The Influence of Lichen Extracts on *Vicia faba* and Chinese Hamster Cells

Lichen thallus that contain substances with fungistatic or fungicidic effects have been reported by e.g. TICHÝ and RYPÁČEK[1] and RYPÁČEK[2]. Some of the substances from the lichen thallus have been shown to be watersoluble and these water-extractable substances from lichens have been tested on wood-destroying fungi[3].

Water extracts, prepared according to HENNINGSSON and LUNDSTRÖM[3] by the extraction of 7.5 g of ground thallus from different lichens in 150 ml distilled water in a Sohxlet apparatus for 7 h, have been studied. The extracts were used without any further refining and have been tested for their effects on chromosome structure and mitotic activity in bean root tips and in cell cultures of Chinese hamster. The extracts were obtained from lichens stored for 1 year in a refrigerator.

Experiments with Vicia faba (var. Weibulls åkerböna). The method of cultivation has been described elsewhere[4]. Lateral roots of 9-day-old seedlings were exposed to various concentrations of the different lichen extracts for a treatment period of 2 h at 20 °C (see Table I). The treatment solutions were prepared by diluting the lichen extracts with a mixture of 1 part distilled water and 1 part Uppsala tap water (pH 7.2) of known composition[5]. The pH was adjusted to 7.0–7.2 with 0.1 *N* NaOH. The untreated control beans were handled in the same way as the treated beans except that no lichen extract was added. After the treatment the seedlings were transferred to tubes containing tap water for recovery periods of 4 and 24 h at 20 °C. The last 3 h before fixation, the roots were exposed to 0.05% colchicine. Roots were fixed and stained and slides prepared as described previously[5,6].

The results of some experiments are shown in Table I. At each concentration of the lichen extracts, 100 metaphases were analyzed for chromosomal aberrations. In no case was any effect on chromosome structure observed. The mitotic activity was, however, effected by some of the treatments. This is evident particularly for the treatment with the extract from *Nephroma arcticum*, which reduced the mitotic index to zero or almost zero at a concentration of 12.5 g/l. The extract from *Peltigera aphthosa* completely inhibited mitosis at a concentration of 50 g/l, whereas the extract from *Hypogymnia physodes* hardly caused any reduction of mitotic index at all at the concentrations tested.

Another experiment which was performed with extract from fresh *Nephroma arcticum* gave on the whole the same result as with extract from lichen stored in a refrigerator for 1 year. Thus, storage had not changed the activity of the extract.

Experiments with cell cultures of the Chinese hamster. The cells belonged to a diploid cell line Cl 1, a clonal derivative of male embryonic lung cells[7]. The cultures were cultivated at 37 °C in Eagle's medium with Hank's salt solution (pH 7.2–7.4), supplemented with 20% calf serum. The cells were subcultured the day before treatment. The hamster cells were exposed to various concentrations of the different lichen extracts for 2 h (see Table II). The treatments were followed by 18 h of recovery in extract-free medium at 37 °C. In the case of *Nephroma arcticum*, the treatment media were prepared by adding 0.5, 1.0, 2.0 or 2.5 ml of extract solution to 9.5, 9.0, 8.0 and 7.5 ml of medium, respectively. In the controls, the extract solution

Table I. The effect of 2-hour treatments with different lichen extracts on the mitotic activity in root tips of *Vicia faba*

Lichen extract	Concentration (g/l)	Recovery (h)	Mitotic index %
Nephroma arcticum	25	4	0
	12.5	4	0.2
	6.3	4	3.3
	3.1	4	6.2
	1.6	4	8.6
	0.8	4	9.8
	12.5	24	0
	6.3	24	7.6
	3.1	24	10.5
Cetraria islandica	50	4	1.7
	37.5	4	3.0
	25	4	8.4
	12.5	4	9.8
	50	24	10.2
Hypogymnia physodes	50	4	7.4
	37.5	4	10.1
	50	24	9.8
Peltigera aphthosa	50	4	0
	37.5	4	0.6
	25	4	1.5
	12.5	4	4.3
	6.3	4	7.3
	3.1	4	9.8
	50	24	0
	37.5	24	8.1
	25	24	10.3
Controls	—	—	9.8

The mitotic indices are based on 2000 cells.

Table II. The effect of 2-hour treatments with different lichen extracts on the mitotic activity in cell cultures of Chinese hamster

Lichen extract	Concentration (g/l)	Recovery (h)	Mitotic index %
Nephroma arcticum	12.5	18	0.4
	0	—	3.6
	10	18	1.1
	0	—	3.6
	5	18	2.9
	0	—	3.3
	2.5	18	3.5
	0	—	3.5
Cetraria islandica	10	18	3.3
	0	—	3.6
Hypogymnia physodes	10	18	3.5
	0	—	3.6
Peltigera aphthosa	10	18	3.4
	0	—	3.6

The mitotic indices are based on 2000 cells.

