C-2.2.1 Studies on the effect of environmental acidification on bryophytes and lichens

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Abstract In the 1980's, the effect of acidic rain on plants became a topic of increasing concern. Some authors have reported the effects of simulated acid rain on lichens and bryophytes. However, the relation between tolerance to acid and the field distribution of examined species has not been discussed well. The tolerances of some epiphytic bryophytes to simulated acid rain, activity of superoxide dismutase (SOD), and the relation to their respective distribution patterns were studied. Twenty species of bryophytes collected in urban, rural and forested areas were treated by sulfuric and nitric acids and the acid mixture (simulated acid rain) to detect the tolerance of species. The tolerances were judged by the death or new flush of treated shoots. Most of the species could tolerate 1.0 m eq/l (pH around 2.6) but died at 10.0 m eq/l. Low concentration of acid solution affect the growth of shoots and rhizoids. SOD activity in increased evidently in the case of acid tolerant species treated with low concentration of sulfuric acid. The tolerance of bark epiphytes seems to be correlated to the nature of the bark. However, the correlation between the distribution pattern and the tolerance to the acidity of simulated rain water is not clear.

Key Words bryophyte, lichen, simulated acid rain, superoxide dismutase

1. Introduction

Epiphytic lichens and bryophytes have been observed as indicators of air pollution because of their intolerance to pollutants. One reason for this intolerance is that bryophytes and lichens have no cuticle and absorb water and dissolved pollutants over of their entire surface [1)]. Though this character is common among the epiphytic cryptogams, sensitivity to air pollution differs among the species. Fumigation experiments show that tolerances of epiphytic bryophytes correlate with distribution patterns in polluted areas[11,12)]. Hawksworth and McManus [4)] reported the recolonization of epiphytic lichens in London under conditions of rapidly falling sulfur dioxide levels. Recovery of epiphytic vegetation has also been observed in Tokyo where the concentration of sulfur dioxide has been remarkably reduced and dry-weather has been enhanced by the heat island effect[10)14)]. In the 1980's, the effect of acidic rain on plants became a topic of increasing concern. Some authors have reported the effects of simulated acid rain on lichens and bryophytes. However, the relation between tolerance to acid and the field distribution of examined species has not been discussed well. This paper refers to the tolerances of some epiphytic bryophytes to simulated acid rain and the relation to their respective distribution patterns.

2. Materials and Methods

Experiments were conducted with simulated acid rain on twenty species of bark epiphytes, including some saxicolous and egigeouse ones, growing in and around urban areas.

Epiphytic species can be classified into several groups according to their tolerance to ambient air pollution. It has been reported[12] that the orders of tolerance estimated by fumigation experiments with sulfur dioxide and those based on the distribution around urban areas are similar. In the case of saxicolous or epigeouse bryophytes growing in forests, the chemical property of substratum seems to be the principal environmental factors.

Materials collected in the field were stored in a refrigerator after air drying. The vivid parts of stored shoots, and newly elongated young shoots obtained from cultivated plants were used in the experiments. They are put on cotton wool in a petri dish and treated with acidic solution in every two or three days. Closed chambers were used in the case of constant concentration. Open chambers were also used to detect the concentrating effects of sulfuric acid by drying.

Acid solutions, used to simulate acid rain, were sulfuric acid, nitrous acid and a mixed solution (of 2:2:1 of sulfuric, nitrous and chloric acids in metric equivalent). Rain water collected in an industrial area and deionized water were used as the controls. Activity of superoxide dismutase (SOD) are determind by the nitrite method[2].

3. Results

Plasmolysis was the first symptom of acute injury, and was followed by chrolosis or bleaching of shoots next day after treatment with the acid solution. In many cases, the chrolosis of shoots resulted in complete death, that is, newly developed young shoots were not observed after one or two weeks.

The weight of newly developed shoots (dry weight measured after the experiment) of *Entodon challengeri* correlated with the concentration of acid components, as shown in Table 1. *Entodon challengeri* is thought to be intolerant to air pollution [11,12]. Growth of rhizoid of *Brachymenium exile* also show the same relation. This species is usually growing on alkalized soil in the urban area. The actual tolerance to simulated acid rain was determined by the viability of shoots after the continuous treatment of one month or m ore.

Table 1. Effect of simulated acid rain on the growth of shoot and rhizoid.

Concentration of total acid (pH)	Dry wight* of new shoots of *Entodon challengeri*	Mean length** of rhizoids of *Brachymenium exile*
Control (5.8)	1.6	9.0
0.1 meq/l (3.6)	1.5	6.0
0.5 meq/l (3.2)	1.0	4.0
1.0 meq/l (2.6)	0.7	4.0
5.0 meq/l (2.2)	0.1	1.0
10.0 meq/l (1.8)	0.0	0.1

*Ratio of newly developed shoots to old (initial) shoots.

**Radius of elongated rhizoids in the medium (cotton).

The pHs of sulfuric and nitric acids of 1.0 meq/l were around 2.6 - 2.9. Most of the moss species examined were tolerant to this concentration. On the other hand, *Marchantia polymorpha* was one of the most intolerant species to acidic conditions. Gemmae of the species could not grow and died in a few days after treatment. Sulfite solution, distilled into the same EC (pH 3.4) is more toxic. All of treated materials died within a day. About half of these died at a pH of 3.7.

