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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.

II. Leaf Surface Area, Visible Leaf Injury, Leaf Chlorophyll Content and Photosynthetic Ability of the Leaf Tissue[1]

Gab Tae Kim[2]

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

II. 葉面積, 可視的 葉被害, 葉綠素含量 및 葉組織의 光合成能[1]

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ABSTRACT

Half-sib seedlings of *Ginkgo biloba* (one-year-old) were treated with various simulated acid rains(pH2.0, pH3.0, pH4.0 and pH5.0) to examine the effects of acid rain on leaf surface area, leaf injury, leaf chlorophyll content and photosynthetic ability of the leaf tissue. The seedlings were grown in a pot(4500㎤) containing one of three different soils(nurseryy soil, mixed soil and sandy soil). 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 5㎜ each time for three minutes during the growing seasons(April to October 1985). Acid rain treatments were done three times per week to potted seedlings by spraying the solutions. The results obtained in this study were as follows :

1. Leaf surface area per seedling at pH2.0 level was the lowest among the levels of pH, but those at other pH levels were not significantly different.
2. Leaf injury(injured leaf rate and injured leaf area)increased with decreasing pH levels of acid rain.
3. Leaf chlorophyll content measured during the period June through October was significantly different among the soil types, and that of the seedling in nursery soil was the highest. The lower pH levels of simulated acid rain was treated ; more leaf chlorophyll content was measured at the beginning of treatment, and the more it severely decreased at the late growing period.
4. Photosyntetic abilities, and the highest value was shown in nursery soil. Significand difference in photosynthetic ability among the levels of pH was observed only in August. Photosynthetic ability increased with decreasing pH levels at the beginning of treatment, but decreased rapidly after July.

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要　約

人工酸性雨가 銀杏나무의 葉面積, 葉被害, 葉綠素含量 및 葉組織의 光合成能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 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 pH2.0, 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)에 週 3回, 每回 5mm씩 處理하여 얻어진 結果는 다음과 같다.

1. 酸性雨處理에 의한 個體的 葉面積의 變化는 土壤別로 相異했으며, pH2.0 處理區에서는 급격히 減少했으나, pH3.0이상의 處理區들에서는 큰 差異가 없었다.
2. 葉被害率은 處理酸性雨의 pH 값이 낮을수록 被害葉率과 被害面積이 增加하였다.
3. 葉組織의 葉綠素含量(總葉綠素, 葉綠素 a 및 葉綠素 b)은 6月부터 10月까지의 5回 測定에서 土壤間에 有意性이 認定되었으며, 苗圃土壤에서 가장 높았다. 處理酸性雨의 pH 값이 낮을수록 處理初期에는 葉綠素含量이 높아졌으나, 7,8月부터는 급격히 減少하였다.
4. 葉組織의 光合成能은 7月, 8月 및 9月 測定에서 土壤間에 差異가 있었으며, 苗圃土壤에서 가장 높았다. 處理酸性雨의 pH 값이 낮을수록 處理初期에는 높아졌으나, 7月부터는 급격히 減少하였으며, 8月 測定에서만 有意性이 認定되었다.

INTRODUCTION

Leisions produced by simulated acid rain occur mostly on leaves and reproductive structures. Visible leaf injury is mostly pronounced on foliage of some species just prior to full leaf expansion.[4,8,9,10] For angiosperms, the size of necrotic spot increased with decreasing pH values,[4,24] and surface wettability must be in volved in leaf visible injury.[3,13] Lang *et al.*[23] showed that acute injury due to acid mist was marginal necrosis of leaf and chronic injury was interveinal necrosis.

