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3.9 Allelopathic Interactions as a Possible Determinant in the Structure and Composition of Antarctic Plant Communities

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Summary: Using the paper disc-plate method, crude extracts of Antarctic terrestrial plants and soils including penguin rookery guano were examined for their allelopathic effects on the growth of some test-algae. The extracts of *Ceratodon purpureus, Pottia heimii, Grimmia lawiana, Usnea sphacelata* and the soil of penguin rookery showed remarkable inhibition of algal growth: also culture filtrates of Antarctic *Ceratodon purpureus* clearly inhibited the growth of *Chlorella* sp., *Koliella helvetica* and *Stichococcus bacillaris*. These results suggest that the ecological features of algal occurrence and species diversity in both epiphytic and terrestrial vegetation in situ must be influenced by the allelopathic effects of host plants and by penguin excrement respectively

Zusammenfassung: Mit Hilfe der Papierscheiben-Platten-Methode wurden die allelopathischen Effekte von Rohextrakten antarktischer Landpflanzen und Böden von Pinguinguano auf das Wachstum von Test-Algen untersucht. Die Extrakte von *Ceratodon purpureus, Pottia heimii, Grimmia lawiana, Usnea sphacelata* und die Böden von Pinguinkolonien schwächten das Algenwachstum erheblich. Auch Kulturfiltrate von *Ceratodon purpureus* aus der Antarktis schwächten deutlich das Wachstum von *Chlorella* sp., *Koliella helvetica* und *Stichococcus bacillaris*. Diese Ergebnisse zeigen, daß die ökologischen Faktoren für das Vorkommen von Algen und deren Artenzusammensetzung in epiphytischer und terrestrischer Vegetation auch allelopathische Effekte von Wirtspflanzen und Pinguinexkrementen einschließen.

1. INTRODUCTION

For an understanding of the structure and diversity of Antarctic terrestrial plant communities, it is necessary to investigate the environmental factors which determine the occurrence and survival of the dominant plants. It is also important to identify the allelopathic interactions of these plants. Although there has long been considerable interest in the toxic effects of plant extracts on other plants and the possible role of allelopathic interactions in the composition of the plant community, comparatively little is known of such phenomena in the Antarctic terrestrial biota.

BOYD et al. (1966) and BOYD (1967) recognized that, despite the eutrophic condition of Adelie penguin rookeries, bacteria and fungi are scarce in the ornithogenic rookery soils in comparison with the abiotic soils of both the polar regions. They assumed that the presence of certain antibiotic substances in the rookery soil originated from penguin excrements, SIEBURTH (1960, 1963) had demonstrated the antibiosis of acrylic acid, a three-carbon unsaturated aliphatic acid produced by a common Antarctic marine phytoplankton, *Phaeocystis pouchetii*. This indigestible substance remains stable when the alga is eaten by krill *Euphausia superba*, and when consumed by penguins it becomes concentrated in the penguin's intestinal tract and in the rookery soil from their excrement. Recently, AKIYAMA et al. (1986) demonstrated the existence of not only acrylic acid but also oxalic acid which may be the main decomposition product of penguin excrements in the rookery soil in the Antarctic.

It is well known that the secondary metabolites of host plants occasionally have an important role in the control of their epiphyte. For example, the branch tips of actively growing fronds of the marine brown alga *Sargassum* are almost devoid of bacteria and other epiphytes because of the secretion of antibiotic agents from the tips of the host plant (CONOVER & SIEBURTH 1963, 1964, SIEBURTH & CONOVER 1965, SIEBURTH 1968).

It has been recognized that Antarctic epiphytic algae on bryophytes frequently exhibit distinct host preferences and degree of development in their community, although the different host species grow under similar ecological conditions (BROADY 1979a, b, OHTANI 1987). These results are suspected to be caused by allelopathic interactions between algae and mosses. Because of the ecological significance of the allelopathic interaction between algae and other plants in Antarctica, the present work was initiated on the screening of the terrestrial plants for their antialgal toxity.

