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Leaf-Conditioning by Microorganisms

Felix Bärlocher and Bryce Kendrick
Department of Biology, University of Waterloo, Waterloo

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Summary. Many detritus-feeders prefer dead leaves which are colonized by microorganisms-*i.e.* conditioned leaves, over freshly fallen or sterile leaves. Traditionally, this has been attributed to the build-up of microbial cells on the substrate. Two experiments show that changes in the leaf itself, brought about by microbial excretions and secretions, or by a hydrolytic agent (hot HCl), can also increase its palatability to the detritus-feeding amphipod *Gammarus pseudolimnaeus*.

Introduction

Many stream-dwelling invertebrates prefer to eat partly decomposed, rather than freshly fallen or sterile, autumn-shed leaves (Kaushik and Hynes, 1971; Mackay and Kalff, 1973; Triska, 1970). Such leaves are now described as having been "conditioned" (Cummins, 1974). Conditioned leaves may consist of much unchanged leaf material, some partly broken down leaf material, microbial excretions and secretions (*e.g.* enzymes), and microbial cells. Fungal cells alone have been shown to be both palatable and highly nutritious to *Gammarus pseudolimnaeus* Bousfield, a detritus-feeding amphipod (Bärlocher and Kendrick, 1973a, b). But the question remains: would the changes in the leaf's composition brought about by fungal attack increase palatability even in the absence of the concurrent build-up of fungal cells? To answer it, *Gammarus* was given a choice between leaf discs which had been exposed to fungal excretions and secretions, and untreated discs, and between leaf discs or pieces of filter paper treated with a hydrolyzing agent (hot HCl), and untreated discs or pieces of filter paper.

Materials and Methods

Specimens of *Gammarus pseudolimnaeus* were collected from the Credit River near Belfountain, Ontario. Dead leaves were taken from a single maple tree (*Acer saccharum* Marsh.). They were cut into discs of 1 cm diameter, leached for four days in running tap water at 12° C, dried for two days at 40° C, then stored in polyethylene bags at room temperature until needed. The two fungi used in the experiments (*Humicola grisea* Traaen and *Heliscus lugdunensis* Saccardo et Therry) had previously been isolated from maple leaves taken from the Speed River near Fergus, Ontario. They were cultured separately on sterilized maple leaf discs submerged in sterilized stream water in 1 l Erlenmeyer flasks. One of these discs colonized either by *Humicola* or *Heliscus* served as inoculum for the experiments described below.

Polyvic Millipore Filters (PVC DDWP, pore size 0.6 μm) and Scotch plastic tape were used to prepare 5 × 5 cm envelopes. Five pre-weighed leaf discs were placed in each envelope which was then completely closed with tape and afterwards sterilized with β-propiolactone (Gauger *et al.*, 1967). This first envelope was placed inside a second, slightly larger one, which was again closed with tape and then sterilized with β-propiolactone. As a result, sterile maple leaf discs were effectively enclosed in a double layer of sterile membrane filter.

Each of these envelopes was then introduced under sterile conditions into a 1 l Erlenmeyer flask containing 250 ml autoclaved stream water (10 mg $(NH_4)_2SO_4$ and 2.5 mg KH_2PO_4

added per litre) and ten gas-sterilized maple leaf discs. The flask was then inoculated with *Humicola* or *Heliscus*, or remained sterile, and was incubated for seven days at room temperature. This period was too short to allow either of the two fungi to penetrate the double layer of filter membrane (sterility was checked by plating out the contents of 10 envelopes on Malt Extract Agar) but is was shown in a similar experiment that fungal exoenzymes could freely enter the envelopes (Kilbertus *et al.*, 1973). After the incubation period the discs from inside the envelopes were used for feeding experiments with the purpose of finding out whether thee was any difference in palatability between discs which may have been exposed to excretions and secretions of either *Humicola* or *Heliscus*, and those which had not. The identification of the discs during these experiments was based on various notches and holes with which they had been marked from the beginning.

In the second set of experiments, the influence of a hydrolysing agent (hot HCl) on the palatability of leaves and filter paper (Whatman No. 1) was examined. 50 ml beakers were filled with HCl (1 N for filter paper and 2 N for leaves) or distilled water, and supplied with either 10 pre-weighed discs or 10 pre-weighed pieces of filter paper, again characterized by various holes. The beakers were put on a waterbath at 90° C (leaves for 4 hrs, filter paper for 45 min). Additional beakers with identical contents were left at room temperature. The discs and filter paper pieces were then rinsed repeatedly in distilled water and used for feeding experiments. Thus, comparisons were made between the palatability of leaves and filter paper treated by hot HCl, cold HCl, hot H_2O, and cold H_2O.

