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THE FATE AND EFFECTS OF INGESTED HYDROLYZABLE TANNINS IN *Porcellio scaber*

MARTIN ZIMMER[1]

Zoologisches Institut – Physiologische Ökologie
Universität zu Köln
Weyertal 119, D-50931 Köln, Germany

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Abstract–When adults of *Porcellio scaber* fed on litter prior to an artificial diet containing 5% of commercially available tannic acid, 55% of the ingested galloylglucose esters was excreted unchanged, about 25% was hydrolyzed, and 20% was oxidized during the gut passage. After reducing the counts of microorganisms in the gut of *P. scaber*, the data obtained indicated an important role of ingested platable microorganisms in hydrolyzing gallotannins. Oxidation of phenolics appeared to be mainly due to the endosymbiotic bacteria of the hepatopancreas. Microbial counts in the hindgut were strongly reduced by ingested galloylglucose esters, while gallic acid in the diet (2%) reduced the number of palatable fungi and bacteria less strikingly, and increased the total number of the gut microbiota. Hepatopancreatic bacteria were only slightly affected by ingested tannic acid, since the hepatopancreas contained only few galloylglucose esters. This may be due to the permeability of the hindgut cuticle: the cuticle of the anterior hindgut was freely permeable to gallic acid, while it was nearly impermeable to larger polyphenols. The cuticle of the posterior hindgut was permeable to only about 4% of the gallic acid present in the hindgut lumen. The results are discussed with respect to potentially harmful effects of ingested hydrolyzable tannins and their digestion in *Porcellio scaber*.

Key Words–Terrestrial isopods, Oniscidea, *Porcellio scaber*, phenolics, hydrolysis, oxidation, detoxification, hindgut cuticle, gut microbiota, hepatopancreatic bacteria.

[1]Present address: Institut für Neurobiologie, AG Zoologie und Didaktik der Biologie, Heinrich-Heine-Universität, Universitätsstr. 1, D-40225 Düsseldorf, Germany.

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INTRODUCTION

Tannins are naturally occurring water-soluble polyphenolic compounds capable of complexing proteins in vitro (Hagerman and Butler, 1991). The effects of hydrolyzable and condensed tannins on consumption and digestion in phytophagous insects have been examined in detail (summarized in Mole and Waterman, 1987; Bernays, 1981; Hagerman and Butler, 1991). Effective physiological counteractions have been described in the gut of caterpillars (e.g., Berenbaum, 1980; Martin et al., 1987). Hydrolysis of tannins may serve as a mechanism of detoxification (Bernays and Chamberlain, 1980; Hagerman and Butler, 1991). On the other hand, oxidative degradation of phenolics may lead to harmful quinones or reactive oxygen species (Appel, 1993).

In contrast to phytophagous insects, little is known about the influence of phenolic compounds in the litter on saprophagous soil animals. Although most phenolics are readily removed from the litter by leaching (Kuiters and Sarink, 1986), effects of condensed tannins–still present in the litter after leaching–on reproduction and mortality of terrestrial isopods (Isopoda: Oniscidea) have been demonstrated recently (Zimmer and Topp, 1997a). However, no data on hydrolytic detoxification or oxidative activation of hydrolyzable tannins in the gut of terrestrial isopods are available.

In phytophagous insects, the uptake of tannin components from the gut lumen may be prevented by the peritrophic envelope (Bernays and Chamberlain, 1980; Bernays et al., 1980; Barbehenn and Martin, 1992, 1994, 1995). The hindgut cuticle of terrestrial isopods (Lane, 1988) appears to have a similar composition as the peritrophic envelope in insects (Brandt et al., 1978; Stamm et al., 1978), and it apparently fulfills similar functions (cf. Fawcett, 1965).

The present study was conducted to determine the fate of ingested hydrolyzable tannins in the gut of the common terrestrial isopod, *Porcellio scaber* Latreille. Commercially available tannic acid served as a simple model compound that has previously been investigated in insects (e.g., Barbehenn and Martin, 1992, 1994).

METHODS AND MATERIALS

Gut of Terrestrial Isopods. The gut of *P. scaber* can be divided into three parts (Figure 1). Two pairs of midgut lobes (hepatopancreas) that secrete digestive fluids and absorb nutrients fill up the body cavity along the alimentary canal (Hassall and Jennings, 1975; Hames and Hopkin, 1989). The hepatopancreas is characterized by abundant endosymbiotic bacteria (Wood and Griffiths, 1988) that are involved in the digestion of cellulose (Zimmer and Topp, 1998a,b) and lignins (Zimmer and Topp, 1998c). In the hindgut, an anterior and a posterior

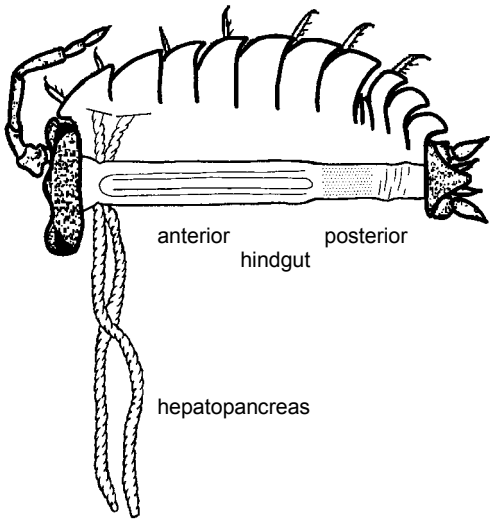

FIG. 1. Gut sections of terrestrial isopods *(Porcellio scaber)* referred to in the text.

part can be distinguished (e.g., Hassall and Jennings, 1975; Hames and Hopkin, 1989; cf. Figure 1). The anterior part is characterized by typhlosole infoldings and contains numerous microorganisms ingested with the food (Reyes and Tiedje, 1976; Zimmer and Topp, 1998b). The posterior part of the hindgut is a straight tube that can be subdivided morphologically, but was considered as one section in this study. It is in this hindgut section that bacterial proliferation takes place (Zimmer and Topp, 1998b).