Neufeld *et al.*[25] reported that gas exchange rate was lower in leaves of four tree species exposed to simulated acid rain, and Lee *et al.*[41] mentioned that chlorophyll contents were reduced in *Pinus koraiensis* and *Pinus densiflora* needles due to artificial acid rain. Similar results were shown in Scots pine needles.[8] Ferenbaugh[12] observed increase in photosynthesis and respiration, and reduction of chlorophyll content in leaves of *Phaseolus vulgaris* exposed to simulated acid rain. Ryan *et al.*[26] also mentioned increase in chlorophyll calcareous soil with sulfuric aicd application. A considerable number of studies on the vegetative effects of acid preipitation have been published in recent days. However, because of limitation in research design, few of these differences in methodologies make 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.[3,17]

The objectives of this study were to determine the effects of differnet pH levels of simulated acid rain on the leaf surface area, visible leaf inury, leaf chlorophyll content and photosynthetic ability of the leaf tissue of *Ginkgo biloba* L. seedling under controlled conditions.

MATERIALS AND METHODS

Materials and Treatments

Plant matericals, soils, simulated acid rain treatment and experimental designs were the same as those used in the previous report.[9]

Measurements

Leaf Surface Area and Leaf Injury Rate

Leaf length and width were measured from early

May to mid October 1985. Because of early leaf-falls due to acid rain treatment, the number of injured leaves and injured leaf area were measured only from early May to late July. These measurements were made biweekly on three seedlings per each of acid rain treatment and its mean values were used for statistical analysis.

Leaf surface area were estimated by regression equation method using leaf length and width.[20] Leaf injury rate was calculated by the methods reported by Gumpertz *et al.*[1-] : injured leaf rate was a proportion of number of injured leaves divided with number of total leaves per seedling and injured leaf areas was calculated on the basis of three severely injured leaves per seedling using dot-grid plate method.

Leaf Chlorophyll Content

Fully expanded leaves were colleted monthly from May to October. Pigments were extracted with 80% acetone, and its absorbances were measured using spectrophotometer(PYE UNICAM, SP8-400) at wave-lengths of 649 and 665nm. Chlorophyll contents(Chl.a, Chl.b and Total Chl.) were determined from Vernon's equations.[34]

Photosynthetic Ability of the Leaf Tissue

In this study, photosynthetic ability was indirectly calculated with the amount of oxygen(μ mole) evolved from unit leaf area(cm^2)per hour under controlled environment. Leaf discs(6mm in diameter) cut from fully expanded leaves were dipped immediately into distilled water and exposed to the tungsten lamp($300\mu E\ cm^{-2}\ sec^{-1}$ in light intensity) at the temperature varying between 24°C and 26°C. Four milliliters of 50mM potassium-phosphat buffer(pH7.2) solution containing 0.5 mM $MgCl_2$ and 0.1 mM $CaSO_4$, and 2ml of 0.625M $NaHCO_3$ solution were used as reaction solution, and their photosynthetic abilities were measured using Oxygen Electode and Meter(YSI Co., Ohio, USA) from late May to mid October.

RESULTS AND DISCUSSION

Leaf Surface Area

Table 1 shows the mean values of leaf surface area(dm^2) per seedling by soil types and by the levels of pH. Leaf surface area per seedling was not significantly different between soil types and between the levels of pH until May 19, but the

Table 1. Changes in leaf surface area(dm^2)per seedling by soil types and by the levels of pH

