Cryptogamie, Bryologie, 2002, 23 (3): 271-275

Antifeeding effects of bryophyte extracts from *Neckera crispa* and *Porella obtusata* against the slug *Arion lusitanicus*

Jan-Peter FRAHM & Klaus KIRCHHOFF*

Botanisches Institut der Rheinischen Friedrich-Wilhelms-Universität, Meckenheimer Str. 170, D 53115 Bonn

(Received 18 March 2002, accepted 7 May 2002)

Abstract – Alcoholic extracts of one moss and one hepatic were tested for the first time for its antifeedant activity against a slug *(Arion lusitanicus)*. In a preference test, the extracts of *Neckera crispa* showed low antifeedant effects in concentrations of 0.5% dry weight and more. In contrast, the extracts of *Porella obtusata* showed moderate effects at concentrations of 0.05% and absolute antifeedant acitivity at 0.25% dry weight.

Bryophyta / *Neckera crispa* / *Porella obtusata* / antiphagic effects / antifeedant effect

INTRODUCTION

Bryophytes contain a variety of secondary compounds. Many of them have antimicrobial effects with which they are defending against fungi and bacteria. Beside this biological activity, also antiphagic effects of bryophytes have been reported (Ando & Matsuo, 1984). This is based on the field observation that bryophytes are not eaten by many insects and snails. This sounds at first surprising, since bryophytes seem to be a soft and tender food for insects and snails. In contrast to most flowering plants, however, bryophytes possess no mechanical protection against feeding animal such as bark, thorns, hairs or just firm leathery leaves. The presence of antifeedant agents in bryophytes seem therefore necessary to survive. Bryophytes would else be "eaten up" by herbivorous animals and get extinct. Gerson (1982) reports that mosses are eaten by beetles and grasshoppers, however, mentions that some compounds are known to affect arthropods, "but this is barely the tip of the iceberg, and many additional substances will be found which repel, deter, inhibit or poison invertebrates". The author supposes that invertebrates in general avoid bryophytes because of their chemical defense but certain specialists have managed to adapt. Jennings & Barkham (1995) observed the food of eight species of slug in a woodland in Britain and stated that "bryophytes, by contrast "to tree leaf litter, all gave low palatability scores".

Davidson & Longton (1987) performed feeding experiments with the slug *Arion hortensis*. The consumption of moss shoots was negligible in contrast to that of *Lactuca sativa* or *Taraxacum officinale* except for capsules and especially

* Correspondence and reprints: frahm@uni-bonn.de

developing spores. The observations suggested the presence of chemical barriers for consumption of mosses. In further experiments. Davidson *et al.* (1990) offered slugs (*A. rufus*, *A. subfuscus*) different stages of the moss life cycle: protonemata, shoots and capsules. The slugs showed a preference for protonemata and immature capsules but the consumption of shoots of *Mnium hornum* and *Brachythecium rutabulum* was neglible even after seven days of starvation. Only some shoots of *Funaria hygrometrica* were eaten.

As antifeedant agents, sesquiterpenoids were isolated from the hepatics *Aneura pinguis* and species of *Porella* and *Plagiochila* and tested against insects by Wada & Munakata (1971), Asakawa et al. (1980), and Kubo *et al.* (1976). Due to the lack of any good effective agent against snails and slugs, the use of bryophytes against these animals could have an importance for agriculture and horticulture. There are principally two possibilities: (1) to isolate the biological active compound or (2) to use extracts of bryophytes. The latter requires the cultivation of bryophytes for extraction. The advantage of the use of whole bryophyte extract is in addition, that the efficacy of whole extracts is usually much greater than that of single compounds because of synergistic effects of the whole "cocktail" of compounds extracted from the plants.

Hitherto either single extracted compounds have been tested for their antifeedant activity or slugs have been fed (unsuccessfully) with bryophytes, but no practical tests using bryophyte extracts have been performed so far. Therefore preliminary experiments have been performed to test the antifeedant activity of bryophyte extracts and to see in how far bryophyte extracts could be used for the control of snails and slugs in horticulture.

MATERIALS AND METHODS

For the tests two species of bryophytes, one moss and one hepatic were collected:

Neckera crispa Hedw. (Bryopsida), Italy, Lago di Ledro, on limestone rocks, *leg. Frahm* 22.3.00.

Porella obtusata (Tayl.) Trev. (Hepaticopsida), France, Dépt. Pyrénées Orientales, between Corvavy and Col de la Descargne NW Arles sur Tech 762 m, on granite rocks, *leg. Frahm* 19.3.00.