Experimental Design. Individuals of *P. scaber* (Isopoda, Oniscidea) were collected in a floodplain forest near Cologne, Germany. In the laboratory, the woodlice were kept separately in small Petri dishes. Only adult specimens of about 20–30 mg fresh wt were used in experiments. All experiments were carried out at 15 °C and a photophase of 16L: 8D.

Prior to the experiments, the isopods were fed with litter from the forest in which they were captured. By this method, the natural populations of gut microbiota were expected to be maintained. Microorganisms in the guts of terrestrial isopods have been described in detail (Reyes and Tiedje, 1976; Griffiths and Wood, 1985; Ullrich et al., 1991). Populations of gut microbiota are influenced when woodlice feed on sterile artificial diet or on diet containing antibiotics (Zimmer and Topp, 1998b). Thus, to alter the microbial counts and their activity in the gut of *P. scaber,* specimens were fed with a sterile chemically defined artificial diet modified after Carefoot (1984). This diet was rich in cellulose (30%), starch (25%), and simple carbohydrates (10% sucrose, and 5% each

of glucose, lactose, and maltose). Casein (15%) served as a nitrogen source. A mineral mix (5%) was added according to Retnakaran and Beck (1968).

The diet (1 g) was mixed thoroughly with agar (0.3 g) and suspended in hot distilled water (2 ml). After cooling, the diet (pH 5.4) was kept at 4°C for up to five days. To reduce microbial counts in the gut, antibiotics [1% w/w of a mixture of oxytetracycline (Terramycin), Tegosept, Dithane and sorbic acid] were used (Sigma, St. Louis, Missouri).

Gut Microbiota. After feeding on the described food sources, some specimens were examined to determine the influence of the food source on microbial counts in the gut. Counts of platable bacteria and fungi (platable microbial counts, PMC) were determined as described in Treves and Martin (1994). Since endosymbiotic bacteria of the hepatopancreas cannot be cultivated by this plating technique (Wood and Griffiths. 1988; Zimmer, 1998), platable microorganisms can be considered as ingested microbiota. Total microbial counts (TMC) that reveal additional information on nonplatable microbiota, including hepatopancreatic endosymbionts, were estimated by the acridine orange method (Francisco et al., 1973).

Phenolics in the Diet. For studies on the degradation of hydrolyzable tannins in the gut of *P. scaber*, the artificial diet was enriched with 5% of commercially available tannic acid (Sigma), resulting in a pH value of 5.5. A budget for gallic acid was determined with a diet containing 2% gallic acid (Sigma), pH 5.4.

During the feeding experiment, artificial diet (30 mg fresh wt; 10 mg dry wt) containing tannic acid was offered to individuals of *P. scaber* for five days. Feces from the previous food source (litter or artificial diet without phenolics) were removed from the Petri dishes twice a day. The feces derived from the experimental diet were collected quantitatively twice a day and were stored at –20°C until they were lyophilized and weighed. After five days of feeding, the remaining diet was removed, lyophilized, and weighed to estimate the amount of ingested diet (dry wt). From these values the amount of ingested gallotannins could be calculated by using a standard curve of fresh diet (dry wt). As a comparison between fresh diet and diet after five days of experimental treatment indicated, changes in phenolic content that were due to chemical breakdown and autoxidation were negligible. Litter was offered as subsequent food to collect further feces derived from the tannin containing diet that were easily distinguished by their color. Data of ingested diet and egested feces allowed the estimation of consumption rates (RCR) and digestibility (AD) according to Waldbauer (1968).

Specimens of *P. scaber* were dissected to extract the described sections of the gut. After lyophilizing the samples of diet, gut contents, and feces, the gallotannins were extracted three times in 1000µl of 70% acetone containing 0.001 M ascorbic acid at 22 ± 1°C. Extracts were centrifuged (4 min; 13,600 g), and the supernatant solutions were pooled, concentrated under a stream of nitrogen,

and lyophilized. Lyophilized extracts were resolubilized in 500 µl of 23% v/v acetronitrile in double-distilled water containing 1 % acetic acid. After filtration (0.45 µm, Gelman GHP), the qualitative and quantitative determination of the compounds of tannic acid was performed by reverse-phase HPLC with a Waters 10-µm C-18 column (4.6 x 250 mm). The mobile phase was 18% v/v acetonitrile in double-distilled water containing 1% acetic acid. The flow rate of the mobile phase was 1 ml/min. Samples (25-µl aliquots) were injected with an autoinjector (Shimadzu). Peaks were detected with a Shimadzu UV-visible detector (280 nm, 0.002 AUFS) and quantified with a Shimadzu C-R4A chromatopac. After correcting peak areas for interfering substances present in control samples, the amount of phenolics was calculated from the peak area by using standard curves of tannic acid and gallic acid. In this study, the fate of four tannic acid compounds that eluted in the range of 3–10 min was noted (Figure 2a). One of these compounds (peak A, Figure 2a) was identified as gallic acid by standards. According to Barbehenn and Martin (1994), gallic acid is present in commercially available tannic acid as an impurity. The other compounds of tannic acid are considered as galloylglucose esters (cf. Barbehenn and Martin, 1992), and are named peaks B, C, and D (Figure 2a). According to Barbehenn et al. (1996), the number of galloyl groups, and thus, the molecular weight of these compounds is positively correlated with their retention time.