Soil types (main plot)	pH levels (sub-plot)	Date											
		May 5	May 19	Jun. 1	Jun. 16	Jun. 30	Jul. 17	Jul. 27	Aug. 18	Sep. 1	Sep. 21	Oct. 9	Oct. 20
Nursery soil	Control(pH6.4)	.41	1.13	1.73	1.79	2.22	2.32	2.42ab	2.98	3.17a	3.37a	3.20a	2.71a
	PH5.0	.40	1.05	1.28	1.73	1.92	1.99	1.92b	2.20	2.29ab	2.47ab	2.48a	1.99b
	pH4.0	.29	1.02	1.66	2.00	2.40	2.61	2.77a	2.97	3.06a	3.42a	3.32a	2.80a
	pH3.0	.38	1.24	1.62	1.86	2.32	2.44	2.74a	2.96	3.01a	3.25a	3.20a	2.74a
	pH2.0	.34	.98	1.52	1.74	2.06	2.31	2.52ab	2.40	1.95b	1.48b	.89b	.26c
Mixed soil	Control(pH6.4)	.48	1.24	1.94	2.10	2.47	2.79	3.05	3.10a	3.24a	3.09a	2.00a	
	pH5.0	.46	1.11	1.49	1.77	1.99	1.93	2.23	2.63	2.68a	2.65a	2.50a	2.05a
	pH4.0	.41	1.25	1.71	2.02	2.08	2.06	2.45	2.60	2.74a	2.82a	2.82a	2.29a
	pH3.0	.34	1.09	1.43	1.95	2.17	2.17	2.39	2.54	2.72a	2.49a	2.49a	1.81a
	pH2.0	.48	1.23	1.66	2.15	2.44	1.99	2.01	1.92	1.08b	.63b	.36b	.07b
Sandy soil	Control(pH6.4)	.54	1.06	1.37	1.55	1.77	1.84	2.01	2.15a	2.10a	2.06a	1.82b	1.17a
	pH5.0	.44	1.15	1.43	1.88	2.22	2.22	2.42a	2.54a	2.54a	2.91a	2.86a	1.36a
	pH4.0	.46	1.22	1.56	1.73	1.95	2.01	2.26a	2.22a	2.31a	2.32a	2.28ab	1.63a
	pH3.0	.41	1.08	1.40	1.59	1.81	1.89	1.89a	2.05a	2.17a	2.25a	2.20ab	1.42a
	pH2.0	.45	1.14	1.45	1.88	1.83	1.38	.91b	.83b	.41b	.23c	.09b	
F-value													
between main-plot		1.723	.787	10.557*	11.010*	6.959*	12.745*	14.876*	16.176*	18.925**	27.517**	30.637**	26.181**
between sub-plot		1.763	.157	2.205	.443	.130	1.137	3.354*	6.102**	17.968**	28.923**	50.370**	99.786**
interaction		.457	.760	.750	.637	.911	1.292*	1.931	1.090	1.180	1.558	1.871	3.435**

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

Differences in letters in vertical columns indicate significant difference at 5% level for Duncan test difference was only significant at 5% level between soil types from June 1 to July 17. The differences in leaf surface area were significant at both 5% and 1% levels between soil types and between the levels of pH since July 27. Changing patterns in leaf surface area per seedling fore each soil type was shown in Fig 1. There were no differences in leaf surface area between the soil types upto June 16, but they were significantly different since July 17. Nursery soil showed the highest leaf surface area, followed by the mixed soil and sandy soil. This result was probably due to high soil fertility in nursery soils.

At pH2.0 maximum values of leaf surface area per seedling in most of soil types were observed on June 16, six weeks after simulated acid rain was fairly treated. Thereafter the area dropped rapidly. In nursery soil, maximum value at pH2.0 was observed on July 27 when it occurred later than