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2. MATERIAL AND METHODS

The soil and plant samples used in this study were collected from the vicinity of Syowa Station, Antarctica (i. e., on East Ongul Island; Yukidori Valley, Langhovde; Mukai Rocks), in the austral summers of 1983—84. Fresh material was kept at 20° C until analysed. Dried plant material, which had been stored at room temperature was also used in the experiments.

Ten grams of each dried sample was extracted with 20 ml of acetone for 24 hours at room temperature. The extracts were tested for antialgal activity using a diffusion technique on solid media seeded with cultures of selected strains of test algae. 200 µl of crude acetone extracts were applied to a sterilized glass paper disc. After drying, the discs were placed on agar plates seeded with test algae. Pastes made of homogenized plant material and distilled water were also applied directly to the seeded agar plates.

Bold's basal medium (BBM) was used as the standard culture medium. All cultures were incubated at 20° C under illumination of ca. 2 klux in a 12—12 h diurnal light-dark cycle. After incubation (2—3 weeks) the inhibition zone was measured. The algal growth in liquid culture medium was measured as turbidity by using a turbidimeter (Corona type Ut-11, front scatter method turbidimeter).

Of the test algae used in this study, three *Koliella helvetica*, *Stichococcus bacillaris* and *Heterothrix* sp.) were isolated from Antarctic sandy soil, and two *Chlorella* sp. and *Fritschiella tuberosa* were isolated from Japanese soil. Other methods are described later in connection with specific experiments.

3. RESULTS AND DISCUSSION

Acetone extracts and aqueous pastes made of fresh and dried plants and soil were tested for their inhibitive activity against the selected test algae. Of these 3 lichens, 4 bryophytes, 4 naturally occurring algal deposits and soils of penguin rookery clearly inhibited the growth of algae. We observed the remarkable activity in the lichen *Usnea sphacelata*, in the mosses *Grimmia lawiana*, and *Ceratodon purpureus*, in the naturally ocurring plant deposits marine brown algal residue, and in the penguin rookery soil (Tab. 1).

Among the lichens, *Usnea spacelata* deserves special attention as it was rich in agents active in inhibiting algal growth. The ability of secondary lichen substances to inhibit the growth of both. bacteria and fungi. and higher

Plants	Inhibition zone (mm)			
	Pastes*		Acetone extracts**	
	Mean	s.d.	Mean	s.d.
Lichens				
Usnea sulphurea	3.83	1.65	(0.5)	
Umbricaria aprina	0.4	0.43	(0.5)	
Buellia frigida			(0)	
Unidentified lichen encrusted on *Prasiola*	(0)			
Unidentified lichen encrusted on *Ceratodon*	0.4	0.43		
Mosses				
Pottia heimii	(10.0)***			
Grimmia lawiana	3.0	0.0		
Ceratodon purpureus (Antarctic material)	0.64	0.94	2.2	1.61
Ceratodon purpureus (Japanese material)	0.27	0.21		
Bryum argenteum	(0.5)			
Bryum pseudotriquetrum	0	0	0	0
Naturally occurring algal deposits				
diatomaceous sediments	0	0	1.15	1.65
cyanophytan deposits	0.2	0.22		
phaeophytan deposits			5.6	0.43
rhodophytan peat			0.17	0.24
Rookery soil	(2.0)		0.97	0.54

Tab. 1: Antialgal activity of Antarctic plants, and their naturally occurring deposits and rookery soil. Results were calculated on the basis of values obtained from 3 replicate samples. (s.d.: standard deviation) * ca. 0.2g dry weight. **extracts of ca. 0.06g dry weight. *** indicates weak reaction. ( ) shows a value obtained from a single result.

