韓國林學會誌 82(4) : 347-353. 1993
Jour. Korean For. Soc. 82(4) : 347-353. 1993

Contents of Sulfur, Fluorine, Wax and Chlorophyll in Needle Tissue and Needle Growth of *Pinus thunbergii* as Bioindicators of Air Pollution.[1]

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大氣汚染 지역의 海松葉內 水溶性 黃, 弗素, WAX, 葉綠素 含量 및 葉 生長의 변화와 生物指標性.[1]

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ABSTRACT

Accumulation of air pollutants such as SO_2 and HF, chlorophyll contents, wax concentrations in needle tissue and needle growth of *Pinus thunbergii* were studied to verify possibilities of bioindicators of air pollution in Yochon Industrial Complex from July to October, 1991. The concentrations of water-soluble sulfur and fluorine increased nearer to the pollution sources regardless of sampling time, but water-soluble sulfur varied little during investigation and fluorine accumulated more with the passage of time. Total chlorophyll contents decreased a little in the slightly polluted site but sharply in the heavily polluted site. The higher the pollution level was, the more decreased chloroform-extractable wax. Air pollution exposure inhibited needle growth of *Pinus thunbergii* during the latter part of growth period. Water soluble sulfur, fluorine and wax concentrations in needle of *Pinus thunbergii* were rather good sensitive indicators of susceptibility to ambient air pollution from early July to early October. Chlorophyll contents seemed to be able to be used as a bioindicator of air pollution in early stage of needle development and in the severely polluted area. In the meanwhile needle length must be used as a bioindicator in latter part of growing season.

Key words : Air pollution, bioindicator, Pinus thunbrgii, water-soluble sulfur, fluorine, chlorophyll, wax, needle growth.

要 約

麗川工業團地에서 배출되는 大氣汚染物質이 海松의 생육에 미치는 영향을 구명하기 위하여 3개 지역에서 海松葉內 汚染物質, 葉綠素 및 wax함량과 針葉의 생장량을 1991년 7월 초에서 10월 초까지 4주마다 조사하였다. 水溶性 黃과 弗素는 채취시기에 관계없이 汚染源에 가까운 지역일수록 높았으며 水溶性 黃은 7월 초에서 10월 초까지 큰 변동이 없었으나 弗素는 지속적으로 증가하였다. 葉綠素含量은 大氣汚染이 경미한 지역에서는 對照地域과 큰 차이를 보이지 않았으나 大氣汚染이 심한 지역에서

[1] 接受 1993年 6月 24日 Received on June 24, 1993.
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는 급격히 감소하였고 針葉의 wax함량은 汚染度가 높을수록 점차 감소하였다. 針葉의 생장은 7월 초까지는 각 조사지간에 차이가 없었으나 시간이 흐를수록 오염지역내 海松 葉의 生長은 둔화되었다. 따라서 海松葉內 水溶性 黃 및 弗素含量의 증가와 wax함량의 감소는 여천지역의 大氣汚染에 의한 것으로 생육중반 이후에는 大氣汚染에 대한 生物指標로 이용될 수 있는 것으로 판단되었다. 葉綠素含量의 감소는 汚染이 심한 지역에서 비교적 이른 시기에 生物指標性을 보이는 것으로 생각되었다. 한편 針葉의 矮小化는 장기간의 大氣汚染에 의한 영향으로 생육기 후반부에 나타나기 때문에 초기에 針葉의 길이만으로 大氣汚染을 파악하는 것은 적절하지 못하였다.