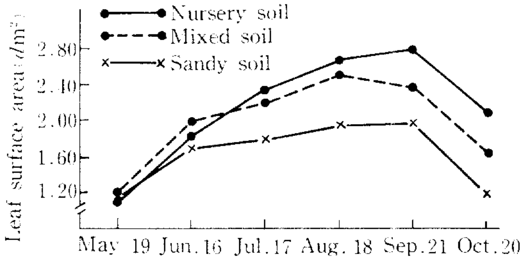

Fig 1. Leaf surface area per seedling for each soil types

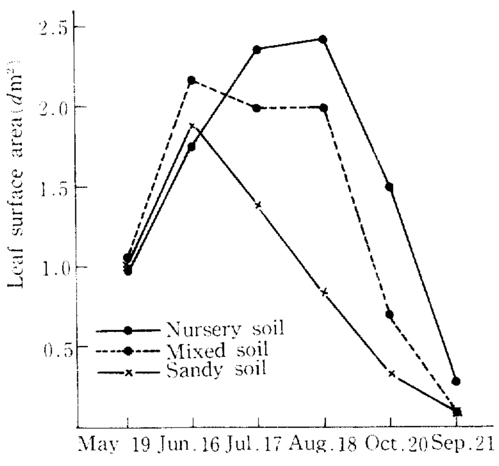

Fig 2. Leaf surface area per seedling for each soil type at pH2.0 levels

other soil types (Fig. 2). At other levels of pH, maximum values of leaf surface area per seedling were represented on Sep.21. Maximum value of leaf surface area per seedling at control (pH6.4) was also shown on Sep.21. The time of reaching to maximum leaf surface areas differed among the levels of pH for each soil types. This may be resulted from the differences in buffering capacity and adaptation capacity to simulated acid rain among soil types.

In general, the levels of pH2.0 in sandy, mixed and nursery soils showed the maximum leaf surface area per seedling on June 16, June 30 and July 27, respectively, whereas other levels did on Sep.21. These results were caused by early leaf-falling time in the following order : the earlist leaf-falls in sandy soil, followed by mixed and nursery soils, which is due to the difference in buffering capacity among soil types. Therefore, the size of leaf surface area[20] and duration of leaf retention[3] affect the growth of *Ginkgo biloba* seedling, which was reduced remarkably at pH2.0. It was suggested that soil responsed to highly acidified rain (pH2.0) differed among soil types different in soil buffering capacity. These agreed withe the explanation that non-calcareous soils with low C.E.C. are the most sensitive to acidification.

Visible Leaf Injury and Leaf Injury Rate

Visible leaf inury detected two weeks after simulated acid rain had been treated, the symptom of which was leaf deformation Regardless of soil types, more serious leaf deformation occurred as pH decreased. The lower pH level of simulated acid rain was treated and the more treatment was done, the more levels showed necrotic spot and the larger size of necrotic spot was observed. Because early leaf-falls had begun, injured leaf rate and injured leaf area were not able to be measured after July 27 on.

At the levels of pH2.0 and pH3.0, chlorosis in leaf margin was observed from early June, and it became severe, as the levels of rain pH decreased. This fact was even more obvious in sandy soil, but was not distinct in nursery soil.

Simulated acid rain drops did not stick to leaf surface, rater rolled down in the beginning of all the levels of pH treatment, but they did well to leal surface in six weeks after acid rain had been treated. However, it was observed at the level of pH2.0 that rain drops have failed to stick to leaf surface since the middle August. Refering to the report[3,13] that leaf injury due to acid rain depended on wettability of the leaf surface and leaf contact angle, it was an interesting result. However, the reaction mechanism of leaf surface to acid rain and the change in epidermal tissues were not known.

Differences in injured leaf rate were highly significant between the levels of pH since simulated acid rain was treated. Significant difference was even higher between soil types on July 27. The most severe injury was shown at the level of pH2.0 and its degree of the rates was highest in sandy soil, followed by in mixed and nursery soils. In general, injured leaf rate became higher as the

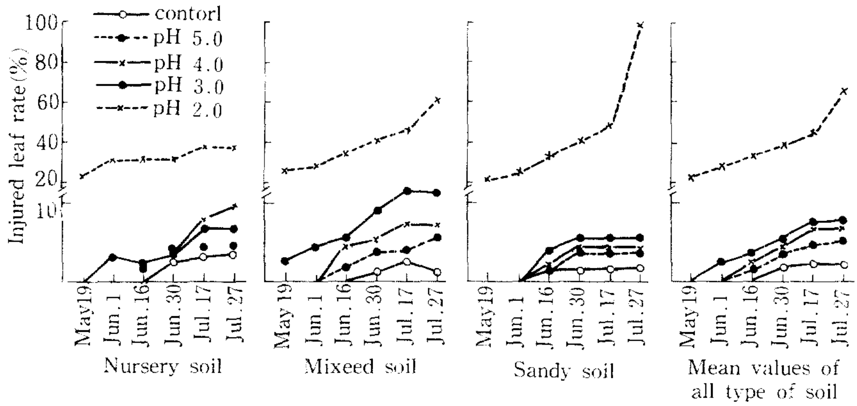

Fig. 3. Injured leaf rate for each level of pH for each soil types

Table 2. Mean values of injured leaf area (㎟) by soil types and by the levels of pH