Substances	Inhibition zone (mm)
Usnic acid	3.8
Lichesterinic acid	0.6
Fumarprotocetraric acid	0.3
Stictic acid	0
Ursolic acid	0
Orcinol	0

Tab. 2: Antialgal activities of lichen substances. on *Chlorella* sp. 0.2 ml of 0.001 M acetonic solution was charged. *products of Roth Co.

seed plants has been widely demonstrated (LAWREY 1986). There is, however, a general lack of data concerning antialgal effects of lichen substances. KINRAIDE & AHMADJIAN (1970) demonstrated that sodium usnate, in concentrations between 1 and 10 µg/ml, inhibited the growth of two species of *Trebouxia* which were isolated from the lichens *Acarospora fuscata* and *Cladonia boryi*. STEPHENSON & RUNDEL (1978) also suggested that atranorin, a β-orcinol depside commonly found in many lichens, may act as a growth-regulating substance on the algal symbiont. In this connection, the inhibitory effects of certain secondary lichen metabolites on *Chlorella* sp. were examined (Tab. 2). It is evident that substances such as usnic acid, lichesteric acid and fumarprotocetraric acid posses antialgal activity. The principal antialgal effect of the lichens used in the present study is probably caused by the toxicity of usnic acid, which is the main component of their lichen substance.

Among the mosses, *Grimmia lawiana*, and *Ceratodon purpureus* showed a clear inhibitory effect on algae (Fig. 1). There is a dearth of information regarding the occurrence of antialgal substances in mosses, though the antibacterial and antifungal substances in bryophytes, particularly in liverworts, have been extensively documented (BANERJEE & SEN 1979, ASAKAWA 1981). Little research on the antialgal activity of mosses has been undertaken with the exception of that by WOLTERS (1960). He demonstrated the presence of antialgal activity in the culture filtrates of several moss species. In this connection, we examined the antialgal properties of several Japanese moss species, employing the same methods as described for the Antarctic material to assess antialgal activity (Tab. 3). Three of the mosses showed different degreees of antialgal activity.

Fig. 1: Antialgal inhibition zone produced by aqueous plant paste of *Grimmia lawiana* on assay agar plate seeded with *Chlorella* sp.

In other experiments, actively growing liquid cultures of Antarctic *Ceratodon purpureus* were harvested after 1 month. The culture filtrates were treated in the following ways: 1. to determine the degree of heat-lability of active agents the culture filtrates were autoclaved for 30 minutes; 2. to determine if the active agent is capable of being eliminated from the filtrate by treating with an adsorbent, the culture filtrate was passed through a lipophilic

Moss species	Inhibition zone (mm)
Bryum capillare	2.5
Dicranum japonicum	1.2
Racomitrium canescens	0.5
Bartramia pomiformis var. *elongata*	±
Tradycystis microphylla	±

Tab.3: Antialgal activities of several Japanese moss pastes on *Chlorella* sp. ± indicates a slight reaction less than 0.1 mm.

column (Sep-pak cartridge; Waters Associates Inc.) made of C18 (octadecyl silane). The algal growth, both in the autoclaved and the C18 treated filtrates was evidently better than that of control (Tab. 4). Accordingly, the result suggests that the active agent is clearly heat-labile and removable from the original filtrate by the C18 treatment. This active agent proved to be lipophilic; the inhibitory effect migrates to the chloroform fraction when the extractible solvent of chloroform and methanol mixture was fractionated by addition of water.

Among the scondary bryophyte metabolites, various sesquiterpenoids have been detected as biologically active agents particularly in liverworts, but no sesquiterpenoids have been detected in moss species so far (ASAKAWA 1981, ASAKAWA et al. 1979, HUNECK 1983): Recently, ICHIKAWA et al. (1983, 1984) detected a new cyclopentanoyl fatty acid and several related precursors possessing an antibiosis in several Japanese moss species, including *Dicranum scoparium* and *Fissidens areolatus*.

	Test algae		
	Chlorella	*Koliella*	*Heterothrix*
Inhibitory activity (%)*			
Autoclaved	51.9	11.6	40.5
Filtered through S.P.C.**	24.4	6.1	37.4

Tab. 4: Antialgal effects of cultural filtrates of Antarctic *Ceratodon purpureus*. * Inhibitory activity (%) was culculated according to the following HARRIS (1971) formula. Inhibitory activity (%)=100–(control/treatedX100). ** S.P.C.: (Sep-pak cartridge) a lipophilic column made of C18 (octadecyl silane).