Sulfuric acid, nitric acid and simulated acid rain of the 10 meq/l were very toxic, and most of materials died at this concentration. However tolerance differed among the species at a concentration of 5.0 meq/l, as shown in Table 1. The toxity of sulfuric and nitric acids were the same at low pH.

The relationship between the growth of newly developed shoots and the concentration of total acid is shown in Table 2. The shoot growth decreases with increasing the acidity in the cases of *Hypnum plumaeforme* and *Pylaisiadelpha tenuirostre.* The growth ratio to control (ion free water) show difference in tolerances of these two species. *P. tenuirostre* may be more tolerant than *H. plumaeforme*. This tendency is the same to the fumigation experiment by sulfur dioxide[13] and not to contradict their distribution patterns[11].

Table 2. Length of newly developed shoots after two months.

Concentration of hydrogen ion	*Hypnum plumaeforme*	*Pylaisiadelpha tenuirostre*	*Haplohymenium pseuto-triste*	*Barbella pendula*
Control	20.0±0.6	6.8±0.7	4.2±0.5	7.8±0.4
0.1 meq/l	17.0±1.9	7.4±0.9	4.2±0.2	12.4±1.5
0.5 meq/l	13.0±0.3	3.2±0.2	2.0±0.4	12.0±2.1
1.0 meq/l	9.0±0.0	2.8±0.2	0.1±0.0	9.8±1.2
5.0 meq/l	5.8±0.7	2.1±0.2	0.0±0.0	4.8±2.5
10.0 meq/l	2.3±0.4	1.0±0.0	0.0±0.0	1.3±0.3

Figures show mean length (mm) ± standard error

Haplohymenium pseuto-triste, which seems to be intolerant to air pollution, is severely damaged by strong acidity. In the case of *Barbella pendula*, which grows in the humid valley and never found in the urban area, shoot growth may be accelerated by low concentration of acid. This must be due to the nitric component of simulated acid rain. Attenuated nitric acid (pH of 4 or more) acted as a nitrogen fertilizer. The color of developed shoots treated in nitric acid sometimes showed a deeper green in those treated in sulfite acid.

The tolerance of species was compared by two methods. The absolute tolerance was determined by the acidity of the solutions that were always lethal to the species in the constant condition. The second method used relative tolerance determined by the reduction of growth after acid treatments. In the case the epigeouse species, *Brachymenium exile*, the growth of rhizoid differed according to the treatment. The growth of shoots, however, was not so affected by the different treatments.

Many species were tolerant to strong acid (pH less than 3.0) and developed new shoots. The most tolerant species was *Drepanocladus fluitans,* which was collected in an acidic stream at Kusatsu Hot Spring. Species growing on rocks and soils are relatively tolerant except for *Brachymenium exile* which grows on alkaline soils (pH of 6 or more) in urban environments.

The activity of superoxide dismutase is evidently increased in the case of *Hypnum plumaeforme* treated with 1.0 m eq/l of sulfuric acid and not increased with 10 m eq/l. In the case of *Pylaisiadelpha tenuirostris,* the activities are constantly high enough in both concentrations.

Table 3. Materials and the lethal level pH of simulated acid rain.

Species of bryophyte	Habitat	Maximum pH* of lethal level	Mimimum pH of survival level
Group 1. Species penetrating far into the center of urban areas**			
Glyphomitrium humillimum	bark of broadleaf tree	1.8	2.6, 2.8
Pylaisiadelpha tenuirostris	bark of broadleaf tree	1.8, 2.2	2.6, 2.8
Groupe 2. Species penetrating into suburban areas			
Entodon challengeri	bark of broadleaf tree	1.8	2.6, 2.8
Hypnum plumaeforme	bark of broadleaf tree	2.2, 2.6	(1.8), 2.8
Group 3. Species occurring at the edge of an urban area			
Venturiella sinensis	bark of broadleaf tree	1.8, 2.2	2.6, 2.8
Group 4. Species occuring optimally in the rural area			
Frullania muscicola	bark of broadleaf tree	2.2	2.6
Orthtrichum consobrinum	bark of broadleaf tree	2.2	2.6
Fauriella tenuis	bark of broadleaf tree	2.2	2.6
Haplohymenium pseudo-triste	bark of broadleaf tree		(1.8), 2.8
Haplohymenium triste	bark of broadleaf tree		2.9
Species growing in the mountainous area			
Boulaya mittenii	bark of broadleaf tree	2.9	(2.9)
Hypnum plicatulum	bark of conifer		2.9
Species found on rock or soil			
Drepanocladus fluitans	in acidic stream	(1.8)	(1.8), 2.8
Plagiomnium vesicatum	on wet rock	1.8	2.6, 2.8
Marsupella sphacelata	in highmoor		2.9
Sphagnum girgensohnii	in highmoor		2.9
Plagiochilla fruticosa	on wet rock	1.8	(1.8), 2.8
Brachymenium exile	on soil under street tree	1.8, 2.2	2.6
Scopelophila cataractae	on soil under copper roof	1.8	2.6, 2.8
Myuroclada maximowiczii	on soil with grass	2.2	2.6