Soil types (main plot)	pH levels (sub-plot)	Date May 19	June 1	June 16	June 30	July 17	July 27
Nursery soil	Control (pH6.4)	.00a	.00a	.00a	1.33a	1.33a	1.33a
	pH5.0	.00a	.00a	.67a	.67a	.67a	.67a
	pH4.0	.00a	.00a	1.00a	1.67a	1.67a	2.00a
	pH3.0	.00a	.33a	.33a	.33a	1.33a	1.67a
	pH2.0	1.67b	2.67b	3.67b	7.00b	21.67b	41.00b
Mixed soil	Control (pH6.4)	.00a	.00a	.00a	.67a	1.00a	1.00a
	pH5.0	.00a	.00a	.00a	.67a	1.00a	1.00a
	pH4.0	.00a	.00a	1.33ab	2.33ab	3.00ab	3.00a
	pH3.0	.33a	.67a	2.33bc	2.33ab	2.33a	2.33a
	pH2.0	2.00b	2.67b	4.00c	5.67b	16.00b	163.33b
Sandy soil	Control (pH6.4)	.00a	.00a	.33a	1.00a	1.33a	1.33a
	pH5.0	.00a	.00a	.67a	1.33a	1.33a	2.00a
	pH4.0	.00a	.00a	.33a	.67a	.67a	.67a
	pH3.0	.00a	.00a	.33b	.67a	.67a	.67a
	pH2.0	1.67a	2.67b	4.33b	20.00b	57.33b	216.67b
F-value							
between main-plot		4.000	1.000	2.235	.563	.644	7.033*
between sub-plot		110.667**	146.400**	38.575**	4.823**	6.790**	52.860**
interaction		.500	.600	1.359	.872	.795	5.567**

*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 of pH decreased. These results of increasing injured leaf rate with decreasing pH levels were similar to the reports on seven plant species,[4] three tree species[39] and soybean.[15] Lang et al.[23] also reported leaf injury results due to acid mist.

Table 2. gives the mean values of injured leaf area by soil types and by the levels of pH. Injured leaf area was also more severe at pH2.0 level than at other pH levels, which was the same as the injured leaf rate. Its severity was the highest in sandy soil, followed by mixed and nursery soils. Other pH levels showed different trends from pH2.0 level for each of the soil types. In general, injured leaf area became larger as the levels of pH decreased.

The lower the pH levels of acid were treated, the more visible leaf injury was observed.[4,14,15,39] However, considering the acidity of rain fallen in Korea,[35,37,38,40] visible leaf injury of *Ginkgo biloba* seedling in the field may not be serious. The tolerance acidity determining visible leaf injury differed among three species,[4,8,10,39] and reproductive growth of trees was influenced by acid rain. Remarkable decrease in fertilization or survival rates of fern[3,5,6,7] and the decoloration and formation of necrotic spot in the petal occurred due to acid rain,[43,44] Considering above research reports and the acidity of rain in Korea. Visible leaf injury might occur on sensitive pland species and reproduction of some plant speices might be influenced by acid rain.

Leaf Chlorophyll Content

Total chlorophyll, chlorophyll a and chlorophyll b contents were significantly different between soil types during the period June to October(Figure4). This ratio of chlorophyll a to b was significantly diffenent only in October between soil types. Total chlorophyll, chlorophyll a and chlorophyll b contents were significantly different between the levels of pH during May to June, but not since July (Figure5).