INTRODUCTION

It is well known that gaseous air pollutants accumulate in plant tissues via foliar absorption and have adverse effects on some physiological processes of tree species. Typical symptoms such as chlorosis or necrosis of leaves exposed to air pollutants, are often followed by reduced leaf growth and prematured defoliation(Jensen and Patton, 1980 ; Axelrod et. al., 1980). The reduction in leaf growth was often restricted to the upper part of the crown, where annual shoots tended to be shorter and the needles smaller than normal. Accordingly needle growth of conifer species was often used as a bioindicator to air pollution(Manning and Feder, 1980). Meanwhile damage of protective surface structure such as cuticle has been studied to investigate the effects of acid deposition on terrestrial vegetation. The eroded appearance of surface wax was reported by field observations in polluted environments(Percy and Riding, 1978 ; Karhu and Huttenen, 1986 ; Cape and Fowler, 1981 ; Cape, 1983). Changes in the contents of pigments have often been used as bioindicators of air pollution injury. Horsman and Wellburn(1976) listed the reports of decreased chlorophyll contents in plants fumigated with SO_2, O_3 and HF.

In recent years accelerated air pollution has caused dramatic forest morbidity in industrial complexes such as Yochon(Kim, 1985 ; Kim and Kim, 1986), Ulsan(Kim et. al., 1982) and Onsan district(Kim, 1991). Chlorosis, necrosis, reduced volume growth, high accumulation of air pollutants, shift of species composition and decreased species diversity were often reported in the above mentioned areas(Kim, 1985 ; Kim and Kim, 1986 ; Kim, 1992). The absorbed acidic substances might induce biochemical changes in plant cells and cause injuries by physiological and metabolic changes in whole plant. Those injuries have been reported as needle chlorosis, necrosis, senescence and premature defoliation in *Pinus thunbergii* forest around Yochon district(Kim, 1985). But physiological responses of leaves to accumulation of acidic substances have not been investigated in trees growing in those industrial complexes.

The study was carried out at *Pinus thunbergii* forest exposed to SO_2 and HF gas in Yochon Industrial Complex, Chonnam. The objectives of the study were to determine ; (i) how much water-soluble sulfur and fluorine accumulated in needles of *Pinus thunbergii*, (ii) whether chlorophyll content, wax concentration and needle growth have been affected by aicdic deposition, and (iii) whether those measures could be used as bioindicators of ambient air pollution.

MATERIALS AND METHODS

The study area locates in Yochon city, Chonnam(Long. 127° 40′ E., N. L. 34° 48′), where more than 50 petrochemical plants are situated along the coast of Kwangyang bay. The major forest type in the area is conifer community comprised of *Pinus thunbergii* and *Pinus densiflora*. Adjacent to the pollution sources, especially Namhae Chemical Co., the injuries of trees by air pollutants are obvious. Morbidity or mortality of trees, reduced growth, shift of species composition, lower species diversity and soil acidification were reported in the area(Kim. 1985 ; Lee and Min, 1989).

Because *Pinus thunbergii* is dominant tree species in Yochon area, three sites of *Pinus thunbergii* forests of similar environmental conditions(site 1 at Sangam-dong, site 2 at Homyoung-dong, and site 3 at Sukjung-dong) were selected in the outside zone of heavy pollution along a transect oriented to southeast from Namhae Chemical Co.. Control site was established at Dolsan-myon, Yochon-gun, Chonnam, which is located about 20km far from the heavily polluted site. Five sample trees of *Pinus thunbergii* were chosen. From each sample tree 4 replicates of needle of the upper crown were taken in all directions at intervals of 4 weeks from July 10 to October 2, 1990. The length of 100 needles for each sample tree was recorded to mm. Water-soluble sulfur was analyzed by gravimetric, fluorine by spectrophotometry with zirconium-eriochrome cyanine red lake(Megregian, 1954). Chloroform-extractable wax was analyzed according to Cape et. al.(1988 . Chlorophyll was determined from Arnon's equations by extracting 5 grams of fresh needle with 96% ethanol(Sestak et. al., 1971).

RESULTS

Water-soluble sulfur and fluorine concentrations.