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[5] B. A. KIHLMAN, Hereditas *49*, 353 (1963).
[6] S. STURELID, Hereditas *68*, 255 (1971).
[7] R. KATO, Hereditas *58*, 221 (1967).

was replaced by the same amount of sterile redistilled water. The other 3 lichen extracts were only tested in concentrations of 5 and 10 g/l. The cells were processed after 1-hour exposure to $5 \times 10^{-7} M$ colchicine. Processing, fixation and preparation of slides have been described previously[6].

The results from some of the experiments are shown in Table II. In each case 100 metaphases were analyzed for chromosomal aberrations. The frequency of chromosomal aberrations was not influenced by the treatments with the different extracts. However, the extract of *Nephroma arcticum* produced a marked reduction of the mitotic index at concentrations of 10 and 12.5 g/l. The other 3 extracts did not have any effect on the mitotic index at the concentrations tested. This finding is in good agreement with that obtained for *Vicia faba*.

The results presented in this paper concerning the mitotic inhibition produced by different lichen extracts may partly explain results obtained in earlier investigations by HENNINGSSON and LUNDSTRÖM[8]. Recently, these authors have shown that low concentrations of extract from *Nephroma arcticum* inhibit the growth of several decay fungi. Extracts from other lichens had not the same inhibiting influence on growth (HENNINGSSON and LUNDSTRÖM, under preparation).

Zusammenfassung. Der Einfluss verschiedener Wasserextrakte aus Flechten auf Chromosomenstruktur und Zellteilung an Bohnenwurzeln und Zellkulturen des chinesischen Hamsters wurde untersucht. Es kam zu keinen Chromosomenaberrationen. Einzig der Extract von *Nephroma arcticum* hatte in schwacher Konzentration einen hemmenden Einfluss auf den Mitoseverlauf. Diese mitosehemmende Wirkung könnte die Ursache sein, dass *Nephroma arcticum* das Wachstum vieler holzzerstörender Pilze hemmt.

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Resistance in Some Wild and Cultivated Grasses to the Phytotoxicity of a Systemic Fungicide

In the last decades, extensive use of chemicals for agricultural and other purposes has subjected the wild as well as the cultivated species of plants to a new evolutionary sieve. Genetic differences in barley and oats for reaction to the insecticides DDT[1] and Toxaphene[2] and a herbicide, Barban[3], have been reported. More recently, different reactions of wheat mutants for resistance to a systemic fungicide, Calixine, have been observed[4]. However, comparative studies on wild plants, particularly those which have made a genomic contribution in the evolution of their cultivated relatives, do not seem to have been reported. Such studies are possible in the tribe Triticeae, in which wild species of *Aegilops* have participated in the evolution of the cultivated species of *Triticum* and where cytogenetic and biochemical information is available on all the related species. This report concerns such comparative studies.

Forty different (Table) lines representing species and varieties of *Aegilops*, *Triticum*, *Secale*, *Hordeum*, and *Avena*, including three synthetic species[5], were grown with 10 replications in a randomized block design in a greenhouse with automatic irrigation, and kept at 22 °C. The systemic fungicide 4-tridecyl-2, 6-dimethylmorpholin (= tridemorph) (Calixine) produced by BASF, Germany, which has been used for the effective control of the mildew, *Erysiphe graminis* in Germany, Great Britain, Denmark, and the Netherlands, was sprayed (0.1% concentration) 24 days after sowing. One week after spraying, chlorosis of leaves was noted on some plants, but many plants appeared unaffected (Table). An extended scale (0–4)[4] was used in the classification of treated plants according to the degree of chlorosis. The horizontal line in the Table gives the distribution of 10 plants in each line for chlorosis of leaves, and the last column records the average character. The Table also includes the genomic contitutions and somatic chromosome numbers of the treated material. Since the application of fungicides began only recently, the pivotal – cum – differentiated genome system[6] does not seem to be operative, as indicated by the reactions of differnet species sharing common genomes.

Inheritance of reaction to DDT is governed by a major gene with susceptibility dominant over resistance[1], and since no gross morphological features are associated with the reaction, there appeared to be no selective advantages or disadvantages for the gene during the evolution of the cultivated forms[1,7]. Species of *Aegilops*, *Secale*, *Hordeum*, and *Avena* were resistant to Calixine, whereas the reaction in the genus *Triticum* was quite variable (Table). Since *Triticum* is also different from the other genera in morphology[8,9], the possibility of an important role of the morphological features in the development of chlorosis cannot be ruled out. *Triticum monococcum* and *Triticum aegilopoides* were more like *Aegilops* in their reactions. This indicates an additional similarity[10,11] of these diploid wheats ($2n = 14$) with *Aegilops*. Of all the hexaploids of *Triticum* studied only the two induced mutants (Mutant No. 11 and Sonora Sharbati) were resistant to Calixine. This might be a coincidence, but the observation presents a possibility of producing tolerant varieties through induced mutations.

Three synthetic polyploid species which have not dunergone genetic diploidization were susceptible to Calixine. The behaviour of *Aegilops ventricosa* × *Triticum aegilopoides*[12] was almost an additive reaction of the constituent species.

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