Monthly changes in total chlorophyll content were similar to those in chlorophyll a and chlorophyll b contents, and the ratio of chlorophyll a to b did not show any tendency. These results were different from those reported by Lee et at.[41] and Oh,[39] who showed more reduction of chlorophyll a than chlorophyll b in *Pinus koraiensis* and *Pinus densiflora* seedling at a greenhouse during winter and in *Ginkgo biloba*, *Abies holophylla* and *Pinus densiflora*, respectively. This may be resulted from the differences in season, experimental conditions ans species.

The changes in total chlorophyll content for each of soil types are shown in Figure 4. Total chlorophyll contents were similar among soiltypes in May, at the beginning acid rain treatment. However, in June to October the highest chlorophyll content was observed in nursery soil and the lowest in sandy soil. Differences in chlorophyll contents were significant. These results would be due mainly to the differences in nutrient status and buffering capacity between soil types, and partly to the significant dificency of Ca Mg caused by simulated acid rain treatment, especially in sandy soils. Sung[30] also reported the reduction of chlorophyll content by more than 30% due to superabundance of Al and the deficiency of Ca.

The changes in total chlorophyll content for each of pH levels are given in Figure 5. At the beginning of acid rain treatment, rater high total chlorophyll contents were observed as rain pH decreased for all soil types. After that time, however, different tendency was shown for each of soil types. In nursery soil, total chlorophyll content at pH2.0 level was higher than those of other pH levels. The highest chlorophyll content 3.21mg. g^{-1} f.w. was

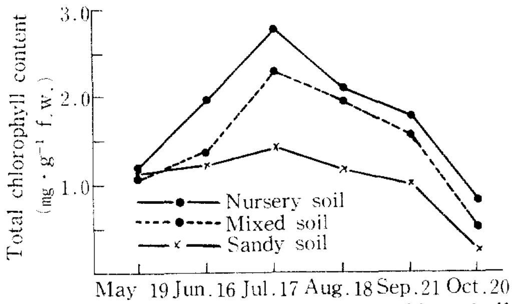

Fig 4. Monthly change in total chlorophyll contents among soil types

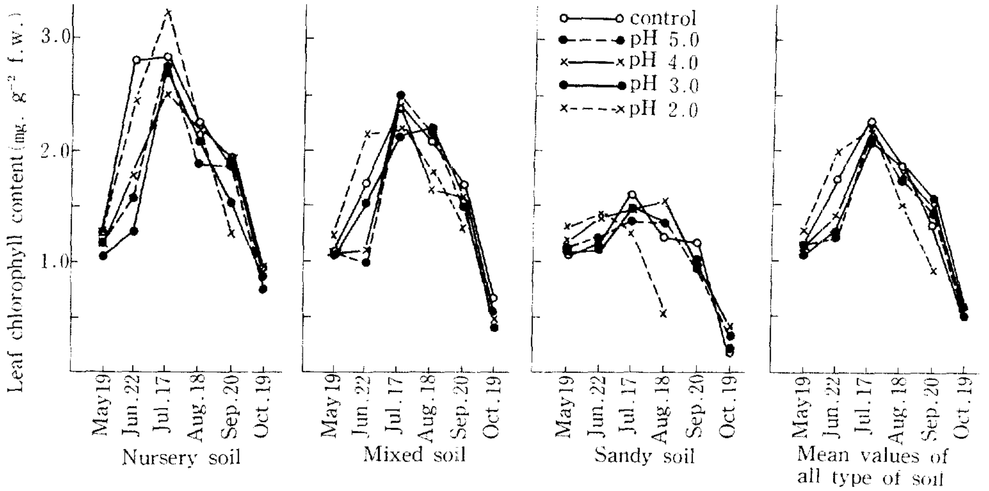

Fig 5. Monthly change in total chlorophyll contents among the levels of pH

measured in July, and then its content decreased rapidly. In mixed soil, the level of pH2.0 showed much higher total chlorophyll than other levels of pH in May and June, and then more remarkable decrease occurred than those in other levels of pH. In sandy soil, total chlorophyll content at pH2.0 level decreased rapidly since June, when it showed the greatest value.