There were significant differences in water-soluble sulfur concentrations among sampling sites regardless of sampling time(Table 1). Water-soluble sulfur in needle tissue maintained higher at the polluted sites than at the control site, and decreased with the distance from the pollution sources. Water-soluble sulfur varied from 0.041% in early October to 0.057% in early September at control site. But water-soluble sulfur, at the polluted sites, accumulated in needle tissues about 2 to 5 times as much as at control site. It varied from 0.101% to 0.108% at site 3, from 0.133% to 0.144% at site 2, and from 0.193% to 0.212 at site 1. These indicated that forest in and around Yochon Industrial Complex had been under the chronic influence of SO_2 emission. Despite the differences among sites, water-soluble sulfur varied little during investigated period.

Table 1. Water-soluble sulfur concentrations(%) in current needles of *Pinus thunbergii*.

Site	July	Aug.	Sep.	Oct.
S1	0.212[a]	0.198[a]	0.207[a]	0.193[a]
S2	0.141[b]	0.144[b]	0.143[b]	0.133[b]
S3	0.102[c]	0.106[c]	0.108[c]	0.101[b]
Control	0.045[d]*	0.048[d]	0.057[d]	0.041[c]

* The same letter in a column indicates non-significance at the 5% level by DMRT.

Fluorine concentrations differed significantly among sites(Table 2). The nearer to the pollution sources, the more fluorine accumulated. But there were little differences in fluorine between site 3 and control site. Whereas, despite the high accumulation of fluorine in the severe zone, HF gas seemed not to disperse farther than SO_2 gas. In the meanwhile fluorine accumulated steadily as time went by. Although fluorine concentrations ranged from 14.4ppm to 21.6ppm during growth period at control site, it ranged from 22.2ppm to 32.4ppm at site 3, from 42.1 to 53.9ppm at site 2, from 80.7ppm to 92.3ppm at site 1.

Table 2. Fluorine concentrations(ppm) in current needles of *Pinus thunbergii*.

Site	July	Aug.	Sep.	Oct.
S1	80.70[a]	84.98[a]	88.26[a]	92.32[a]
S2	42.08[b]	46.94[b]	51.20[b]	53.94[b]
S3	22.16[c]	27.76[c]	31.06[c]	32.40[b]
Control	14.38[c]*	16.42[d]	20.80[c]	21.64[d]

* The same letter in a column indicates non-significance at the 5% level by DMRT.

Table 3. Total chlorophyll contents(mg.g^{-1}.f.w.) in current needles of *Pinus thunbergii*.

Site	July			Aug.			Sep.			Oct.		
	Cht	Cha	Chb	Cht	Cha	Chb	Cht	Cha	Chb	Cht	Cha	Chb
S1	0.72^{c}	0.57^{c}	0.18^{b}	0.86^{b}	0.67^{b}	0.19^{c}	1.10^{c}	0.91^{c}	0.22^{c}	1.57^{d}	1.26^{c}	0.41^{bc}
S2	0.82^{bc}	0.61^{c}	0.21^{b}	1.27^{a}	0.95^{a}	0.26^{b}	1.46^{b}	1.18^{b}	0.29^{b}	1.99^{c}	1.62^{b}	0.39^{c}
S3	0.98^{b}	0.78^{b}	0.21^{b}	1.24^{a}	0.95^{a}	0.30^{a}	1.78^{a}	1.44^{a}	0.36^{a}	2.40^{b}	1.91^{a}	0.52^{b}
Control	1.34^{a*}	0.96^{a}	0.38^{a}	1.38^{a}	1.10^{a}	0.33^{a}	1.84^{a}	1.52^{a}	0.34^{ab}	2.88^{a}	2.11^{a}	0.75^{a}

* The same letter in a column indicates non-significance at the 5% level by DMRT.
note : Cht, Cha and Chb means total chlorophyll, chlorophyll a and chlorophyll b, respectively.

Chlorophyll contents.