It is well known that long-chain fatty acids have been implicated in allelopathy of algae as well as of angiosperms (PROCTOR 1957, SCUTT 1964, McGRATTAN et al. 1976, ALSAADAWI et al. 1983). Recently, McGRATTAN et al. (1980) purified the inhibiting extracts from the same strain of *Chlamydomonas reinhardii* as used by PROCTOR (1957), and detected at least 15 free fatty acids ranging in chain-length 14 to 20 carbon atoms in the active extract. Seven of the identified substances were tested against four test algae, and it was found that unsaturated free fatty acids such as linoleic (18:2), linolenic (18:3) and palmitoleic (16:1) acids were the most inhibitory, and that toxicity increased with an increase in double bonds.

The presence of highly unsaturated fatty acids (mostly as triglycerides), particularly in plasmic oil droplets of certain moss species including *Ceratodon purpureus* has been reported (SWANSON et al. 1976). In our preliminary chemical analysis on *Ceratodon purpureus* we detected several fatty acids ranging in chain-length from 12 to 18 or more carbon atoms in the active extracts of chloroform fractions obtained from Antarctic and Japanese materials. There was also some evidence of a relationship between the relative content of fatty acids and the inhibitive activity of each extract. However, the prsent result has not yet elucidated whether the active agent served as the free fatty acids themselves or the triglycerides composed of those fatty acids. The investigation of these details must be the subject of future research.

In general, allelopathy may be of widespread significance in plant communities. In both successional and climax communities strongly dominated by a single species, the chemical products of that species may be leached or exuded into the associated soil and soil water and may be significant in limiting the number of composition of other species with it. Besides, among the allolpathic interactions, it is quite possible that the allelochemical substances of host plants are still more effective directly against the epiphytes than the other peripherial plants. A comparison was made of the richness in epiphytic algal species of several Antarctic moss species (Tab. 5). It seems that the critical factor determining the abundance of epiphytes appears to be principally moisture conditions (e. g. *Bryum pseudotriquetrum*), however, in the case of *Grimmia lawiana*, the abundance of epiphytic algal species is scarce irrespective of their habitat conditions. We can recognize a close relationship between the abundance of epiphytic algae and the allelopathic potential of bryophyte host plants particularly of *Grimmia lawiana* as indicated in Table 1.

Localities Habitat condition	East Ongul Island Dry		Yukidori Valley Wet				Mukai Rocks Dry
Moss species*	BP	CP	CP	GL	BP	BA	GL
Number of moss samples examined	1	6	4	3	8	2	8
Number of slide samples examined	1	7	22	6	20	2	8
Number of algal species detected	6	6	16	5	19	11	5
Comparison of abundance of algal species found in a slide sample							
Grade of algal species number							
1–3		6	1	5	5		5
4–7	1	1	3	1	6		2
8–11			5		5	2	
12–14			1		4		
Mean and standard deviation ( ) of algal species in a slide sample	6	2.14 (1.64)	2.36 (4.37)	0.83 (1.86)	3.65 (5.04)	5.67 (4.03)	1.75 (1.48)

Tab. 5: Comparison of richness in epiphytic algal species composition of Antarctic moss species and their habitats. * BA = *Bryum argenteum*, BP = *Bryum pseudetriquetrum*, CP = *Ceratodon purpureus*, GL = *Grimmia lawiana*.

It is well known that angiosperm residues and their decomposed substances frequently act on other plants as allelopathic agents (RICE 1984). Of the Antarctic organic deposits formed by algae such as cyanophytic deposits and diatomaceous sediments, most of them possessed more or less antialgal activities in their acetone extracts, as shown in Table 1. The allelopathic effects of these naturally occurring organic deposits have not been recognized so far. However, their active substances have not yet been identified, but these findings may have an important ecological significance.

The allelopathic effect of penguin excrements associated to ornithogenic rookery soils on soil microbes has previousely been demonstrated (SIEBURTH 1963, BOYD et al. 1966, AKIYAMA et al. 1986a and b). In pengin rookeries the increase in allelopathic substances such as acrylic acid and oxalic acids towards the central area causes a dramatic decline in the occurrence of epipsamic and free-living algae.

The general conclusion revealed by this study is that allelopathic interactions may be an important factor in determining species distribution and composition in Antarctic terrestrial plant communities.

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