The plants were cleaned and air dried. For the alcoholic extract, ten g of dry weight was mixed in a kitchen blender in 90 ml of 70% ethanol, kept for 24 hrs on the ground and filtered, which resulted in ca. 50 ml of extract, which was filled up with dist. water to 100 ml. This procedure resulted in an 5% extract (based on plant weight) with an alcohol content of 35%. Blending was necessary as extractions with unblended plant material did not show any activity. The alcoholic extracts were diluted with dist. water to various concentrations.

The aqueous extract was made by the same way but with dist. water instead of ethanol. dist. water.

The feeding tests were performed in small plastic aquariums, in which each five slugs (*Arion lusitanicus*) collected in the surroundings of Bonn were given. In a preference test, two leaves of iceberg lettuce were given over night. One leaf was sprayed with 5 ml of bryophyte extract (which corresponds to 0.05 ml/cm^2), the other with the same amount of solvent (water or diluted ethanol). Next morning the amount of lettuce eaten by the slugs was estimated on both leaves.

Porella obtusata is known to contain several pinguisane-type sesquiterpenoids as well as drimane-type sequiterpenoids such as drimenol, drimeninol, isodrimeninol, drimenin etc., and also polygodial (Huneck, 1984). The latter causes the pungent taste of several *Porella* species.

RESULTS

Preliminary tests revealed that aqueous extracts of Neckera crispa up to a concentration of 5% dry weight showed hardly or only slight effects. Therefore the following tests were performed only with alcoholic extracts.

1. *Neckera crispa*

The alcoholic extracts were diluted with water to 2.5% 1%, 0.5% and 0.05% dry weight (with 17.5%, 5%, 2.5% and 0.25% alcohol contents). Solutions of 0.05% showed no effect, that means leaves sprayed with bryophyte extract and those without were equally fed. Leaves sprayed with solutions of 1 or 0.5% dry weight were less fed than those sprayed with 5% or 2.5% alcohol.

2. *Porella obtusata*

The alcoholic extracts were diluted with dist. water to 2,5%, 0.25%, 0,05% and 0.025% dry weight. The tests failed only with a concentration of 0.025%. First distinct antifeedant effects could be observed at a concentration of 0.05%, whereas the leaves sprayed with a concentration of 0.25% (and higher) were no more eaten.

DISCUSSION

Our results show that the alcoholic extracts of the hepatic *Porella obtusata* showed a much higher activity as compared with extracts of *Neckera crispa* (Tab. 1). The efficacy of *Porella* extracts is ten times higher than those of

Tab. 1. Results of preference tests with alcoholic extracts of the moss *Neckera crispa* and the hepatic *Porella obtusata*. + = sprayed leaves not fed by slugs, +/– sprayed leaves partially fed by slugs, less than control, – = sprayed leaves as well fed as control.

% dry weight	Neckera crispa	Porella obtusata
1		+/–
0.5	+/–	
0.25		+
0.05	–	+/–
0.025		–