To determine the extent of hydrolysis of gallotannins in the gut of *P. scaber*, the ingested and egested amounts of gallic acid and galloylglucose esters were used for a calculation of the tannin budget as described in Barbehenn et al. (1996). The amount of oxidized gallotannins was estimated from the difference between the total tannic acid ingested and the sum of the percentage of gallotannins recovered and hydrolyzed. Furthermore, by measuring the amounts of soluble brown pigments in the feces of isopods fed on tannic acid (Barbehenn et al., 1996) relative data on the extent of tannin oxidation was obtained.

Permeability of Gut Cuticle. Isopods (N = 20) were fed with diet containing tannic acid as described above. An additional 10 adults fed on artificial diet without phenolics served as a control. Either the anterior hindgut and the mid-hindgut, or the mid-hindgut and the posterior hindgut were taken for the subsequent measurements. Under a dissecting microscope, these parts of the hindgut, containing the ingested artificial diet, were ligated with fine silk sutures (Barbehenn and Martin, 1995). Afterwards, a small hole (0.1 ± 0.02 mm²) was cut into the tissue of either the posterior hindgut or the ventral anterior hindgut, where there are no typhlosole canals, to expose the cuticle. To measure the permeation of compounds in the gut lumen, the prepared guts were transferred into 1 ml of an incubation solution (3 mM NaCl, 227 mM KCl, 300 mM fructose, 175 mM NaH_2PO_4, 25 mM Na_2HPO_4) (Giordana and Sacchi, 1978) that was roughly consistent with hemolymph composition (Wright et al., 1997; Ziegler and Scholz, 1997) and stirred for 2 hr at 22 ± 1°C. As controls, indicating that

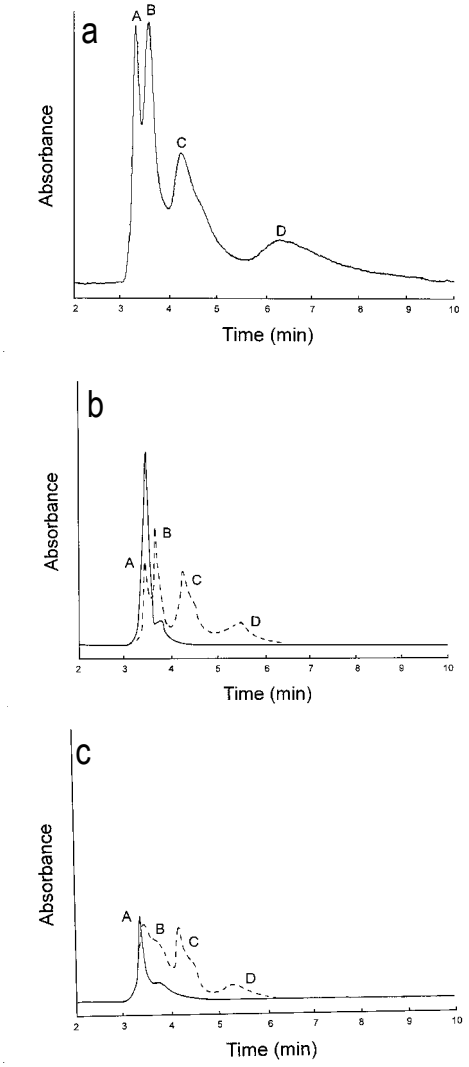

FIG. 2. Chromatogram of a tannic acid standard (a), illustrating the compounds observed by reverse-phase HPLC, and representative chromatograms of (b): the anterior hindgut (dotted line) and the surrounding incubation solution (solid line), and (c): the posterior hindgut (dotted line) and the surrounding incubation solution (solid line), after substracting control chromatograms of specimens fed on diet without tannic acid. Peak A: gallic acid; peaks B–D: galloylglucose esters. No compounds eluted before 2 min.

permeation of phenolics through the hindgut cuticle was observed, but not leaching of phenolics at the sutured ends, five gut samples without exposed cuticles were placed in the incubation solution. The incubation solutions, containing the permeated compounds of tannic acid, were frozen at –20°C and subsequently lyophilized. Phenolics were extracted from the gut contents as described above.

The permeability of the hindgut cuticle of *P. scaber* to gallic acid and different galloylglucose esters was determined as the percent of these TA compounds in the incubating solution, compared to the total amount initially present in the anterior or posterior hindgut (Figure 2b and c) after feeding on artificial diet containing tannic acid: permeability = [content in solution/(content in gut + content in solution)].

Statistics. Most of the data showed deviations from the model of normal distribution. To treat all data in the same manner, generally nonparametric statistics were used. Hence, results are presented as median (M) ± median absolute deviation (MAD). Comparison of different treatments concerning consumption and digestion of food and the formation of brown pigments were conducted by the multivariate Kruskal-Wallis H test. Pairwise differences between treatments were localized with the Mann-Whitney U test. In the case of results given in percentages, data were transformed (x' = arc sin $\sqrt{x}$) and subsequently compared as described above.

RESULTS

Effects of Tannic Acid on Consumption and Digestion. Consumption and assimilation of the offered food are given in Table 1. After foraging on litter, relative consumption rates (RCR) were reduced by feeding on artificial diet with tannic acid (TA) compared to diet without TA ($P < 0.001$). A further reduction of RCR occurred when *P. scaber* had previously fed on artificial diet ($P < 0.001$). Antibiotics did not show any effect on the consumption rate. The effects of ingested gallic acid (GA) on RCR differed only slightly from those of TA, when the isopods had fed on litter ($P < 0.1$), and no differences between the effects of GA and TA on RCR was observed in isopods fed on artificial diet (Table 1).

With respect to the approximate digestibility (AD) of the diet, ingested tannic acid did not influence *P. scaber* when the isopods had previously fed on litter. However, foraging on artificial diet significantly reduced AD when TA was ingested ($P < 0.05$). Antibiotics in the diet did not show any effects on AD.