* Concentration treated in the constant condition. Figures in brakets show the case of partial surviving

** Classification of groups according to Taoda (1972)

4. Discussion

Although, the aim of these experiments was to find differences of tolerance to acidity, the results show that there was little difference among the species. As expected, tolerance of epiphytic bryophytes correlated to their substrata. Species that preferred conifer bark were more tolerant to low pH than those which grew on broad-leaved trees only. *Glyphomitrium humillimum*, *Pylaisiadelpha tenuirostris* and *Hypnum plumaeforme* are examples of the former type, and *Fauriella tenuis*, *Orthotrichum consobrinum* and *Boulaya mittenii* are examples of the latter. One of the acid sensitive species, *Venturiella sinensis,* prefers the bark of street trees that are covered by alkaline dust, and are sometimes found on concrete walls. The difference between tolerances to air pollution and simulated acid rain seems to be smaller than that of their substrata. Even *Orthotrichum consobrinum*, which is one of the sensitive species is, tolerant to simulated acid rain of pH 2.6. These figures cannot explain the present distribution pattern of epiphytic bryophytes, because pHs of actual rain are higher than 4.0.

Hutchinson et al.[6] reported that *Plueridium schreberi* growing the coniferous forest floors are affected by simulated acid rain of pH 3.5 and are killed by a pH of 2.5. The enhanced growth of moss treated with nitric acid was also reported. The present experiments showed the same results. The fatal level of pH was nearly the same and mosses treated by nitric acid of pH 3.0 sometimes showed a deeper green color than those treated by sulfuric acid in these experiments. The concentrating effect by drying was restricted to the treatment with sulfuric acid. The chlorosis of leaves of shoots that was dried after treatment with sulfuric acid was more severe than of the leaves that were kept moist.

The toxity of sulfurous acid compared with sulfuric acid of the same pH has been reported by the present author[13]. Though the toxic effect of sulfuric acid was reduced by the addtion of cations, the toxity of reductive action of sulfurous acid was not weakened by the neutralization.

There are areas in which no epiphytic lichens or bryophytes are found, *i.e.*, the innermost parts of industrial areas or large cities, are called 'epiphytic deserts'. The presence of toxic substances in the atmosphere is detected by the 'filtered air chamber methods'[12]. The rain water in polluted areas also contains some phytotoxic substances as heavy metals[9]. The same experiments using today's rain water show no toxity to the bryophytes. In this experiment, shoot growth of *Barbella pendula*, which grows in the humid valley and never found in the urban area, may be accelerated by low concentration of acid. This must be due to the nitric component of simulated acid rain. Attenuated nitric acid (pH of 4 or more) acted as a nitrogen fertilizer. The color of developed shoots treated in nitric acid sometimes showed a deeper green in those treated in sulfite acid.

The reduction of growth, *i.e.,* chronic or invisible injury, was observed when the concentration of hydrogen ions was not fatal. These damages by acidic precipitation must be effective in the competition among the species in the development of bryophytes communities. However, such principal habit as growth form is more effective to the competition among the species If the acidity is not sufficient to kill a certain species, it appearently acts as just another one of the major environment factors, such as, light condition, humidity, nutrients, which affect the growth of the bryophytes.

The activity of superoxide dismutase is evidently increased only in the case of acid intolerant species treated with low concentration of acid. It is constantly high enough in the case of acid tolerant species. The ability to increase SOD activity may be correlate to the tolerance to acidity.

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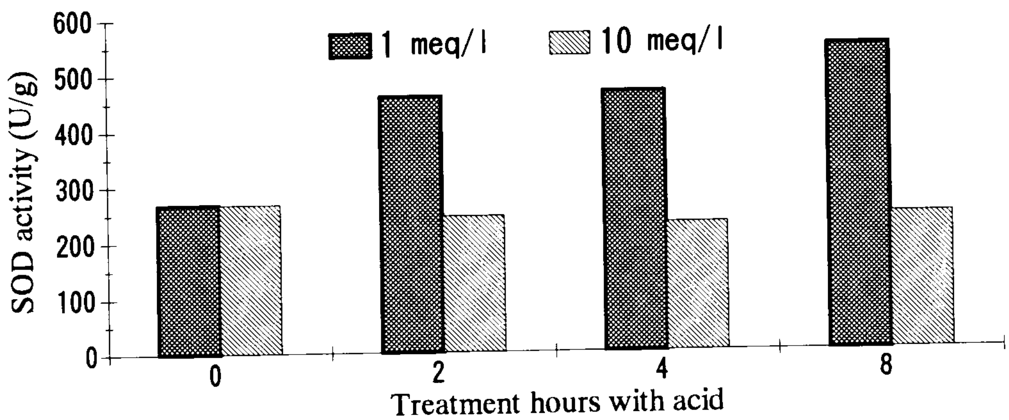

Fig. 1 SOD acitivity of *Hypnum plumaeforme* treated with sulfuric acid.