are more sensitive to acid rain than late growth stages. This is probably due to non-lignified and soft tissues of the plants at earlier growth stages.

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