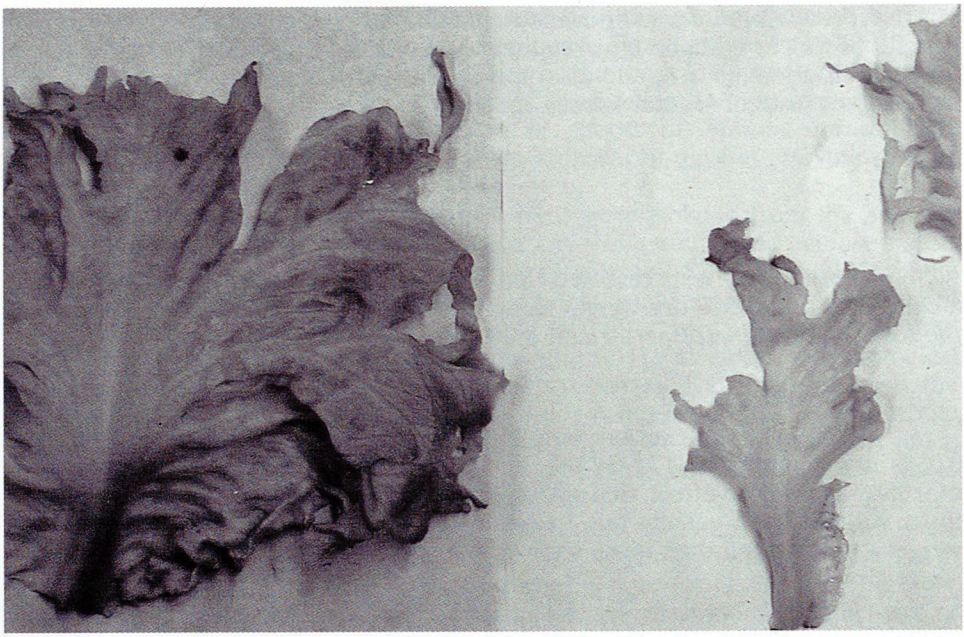

Fig. 1. Leaves of *Lactuca sativa* offered in a preference test as food for the slug Arion *lusitanicus*. Left: leaf sprayed with alcoholic extract of *Porella obtusata* (0.25% dry weight); the leaf was not touched. Right: leaf sprayed with solvent agent (1.7% ethanol); the leaf is almost eaten up over night except for the hard midrib.

Neckera crispa. The slugs absolutely avoided lettuce leaves sprayed with a concentration of 0.25% *Porella* (Fig. 1). Even at a concentration of 1%, the extracts of *Neckera crispa* showed only partial effects. Higher concentrations had a letal effect on the slugs but because of the higher alcohol contents. The activity of the low concentrations of *Porella obtusata* show significantly that the antifeedant effect is not caused by the solvent (ethanol), because the slugs fed lettuce sprayed with 2.5% ethanol without harm but did not feed on lettuce leaves sprayed with extracts with an alcohol content of 1.25%.

The experiments reveal that alcoholic bryophyte extracts, especially such of hepatics, can be successfully be used to control slugs and presumably also snails. The results could be confirmed in field experiments performed by the federal institute of agriculture (Landesanstalt für Pflanzenbau and Pflanzenschutz) in Mainz (Germany) with a bryophyte extract in the same concentration as that of *Porella obtusata*, using the commercially available product "Lebermooser" ®. The latter consists of an alcoholic extract of *Bazzania trilobata* and is sold in Germany for its antifungal effects. In field experiments, this extract proved to be as effective against snails as the best commercially available products sold for that purpose.

The high efficacy of the hepatic extracts (0.25% dry weight) require only few plant material. Furthermore, bryophyte extract is no biocide; snails and slugs are not poisoned as by other available commercial agents, and the extracts are natural compounds which are present in the nature since millions of years without

causing any damages. Thus the shield, which bryophytes developed against herbivorius animals within the long history of their evolution, can be sprayed over flowering plants.

REFERENCES

ANDO H. & MATSUO A., 1982 – Applied Bryology. *Advances in Bryology* 2: 133-224.

ASAKAWA Y., TOYOTA M., TAKEMOTO T., KUBO I. & NAKANISHI K., 1980 – Insect antifeedant secoaromadendrane-type sesquiterpenes from *Plagiochila* species. *Phytochemistry* 19: 2147-2154.

DAVIDSON A.J. & LONGTON R.E., 1987 – Acceptability of mosses as food for a herbivore, the slug *Arion hortensis*. *Symposia Biologica Hungarica* 35: 707-719.

DAVIDSON A.J., HARBORNE J.B. & LONGTON R.E.,1990 – The acceptability of mosses as food for generalist herbivores, slugs in the Arionidae. *Botanical Journal of the Linnean Society* 104: 99-113.

GERSON U., 1982 – Bryophytes and invertebrates. *in* A.J.E. Smith (ed.) *Bryophyte Ecology*. London, Chapman & Hall, pp. 291-332.

HUNECK, S., 1984 – Chemistry and biochemistry of bryophytes. *in*: R.M. Schuster, New *Manual of Bryology*, Nichinan. pp. 1-116.

JENNINGS T.J. & BARKHAM J.P., 1975 – Food of slugs in mixed deciduous woodland. *Oikos* 26: 211-231.

KUBO I., LEE I.-W. PETTEI M., PILKIEWICZ F., NAKANISHI K., 1976 – Potent army worm antifeedants from the East African *Warburgia* plants. *J. Chem. Soc. Chem. Commun*. 1976: 1013-1014.

WADA K. & MUNAKATA K., 1971 – Insect feeding inhibitors in plants. III. Feeding inhibitory activity of terpenoids in plants. *Agric. Biol. Chem*. 35: 115-118.