GA in the diet strikingly increased AD compared to the corresponding TA samples ($P < 0.001$) and in comparison to isopods feeding on artificial diet without phenolics ($P < 0.01$). Again, antibiotics did not alter AD.

Effects of Tannic Acid on Gut Microbiota. Isopods that fed on artificial diet had reduced numbers of gut microbiota compared to those that fed on litter, while

TABLE 1. CONSUMPTION (RCR) AND DIGESTIBILITY (AD) OF DIFFERENT FOOD SOURCES BY YOUNG ADULT (20–30 mg) *Porcellio scaber* [a]

Preexperimental diet	Content of phenolics in diet[b]	RCR	AD
Leaf litter	0 %	0.20 ± 0.10a	30 ± 20a
Leaf litter	5 % TA	0.09 ± 0.05b	23 ± 14a
Artificial diet[b]	5 % TA	0.03 ± 0.02c	17 ± 7b
Diet[b] + antibiotics	5 % TA	0.03 ± 0.02c	10 ± 9b
Leaf litter	2 % GA	0.04 ± 0.02d	65 ± 5c
Artificial diet[b]	2 % GA	0.02 ± 0.01c	86 ± 9d
Diet[b] + antibiotics	2 % GA	0.02 ± 0.01c	78 ± 13d

[a]TA: commercially available tannic acid; GA: gallic acid. Data are presented as median ± MAD ($N = 9$). The results of statistical comparison ($\alpha = 0.05$) are indicated by letters.
[b]Artificial diet after Zimmer and Topp (1998b).

the number of microorganisms in the hepatopancreas was not changed (Table 2). Antibiotics in the diet resulted in further decrease of microbial counts in the hindgut ($P < 0.001$), and in addition, reduced the number of hepatopancreatic bacteria ($P < 0.05$).

By contrast, gallic acid in the diet increased the number of these bacteria ($P < 0.01$), while tannic acid slightly reduced microbial counts of the hepatopancreas ($P < 0.05$). In the hindgut, tannic acid reduced the number of platable microbiota ($P < 0.001$). In the anterior hindgut, total microbial counts were

TABLE 2. INFLUENCE OF ARTIFICIAL DIET (ad/), ARTIFICIAL DIET WITH 1% ANTIBIOTICS (ad/+), 5% TANNIC ACID (ad/TA) OR 2% GALLIC ACID (ad/GA) ON NUMBERS OF MICROBIOTA IN MIDGUT LOBES (Hepatopancreas) AND ANTERIOR AND POSTERIOR HINDGUT OF *Porcellio scaber* [a]

	Total microbial counts			Platable microbial counts		
Food	Hepatopancreas	Anterior hindgut	Posterior hindgut	Hepatopancreas	Anterior hindgut	Posterior hindgut
ad/[b]	99a	71b	109a	-	13b	73b
ad/+[c]	84b	32c	47b	-	3c	12c
ad/TA[c]	72b	151d	57b	-	6c	20c
ad/GA[c]	145c	306e	173c	-	38b	76b

[a]Data of microbial counts after feeding the above-mentioned diets are given as the percent of the mean microbial counts of isopods fed on leaf litter ($N = 15$). The results of statistical comparison of absolute numbers of microbiota ($\alpha = 0.05$) are indicated by letters (microbial counts of isopods fed on litter are indicated as a).
[b]As a proportion of litter-feeding isopods.
[c]As a proportion of ad/-feeding isopods.

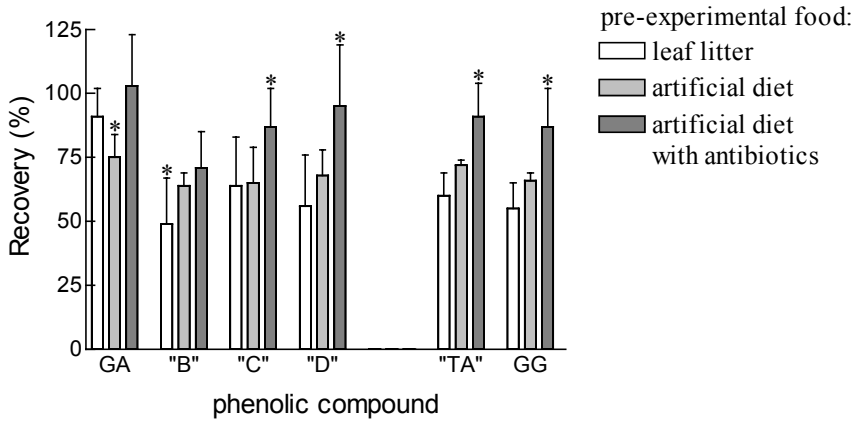

FIG. 3. Recovery of compounds of tannic acid (TA) from the feces of *Porcellio scaber* having fed on TA-containing artificial diet after different pre-experimental diets. GA: gallic acid., B–D: galloylglucose esters (cf. Figure 2a); TA: commercially available tannic acid, including GA; GG: total of galloylglucose esters B–D. Asterisks mark differences between one sample and the two of the comparable samples ($\alpha = 0.05$; $N = 10$).

increased by feeding on tannic acid and gallic acid ($P < 0.01$). The latter compound led to increased TMC in the posterior hindgut, too ($P < 0.001$), while TMC significantly decreased after feeding on tannic acid ($P < 0.001$). Platable bacteria and fungi in the hindgut were affected less strikingly by gallic acid than by tannic acid (Table 2).