Contents of total chlorophyll, chlorophyll a and chlorophyll b differed significantly among sites. Total chlorophyll contents of healthy trees varied from 1.34mg.g^{-1} in early July to 2.88mg.g^{-1} in early October, and increased with the passage of time. While chlorophyll a contents showed a similar trend to total chlorophyll, chlorophyll b varied little until early August and increased strikingly at all sites in early October. Total chlorophyll contents decreased sharply with the approach of pollution sources. It ranged from 0.72mg.g^{-1} to 1.57mg.g^{-1} at site 1, which amounted to 38 to 46% reduction compared with healthy trees at control site. Decrease of chlorophyll a was also notable at the site 1 regardless of sampling time. But at site 3, chlorophyll a contents were not statistically different from those at control site except early July. There were similar trends of decrease of chlorophyll b at site 1 and site 2.

Wax concentrations.

Wax concentrations decreased significantly at all sites compared with those at control site (Table 4). Though wax reduced markedly with the approach of pollution sources, there were little differences between site 2 and site 3. Comparing with control site, average reductions of wax amounted to 47% at site 1, 34% at site 2 and 23% at site 3. Wax concentrations of healthy trees varied little during investigated period, ranging from 3.83% to 3.37%. But they decreased notably with time at the pollution-impacted sites. By early October, wax decreased from 2.20% to 1.60% at site 1, from 2.58% to 2.12% at site 2, and from 3.16% to 2.43% at site 3.

Table 4. Chloroform-extractable wax concentrations(%) in current needles of *Pinus thunbergii*.

Site	July	Aug.	Sep.	Oct.
S1	2.20^{c}	2.00^{c}	1.91^{c}	1.60^{c}
S2	2.58^{c}	2.34^{bc}	2.45^{b}	2.12^{b}
S3	3.16^{b}	2.95^{b}	2.65^{b}	2.43^{b}
Control	3.82^{a*}	3.83^{a}	3.46^{a}	3.37^{a}

* The same letter in a column indicates non-significance at the 5% level by DMRT.

Needle growth.

As shown in table 5, needle length showed no significant differences among sites in early July. But since then, monthly growth of needle decreased at the polluted sites when compared with that at control site. Needle growth apparently retarded in the latter part of growing season, not in the earlier part. In early September and early October needles were the shortest at site 1, which located nearest to pollution sources. Although needles at polluted sites were affected by air pollution, there were no significant differ-

Table 5. Monthly needle length(mm). of current needles of *Pinus thunbergii*.

Site	July	Aug.	Sep.	Oct.
S1	26.6^{a}	50.1^{b}	65.0^{c}	80.3^{c}
S2	29.9^{a}	50.5^{b}	81.1^{b}	97.8^{b}
S3	31.2^{a}	54.6^{b}	88.7^{ab}	102.8^{b}
Control	29.4^{a*}	67.8^{a}	94.7^{a}	119.3^{a}

* The same letter in a column indicates non-significance at the 5% level by DMRT.

ences in needle length between site 2 and site 3 from July to October.

DISCUSSION

Airborne pollutants seemed to have been widespread over investigated area. The increased concentrations of sulfur and fluorine in needles corresponded to other observations that airborne SO_2 or HF gas accumulated in the needle tissues via stomates(Biggs and Davis, 1981 ; Carlson and Dewey, 1967). It was reported that SO_2 was readily absorbed by tree leaves and was rapidly oxidized to sulfate in mesophyll cells. At low uptake rates, SO_2 was presumed to be oxidized about as rapidly as it was absorbed(Bennet and Hill, 1975). As the plants grow, the accumulated sulfur is translocated and diluted in the plant tissues. Sulfur may be lost from the plants by leaching, emission of hydrogen sulfide(H_2S), or leakage from the roots. Biggs and Davis(1981) reported that the immature foliage maintained higher sulfur content than did mature or fully developed leaves, and that this pattern showed the normal cycle of vegetative growth activity for most broadleaved species in the northern United States. Judging from little variation of water-soluble sulfur during growth period, it was evident that SO_2 gas have already accumulated in the immature needles of *Pinus thunbergii* and have translocated or diluted with elapse of time. HF is very water-soluble and reactive pollutant. It can be adsorbed onto plant surfaces, absorbed through stomates and accumulate at the tips and margins of plant leaves. Carlson and Dewey(1971) reported that accumulation of fluorine from 30 to 50ppm could be sufficient to cause visible injury in needles of coniferous trees. Marginal tip necrosis was the typical symptoms of HF and younger needles were more susceptible to HF than older needles (Manning and Feder, 1980). Thus over 40ppm of fluorine in the needles at polluted sites must have injured needle tissue of *Pinus thunbergii* even in early July. But at site 3, remote from pollution sources, fluorine was insufficiently accumulated to cause visible injury such as needle tipburn.