At the beginning of acid rain treatment in May and June, total chlorophyll content increased as rain pH decreased. Such results were rather different from the report[10,36,39,41] that chlorophyll contents decreased with acid rain treatment. However, these results were similar with the report[11] that chlorophyll content of *Phaseolus vulgaris* increased with decreasing the levels of pH. Contrast results between above experiments of acid treatment may be caused by different plant materials and experimental conditions, such as soil properties, rain pH levels, intensity and frequency of the acid rain. The fact at the beginning of acid rain treatment that the more chlorophyll content was measured, as the levels of pH decreased, is comparable to the reports that air pollution reduced chlorophyll content[21,31,42]. Thus, acid rain might give beneficial effects : fertilization on leaf surface and soil nutrient supply. These results in this study and other reports[20,22,32] that the degree of damage on leaf function could be expressed relevantly as chlorophyll content suggest that acid rain might give indirect leaching from leaf tissue and modification of soil nutrient conditions rather than direct damage to leaf function.

Photosynthetic Ability of the Leaf Tissue

At the beginning of acid rain treatment, photosynthetic ability was not significantly different between soil types and between the levels of pH. However, the differences in photosynthetic ability were significant at 1% level between soil types in July, August and September, and at 1% level between pH levels in August.

The changes in photosynthetic ability for each types are shown in Figure 6. The highest value was observed in nursery soil, whereas the lovest in

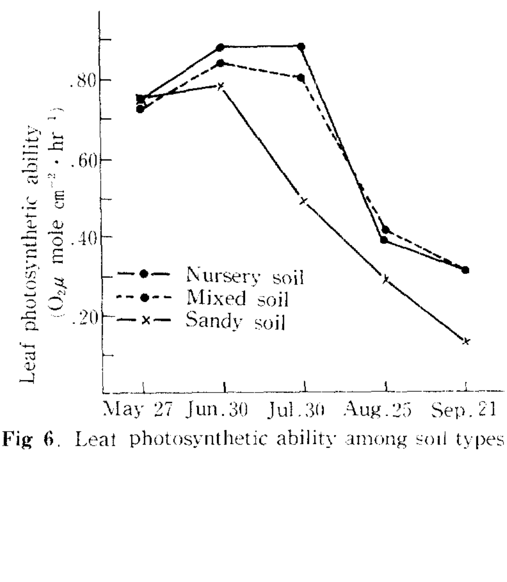

Fig 6. Leaf photosynthetic ability among soil types

sandy soil. The dissimilarities between soil types resulted from the differences in soil nutrient status and soil buffering capacity to acid rain treatment. In May, the measurements were similar among soil types. In July, however, photosynthetic abilities were significantly different between soil types. In general, photosynthetic ability was the highest in June or July, and decreased rapidly after August. This monthly change differed from those shown in *Pinus densiflora and Pinus rigida*[29] and poplar[24] which showed the highest value in August or September. This difference is probalbly due to different conditions of cultivation, such as light intensity, pot soil conditions and existence of acid rain treatment, or different species. In July, the value (0.88~0.49 $O_2\mu$ mole $cm^{-2} \cdot hr^{-1}$) measured by oxygen electrode can be converted into the value (7.7~4.3mg CO_2 $g^{-1} \cdot hr^{-1}$) measured in CO_2 exchange rate. These figures were much lower than those of *Ginkgo biloba* reported by Hodinott,[16] of which values ranged 18.00mg CO_2 g^{-1} hr^{-1}. This difference between two experiments might be due to the differences in measurement device (Oxygen electrode vs. CO_2 gas analyzer) and experimental conditions, such as light intensity, sample preparation methods and conditions of cultivation.