Degradation of Tannic Acid during Gut Passage. When *P. scaber* had fed on litter prior to the experiments, about 60 ± 9% of the ingested tannic acid could be recovered from the feces. However, the observed TA compounds showed remarkable differences concerning the recovery from the feces (Figure 3). About 25% was hydrolyzed in the gut of *P. scaber.*

The distribution of TA compounds in the gut of *P. scaber* is shown in Table 3. The hepatopancreas contained about 12% of the ingested GA, but only small amounts of peak B (Figure 2a). No other compounds of tannic acid could be detected in the hepatopancreas. The anterior and posterior hindguts did not differ in their GA content, but the latter gut section did not contain any peak B, in contrast to the anterior hindgut. The contents of the other galloylglucose esters (peaks C and D in Figure 2a) were similar in both hindgut sections. Foraging on artificial diet reduced the extent of hydrolysis of TA to about 16% of the ingested amount (Figure 3).

The gallic acid content of the hepatopancreas (Figure 4) increased with decreasing counts of ingested (= platable) microorganisms in the hindgut [isopods fed on artificial diet (Table 2; $P < 0.05$)]. A similar effect of pre-ex-

TABLE 3. PERMEABILITY OF VARIOUS REGIONS OF HINDGUT CUTICLE TO COMPOUNDS OF TANNIC ACID [a]

Hindgut	GA (%)	Peak B (%)	Peak C (%)	Peak D (%)
Anterior	85 ± 10a	20 ± 5c	0e	0e
Posterior	4 ± 3b	2 ± 2d	0e	0e

[a]GA: gallic acid; peaks B, C, and D: galloylglucose esters. Data are given as median ± MAD ($N = 9$) of the percent recovered from an incubation solution to the amount of the compounds initially present in the corresponding section of the hindgut. The results of statistical comparison ($\alpha = 0.05$) are indicated by letters.

perimental artificial diet was observed concerning peak B ($P < 0.01$). None of the other galloylglucose esters of tannic acid were detected in the hepatopancreas of *P. scaber* after feeding on artificial diet prior to the experiment. The amount of recovered galloylglucose esters in the hindgut increased with decreasing microbial counts in the gut ($P < 0.05$). However, no peak B was detected in the posterior hindgut. When the isopods fed on diet containing antibiotics prior to the experiments, all of the ingested GA (103 ± 20%) was excreted via the feces (Figure 3). Only 6% of ingested TA was hydrolyzed in this case.

The content of gallic acid and peak B in the hepatopancreas (Figure 4) strongly increased with decreasing counts of bacteria in the hepatopancreas [isopods fed on antibiotics (Table 2; $P < 0.01$)]. Galloylglucose esters other than peak B did not enter the hepatopancreas. Feeding on antibiotics prior to the experiment resulted in a significantly increased content of GA in the anterior hindgut ($P < 0.01$), but caused weak changes in the contents of galloylglucose esters in this hindgut section: only the content of peak B significantly decreased ($P < 0.01$). In contrast to pre-experimental food without antibiotics, about one fourth of the ingested peak B was found in the posterior hindgut. Additionally, a higher proportion of other galloylglucose esters was recovered ($P < 0.05$).

The oxidation of phenolics during the gut passage was indicated by brown pigments present in the feces of isopods feeding on tannic acid. While there were no brown pigments in control diet without phenolics and in feces from isopods fed on this diet, small amounts of brown pigments were detected in the diet containing TA (Figure 5). Significantly higher absorbance was measured in the extract of feces obtained from isopods that had fed on litter ($P < 0.01$) and those that had fed on artificial diet prior to the experiment ($P < 0.01$). After feeding on antibiotics, the feces contained significantly less brown pigments ($P < 0.01$), but still had higher absorbance than was obtained from the diet alone ($P < 0.05$).

Permeability of Hindgut Cuticle. The anterior and posterior hindgut differed remarkably in terms of the permeability of their cuticle (Table 3). While nearly all (75–95%) of the gallic acid initially present in the anterior hindgut

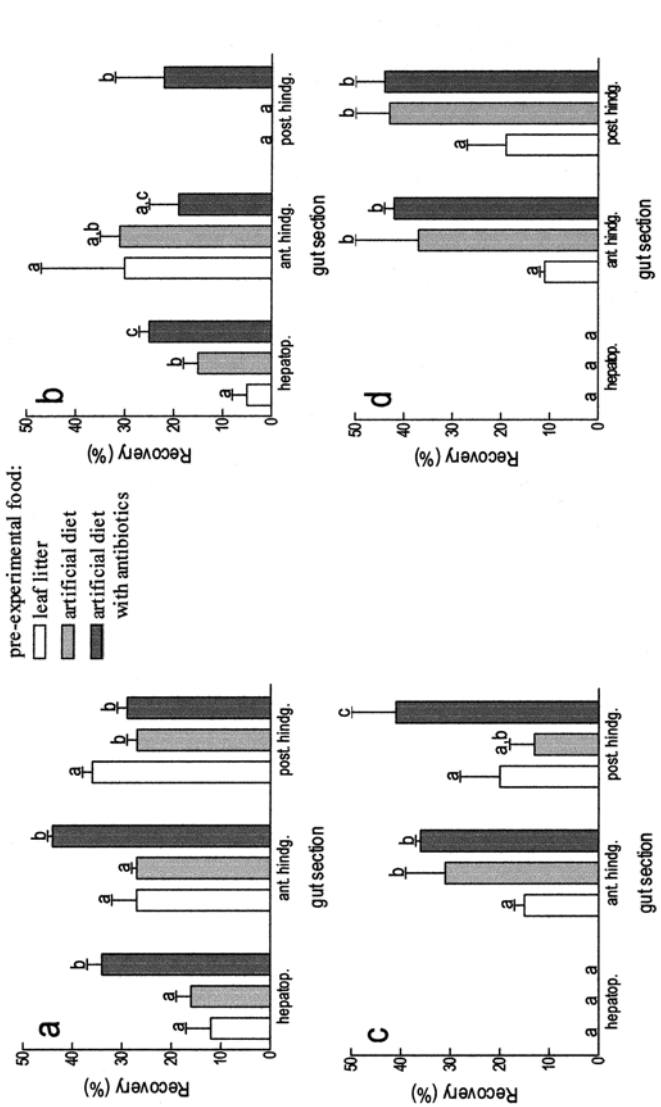

FIG. 4. Recovery of tannic acid compounds (cf. Fig. 2a) (a): gallic acid; (b): peak B; (c): peak C; (d): peak D from the gut of *Porcellio scaber* (hepatop.: midgut lobes; ant. hindg.: anterior hindgut; post. hindg.: posterior hindgut) fed on artificial diet containing 5% tannic acid (TA). Data are presented as median ± MAD of the percent of recovery of the ingested amount ($N = 9$). The results of statistical comparison (comparing samples of the same sections of the gut; $\alpha = 0.05$) are indicated by letters.