Thus foliar accumulation of phytotoxic substances might have resulted in plant injury, such as chlorophyll destruction, wax erosion and shortened needles. Total chlorophyll contents of current needles of *Pinus thunbergii* were reported to increase gradually until August and abruptly in September(Lee, 1990). Though such increasing trends with time were also observed, decreased chlorophyll contents in all the trees growing in the polluted sites were due to absorption of air pollutants. Changes in pigments have been reported to be typical physiological responses to air pollutants(Bell and Mudd, 1976). Constantinidou et. al.(1976) reported that exposure of *Pinus resinosa* seedlings to 0.5 ppm SO_2 for 120 min. decreased chlorophyll content of cotyledons and primary needles. Hällgren(1978) reviewed that chlorophyll destruction resulted from oxidation of pigment molecule and the exact mechanism of oxidation was not clear but it might be due to effects on redox potentials of the pigment carrier complexes. It was also suggested that chlorophylls should be converted to phaeophytin by deposition of acid substances(Linzon, 1978).

The chloroform-extractable wax showed a marked decrease with time and pollution level. The decreased wax concentratins corresponded to other studies that epicuticular wax eroded away from needle surface exposed to air pollution. Cape(1983) discussed that the water on the needle surface could be acidified by gaseous pollutants, and that acidic substances might affect the production and subsequent weathering of epicuticular wax. As epicuticular wax is very important as barriers between leaf surfaces and their surroundings, accelerated wax erosion is most effective in enhancing leaching of calcium, magnesium, potassium, etc. Cape et. al.(1988) observed significant differences of wax contents in needles of Norway spruce and Scots pine collected from 10 sites within Western Europe, and suggested that reduced surface wax might be due to air pollution.

It was evident that ambient air pollution inhibited needle growth of field-growing *Pinus thunbergii*. Retarded needle growth was considered to be attributable to not only direct inhibitory effects of air pollutants but also indirect concequences of physiological impediments due to biochemical changes of absorbed pollutants. There were many reports of indirect effects of air pollutants on leaf growth, i.e. chlorosis, necrosis, premature defoliation without visible injury. Kim(1991) reported that needles of *Pinus thunbergii* were more severely discolored and defoliated in Yochon area. Meanwhile, chronic SO_2 exposures were reported to inhibit the needle growth of coniferous trees due to adverse effects of SO_2 on cell size and cell number(Halbwachs, 1984). There were also reports that low concentrations of HF inhibited length and dry weight of needles of Douglas fir(Treshow et. al., 1967), and that photosynthetic impairment caused by low-level pollution over the growing season might be related to reductions in growth and reproductive parameters(Houston and Dochinger, 1977).

In conclusion, airborne air pollutants such as SO_2 and HF had accumulated in needle tissue of *Pinus thunbergii* and caused some altered physiological responses, that is, chlorophyll destruction, wax erosion and retarded needle growth. These altered responses of *Pinus thunbergii* could be used as bioindicators of air pollution in Yochon Industrial Complex. The results suggested that water-soluble sulfur, fluorine and wax concentrations in needle of *Pinus thunbergii* be rather good sensitive indicators of susceptibility to ambient air pollution than chlorophyll. But shortened needle could be used as a bioindicator in latter part of growing season.

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