Figure 7 shows the changes in photosynthetic ability at each of the pH levels for each of soil types. This pattern was different from that exhibited among soil types. Although there was no clear pattern at the beginning of acid rain treatment, photosynthetic ability decreased, as rain pH decreased since August. The above results were different from those reported by Ferenbaugh,[11] who showed that photosynthesis of *Phaseolus vulgaris* increased with increasing rain acidity, but were similar to the reports that photosynthesis of *Platanus*[25] or Scots pine[8] decreased with increasing rain acidity.

The monthly mean values of photosynthetic ability at pH2.0 level were 0.82, 0.91, 0.54, 0.16 and 0.01 (O_2 μmole $cm^{-2} \cdot hr^{-1}$) in May, June, July, August and September, respectively, which showed the maximum value in June, and then rapid decrease after that time. At the other levels of pH3.0, 4.0, 5.0 and control, there was no regular

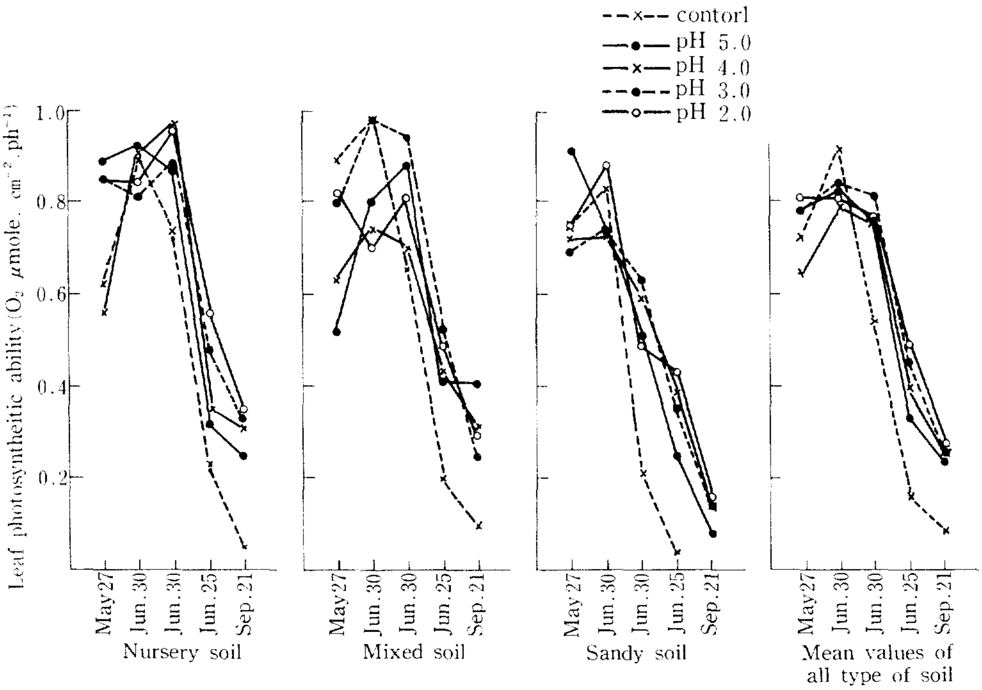

Fig 7. Leaf photosynthetic ability among the levels of pH

patterns in May, June and July. In Augst and September, however, photosynthetic ability decreased, as the rain pH decreased.

In general, photosynthesis was reduced by air pollutants[2,42] and was also inhibited when extracted chloroplast were treated with SO_2[28] or sulfate solution[27] considering these negative effects and high tolerance of *Ginkgo biloba* to air pollutant,[33] acid rain treatment might reduce photosynthetic ability of *Ginkgo biloba* leaf tissue indirectly rater than directly. That is, it would affect photosynthetic ability through nutrient leaching from leaf tissue and from soils, or changes in nutrients which are absorbed and utilized by plants.

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