FIG. 5. Brown pigments, indicating phenol oxidation, in tannic acid-containing diet and the feces of *Porcellio scaber* having fed this food source after different preexperimental diets. Data are presented as mean ± MAD of relative absorbance (λ = 420 nm) per mg (N = 10). The results of statistical comparison ($\alpha = 0.05$) are indicated by letters.

was found in the surrounding incubation solution, significantly less of peak B permeated the anterior hindgut cuticle of *P. scaber* than gallic acid, and higher molecular weight galloylglucose esters did not at all pass through the anterior hindgut cuticle.

Only 2–10% of the gallic acid present in the posterior hindgut permeated the cuticle of this hindgut section. Little of peak B (0–10%) was found in the solution surrounding the posterior hindgut. The higher molecular weight galloylglucose esters of peaks C and D did not permeate the cuticle of the posterior hindgut.

DISCUSSION

Tannins mainly tend to adversely affect species that do not feed on plants containing large quantities of phenolics (Bernays, 1981). Terrestrial isopods are not confronted with high levels of phenolic compounds in their natural food, because many kinds of phenolics are removed from leaf litter by leaching (Kuiters and Sarink, 1986). In particular, gallotannins are present in overwintered litter during the following spring and summer at only low concentrations of 0.2–2.0 mg/g, while total phenolics can be found at concentrations of 1–10 mg/g (Zimmer, 1998). Thus, *P. scaber* can be expected not to have evolved specific mechanisms for counteracting potentially harmful effects of tannins.

Condensed tannins in leaf litter (2–5 mg/g) caused increased mortality of *P. scaber* (Zimmer and Topp, 1997a). Moreover, the development of juvenile *P. scaber* is affected by condensed tannins in the food (Zimmer, unpublished data).

Little is known about the effects of hydrolyzable tannins on isopods. Tannic acid is thought to be toxic to many invertebrates, but not antidigestive (Bernays, 1981). Alkalinity in the gut of many insects can reduce the protein-precipitating effects of tannins (Berenbaum, 1980; Martin and Martin, 1983) and, consequently, their digestibility-reducing features. Furthermore, surfactants in the gut of phytophages prevent tannin binding of digestive enzymes in vivo (Martin et al., 1987; DeVeau and Schultz, 1992). In terrestrial isopods, the gut content is slightly acidic, with a pH level of about 6 (Hartenstein, 1964; Zimmer and Topp, 1997b), but surfactants are present in *P. scaber* (Zimmer, 1997). Since gallic acid, as a product of gallotannin hydrolysis, may be harmless to some phytophagous insects (Bernays and Chamberlain, 1980; Hagerman and Butler, 1991), hydrolysis of gallotannins might serve as a mechanism of detoxification in isopods also.

Deleterious effects of phenolics may be initiated by oxidation (Appel, 1993). On the other hand, enzymatic oxidation of phenolic compounds is required for the degradation of lignin (Ander and Eriksson, 1976; Sinsabaugh and Linkins, 1987; Breznak and Brune, 1994), and this is important with respect to cellulose digestion by saprophagous animals (Ljungdahl and Eriksson, 1985; Breznak and Brune, 1994). Consequently, despite the potentially harmful effects of oxidation products, phenol oxidation can be expected in isopods.

Tannin-Microbiota Interactions. The RCR of *P. scaber* fed on artificial diet (0.20) was similar to that of adults of similar size feeding on poplar litter (0.25–0.34) (Wieser, 1965). Tannic acid and gallic acid significantly reduced the consumption rate of *P. scaber*. No determination was made as to whether this was the result of feeding deterrence or some postingestive feedback mechanism. In some phytophagous insects, similar effects of hydrolyzable tannins on RCR have been observed (summarized in Mole and Waterman, 1987). In saprophagous invertebrates, leaching of phenolics increased consumption of litter (Cameron and LaPoint, 1978; Poinsot-Balaguer, 1993). In contrast to the present results on a saprophagous isopod, TA and GA stimulated feeding in several phytophagous species, although they deterred it in others (Bernays et al., 1980).

When *P. scaber* had been feeding on litter prior to the experiment, the observed digestibility did not show an influence of ingested tannic acid. However, after reducing microbial counts in the anterior hindgut by pre-experimental artificial diet, AD significantly decreased, indicating an important role of gut microbiota in digesting food (Hassall and Jennings, 1975; Reyes and Tiedje, 1976; Griffiths and Wood, 1985; Kukor and Martin, 1986). In contrast to tannic acid, gallic acid only slightly reduced, or even increased microbial counts in the gut and the hepatopancreas of *P. scaber*. Hence, antimicrobial effects of hydrolyzable tannins are mainly due to galloylglucose esters. Furthermore, ingested gallic acid increased digestibility, even if the woodlice had previously fed on antibiotics. Consequently, TA can be expected to exert stronger effects on saprophagous isopods than GA.