All feeding experiments were conducted in a constant temperature room at 16° C. Five replicates were used, each a glass dish (90 mm wide × 100 mm deep) containing 20 animals in 500 ml tap water. Each glass dish received the contents (discs or filter paper) of one Erlenmeyer flask or one beaker per treatment examined. The animals were allowed to feed for one day. Then, the leaves or paper pieces were sorted back into original sets, oven-dried at 40° C for two days and weighed. The weight loss of each set was compared to that of an identical one which had not been exposed to the animals. The difference was assumed to represent consumption by animals.

Results and Discussion

Table 1 shows consumption by *Gammarus* when it was given a choice between sterile leaf discs from inside sterile envelopes which had been immersed either in sterile stream water or in water inoculated with a fungus. In the two experiments conducted, discs which may have been accessible to fungal excretions and secretions were preferred over those which had not (difference significant at 5% level, Student's *t*-test).

When *Gammarus* had a choice between leaf discs treated either with hot HCl, cold HCl, hot H_2O, or cold H_2O it overwhelmingly preferred those which had been exposed to hot HCl (Table 2), that means partly hydrolyzed leaves were preferred over fresh leaves (difference between consumption of leaves treated with hot HCl and any other leaves was significant at 1% level, Duncan's Multiple Range Test).

The same preference for partly hydrolyzed substrates was shown when filter paper instead of leaves were used (Table 2). Again, the difference between the consumption of filter paper treated with hot HCl and any other treatment was significant at the 1% level (Duncan's Multiple Range Test).

It is obvious that changes in the substrate itself, caused by fungal excretions and secretions, or by a hydrolytic agent, can increase its palatability to *Gammarus*. This suggests that detritus-feeders may profit not only from the build-up of microbial cells on detritus, but also from increased digestibility of the detritus itself, presumably due to hydrolytic exoenzymes. Of interest in this respect is the demonstration of enzymes active on cellobiose in the digestive tracts of detritus-feeders which are unable to degrade cellulose (Bjarnov, 1972; Kristensen, 1972;

Table 1. Consumption by *Gammarus* of sterile maple leaves. Sterile: leaf discs from sterile envelopes immersed in sterile water. With fungus: leaf discs from sterile envelopes immersed in water inoculated with *Humicola* (Expt. 1) or *Heliscus* (Expt. 2). Each value mg eaten per day per 10 animals, based on 5 replicates, $\pm$ 95% C.L.

	Expt. 1	Expt. 2
Sterile	1.8 ± 1.1	1.8 ± 0.7
With fungus	5.8 ± 2.5	4.5 ± 1.7

Table 2. Consumption by *Gammarus* of filter paper or maple leaf discs which had been exposed to hot or cold HCl or H_2O. Each value mg eaten per day per 10 animals, based on 5 replicates, $\pm$ 95% C.L.

Treatment	Leaf discs	Filter paper
Hot HCl	15.5 ± 4.4	13.1 ± 3.3
Cold HCl	1.8 ± 0.6	0.8 ± 0.5
Hot H_2O	0.6 ± 1.7	0.5 ± 0.5
Cold H_2O	-0.9 ± 0.6	-0.4 ± 0.4

Nielsen, 1962; Luxton, 1972). Free cellobiose is very rare in nature (Pazur, 1970). By far its most important occurrence is as a subunit of cellulose, and it is an intermediate product of cellulose degradation. Microbiologists have shown that non-cellulolytic fungi and bacteria can thrive on cellulose if the substrate is attacked simultaneously by a strongly cellulolytic fungus whose activity releases an excess of degradation products (Tribe, 1966; Saitô, 1965). But scientists investigating the nutrition of detritus-feeders have ignored this possibility.

In short, we believe that leaf-conditioning by microorganisms is based on at least two crucial mechanisms: a) conversion of plant tissue into microbial materials (microbial production) and b) partial decomposition of plant tissue into subunits digestible by detritus-feeders (microbial catalysis). The relative significance of these two processes will be dictated by the substrate, environmental conditions and the organisms involved.

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Dr. Felix Bärlocher
Department of Biology
University of Waterloo
Waterloo, Ontario, Canada