Hydrolysis of galloylglucose esters results in GA and glucose. Probably, these smaller compounds are drawn to the resorptive hepatopancreas together with fluids containing other products of digestion (Hames and Hopkin, 1989). By contrast, galloylglucose esters that may be more harmful than gallic acid are excluded from entering the hepatopancreas. Presumably, the hindgut cuticle serves as a filter in preventing the transport of these phenolics to the hepatopancreas (see below). While ingested tannic acid reduced the number of microorganisms in the hepatopancreas, GA led to increased microbial counts in the midgut lobes. Considering the potential importance of the hepatopancreatic bacteria in digestive processes (Zimmer and Topp, 1998a–c), excluding galloylglucose esters from entering the hepatopancreas leads to better conditions for food utilization. Consequently, the increase in the number of hepatopancreatic bacteria after ingesting GA appears to be correlated with the increased AD of the diet.

Compared to the results of other studies that have measured the fate of ingested gallotannins in insects, *P. scaber* is intermediate between insects that pass gallotannins through the gut unchanged (Barbehenn and Martin, 1992) and insects that extensively oxidize (Barbehenn and Martin, 1994) or hydrolyze gallotannins (Bernays and Chamberlain, 1980). Hydrolysis of hydrolyzable tannins occurred in the gut of *P. scaber* by microorganisms that could be partially eliminated by antibiotics. For the detoxification of tannins, extensive hydrolysis is required, resulting in nontoxic products, e.g., gallic acid and glucose (cf. Barbehenn et al., 1996). The number of platable (ingested) microorganisms in the hindgut was reduced by feeding on artificial diet in the experiment. Hence, under natural conditions, hydrolysis of gallotannins may be more extensive than was observed in the present study. In woodlice containing high numbers of gut microbiota derived from feeding on litter, hydrolysis of galloylglucose esters resulted in a high level of gallic acid in the anterior hindgut.

The net production of brown pigments in the feces when isopods were fed diet containing TA clearly indicates phenol oxidation in the gut of *P. scaber*. A reduction of platable microorganisms in the hindgut by feeding on artificial diet prior to the experiment did not change the amount of brown pigments in the feces. Thus, phenol oxidation does not seem to depend on ingested bacteria or fungi. By contrast, antibiotics in the diet that reduced the number of bacteria in the hepatopancreas resulted in strongly decreased phenol oxidation. Similar results were obtained from feeding experiments for the estimation of gallic acid digestion. After feeding on litter prior to the experiment, 15–35% of the ingested GA could be recovered from the feces. The difference was assumed to have been oxidized or absorbed. Similar values (15–25%) were obtained after feeding on artificial diet prior to the experiment. Presumably, the reduced number of hindgut microbiota did not influence the recovery of gallic acid. Antibiotics led to 50–80% recovery of ingested GA. In this case, in addition to the platable

microorganisms in the hindgut, microbial counts of the hepatopancreas were reduced. These results suggest an involvement of the hepatopancreatic bacteria in phenol oxidation. By contrast, hydrolysis of gallotannins is mainly brought about by microorganisms ingested with the food.

The terrestrial isopod *Oniscus asellus* Linnaeus is capable of oxidatively metabolizing several phenolic compounds to CO_2 (Neuhauser and Hartenstein, 1976). The authors suggested that the necessary enzymatic activity is attributable to "the gut flora or other internal microbes, . . . since complete catabolism of aromatics has been obtained only for microbes" (Neuhauser and Hartenstein, 1976). Zimmer and Topp (1998c) drew similar conclusions concerning the oxidation of phenolics by hepatopancreatic bacteria in *P. scaber*.

Protective Function of Hindgut Cuticle. In many insects, a membranous peritrophic envelope is supposed to assist digestive processes by allowing compartmentalization of the gut lumen (Terra et al., 1979; Santos and Terra, 1986). Possibly, in terrestrial isopods the hindgut cuticle and an ectocuticular space (Holdich and Mayes, 1975) that is formed by numerous "microvilli-like structures" of the epithelial cells (Lane, 1988) have similar functions. Given that the hindgut cuticle is selectively permeable to certain compounds, fluids containing the products of digestion will be compartmentalized from the gut lumen where digestion takes place, and nutrients can be transported to the resorptive regions of the hepatopancreas via the ectocuticular space or by absorbing nutrients into the epithelial cells of the anterior hindgut (cf. Lane, 1988) and directly passing nutrients to the hepatopancreas via the hemolymph. By contrast, Hames and Hopkin (1989) described gut fluids containing digested nutrients being drawn to the hepatopancreas via the typhlosole canals. In this case, water-soluble compounds of the food would he transported to the hepatopancreas. However, only gallic acid and peak B were detected in these midgut lobes, while none of the higher molecular weight galloylglucose esters was found there. These results are in accord with the permeability of the hindgut cuticle towards TA compounds. Obviously, the hindgut cuticle–besides allowing compartmentalization–serves as a protective filter in keeping galloylglucose esters inside the hindgut lumen, while exhibiting permeability to gallic acid. In some phytophagous insects, the peritrophic envelope serves as a barrier to tannic acid (Barbehenn and Martin, 1992, 1994). These authors proved that the peritrophic envelope of lepidopteran larvae was permeable to gallic acid, but nearly impermeable to galloylglucose esters (Barbehenn and Martin, 1992, 1994). However, the containment of gallotannins inside the gut lumen is not sufficient to protect tannin-sensitive species from the impact of tannins (Barbehenn and Martin, 1994). Gallic acid, which freely penetrated the hindgut cuticle of *P. scaber,* has beneficial effects on the growth of tree-feeding insects (Bernays et al., 1983) and probably is nontoxic to isopods. Toxic effects of galloylglucose esters are prevented by surfactants in the hindgut fluids of *P. scaber* (Zimmer, 1997).

CONCLUSIONS

The present results show that ingested galloylglucose esters are kept inside the endocuticular gut lumen. In contrast to galloylglucose esters, gallic acid penetrates the hindgut cuticle and is transported to the hepatopancreas, where it supports the maintenance of high population densities of hepatopancreatic bacteria involved in cellulose digestion (Zimmer and Topp, 1998a,b) and phenol oxidation (Zimmer and Topp, 1998c; this study). In the hindgut lumen, galloylglucose esters decrease the number of ingested microorganisms thought to be important in digestion (Hassall and Jennings, 1975; Reyes and Tiedje, 1976; Griffiths and Wood, 1985; Kukor and Martin, 1986) and hydrolysis of gallotannins (this study). In phytophagous insects, inhibitory effects on gut microbiota have been suspected to be the main reason for the impact of tannins (Martin et al., 1987). The present results suggest that similar mechanisms are important in terrestrial isopods.

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REFERENCES

ANDER, P., and ERIKSSON, K.-E. 1976. The importance of phenol oxidase activity in lignin degradation by the white-rot funguis *Sporotrichum pulveruletum*. *Arch. Microbiol.* 109:1-8.

APPEL, H. 1993. Phenolics in ecological interactions: The importance of oxidation. *J. Chem. Ecol.* 19:1521-1552.

BARBEHENN, R.V., and MARTIN, M.M. 1992. The protective role of the peritrophic membrane in the tannin-tolerant larvae of *Orgyia leucostigma* (Lepidoptera). *J. Insect Physiol.* 38:973-980.

BARBEHENN, R.V., and MARTIN, M.M. 1994. Tannin sensitivity in larvae of *Malacosoma disstria* (Lepidoptera): Roles of the peritrophic envelope and midgut oxidation. *J. Chem. Ecol.* 20:1985-2001.

BARBEHENN, R.V., and MARTIN, M.M. 1995. Peritrophic envelope permeability in herbivorous insects. *J. Insect Physiol.* 41:303-311.

BARBEHENN, R.V., MARTIN, M.M., and HAGERMAN, A.E. 1996. A reassessment of the roles of the peritrophic envelope and hydrolysis in protecting polyphagous grasshoppers from ingested hydrolyzable tannins. *J. Chem. Ecol.* 22:1911-1929.

BERENBAUM, M. 1980. Adaptive significance of midgut pH in larval Lepidoptera. *Am. Nat.* 115:138-146.

BERNAYS, E.A. 1981. Plant tannins and insect herbivores: an appraisal. *Ecol. Entomol*. 6:353-360.

BERNAYS, E.A, and CHAMBERLAIN, D.J. 1980. A study of tolerance of ingested tannin in *Schistocera gregaria*. *J. Insect Physiol.* 26:415-420.

BERNAYS, E.A, CHAMBERLAIN, D.J, and MCCARTHY, P. 1980. The differential effect of ingested tannic acid on different species of Acridoidea. *Entomol. Exp. Appl.* 28:158-166.

BERNAYS E. A., CHAMBERLAIN D. J. and WOODHEAD S. 1983. Phenols as nutrients for a phytophagous insect *Anacridium melanorhodon*. *J. Insect Physiol.* 29:535-539.

BRANDT, C.R., ADANG, M.J., and SPENCE, K.D. 1978. The peritrophic membrane: Ultrastructural analysis and function as a mechanical barrier to microbial infection in *Orgyia pseudotsugata*. *J. Invert. Path.* 32:12-24.

BREZNAK, J.A, and BRUNE, A. 1994. Role of microorganisms in the digestion of lignocellulose by termites. *Annu. Rev. Entomol.* 39:453-487.

CAMERON, G.N., and LAPOINT, T.W. 1978. Effects of tannins on the decomposition of Chinese Tallow leaves by terrestrial and aquatic invertebrates. *Oecologia* 32:349-366.

CAREFOOT, T.H. 1984. Studies on nutrition of the supralittoral isopod *Ligia pallasii* using chemically defined artificial diets. *Comp. Biochem. Physiol.* 79A:655-665.

DEVEAU, E. J. I., and SCHULTZ, J. C. 1992. Reassassment of interaction between gut detergents and tannins in Lepidoptera and significance for Gypsy Moth larvae. *J. Chem. Ecol.* 18:1437-1453.

FAWCETT, D.W. 1965. Surface specializations of absorbing cells. *J. Histochem. Cytochem.* 13:75-91.

FRANCISCO, D.E., MAH, R.A., and RABIN, A.C. 1973. Acridine orange-epifluorescence technique for counting bacteria in natural waters. *Trans. Amer. Micros. Soc.* 93:416-421.

GIORDANA, B., and SACCHI, F. 1978. Cellular ionic concentrations in the midgut of two larvae of Lepidoptera *in vivo* and *in vitro*. *Comp. Biochem. Physiol.* 59A:17-20.

HAGERMAN, A.E.,and BUTLER, L.G. 1991. Tannins and lignins, pp. 355-388, *in* Rosenthal GA, Berenbaum MR (eds). Herbivores: Their Interactions with Secondary Plant Metabolites–I: The Chemical Participants, 2nd ed. Academic press, New York.

HAMES, C.A.C., and HOPKIN, S.P. 1989. The structure and function of the digestive system of terrestrial isopods. *J. Zool. Lond.* 217:599-627.

HARTENSTEIN, R. 1964. Feeding, digestion, glycogen, and the environmental conditions of the digestive system in *Oniscus asellus*. *J. Insect Physiol.* 10:611-621.

HASSALL, M., and JENNINGS, J.B. 1975. Adaptive features of gut structure and digestive physiology in the terrestrial isopod *Philoscia muscorum* (Scopoli 1763). *Biol. Bull.* 149:348-364.

HOLDICH, D.M., and MAYES, K.R. 1975. A fine-structural re-examination of the so-called 'midgut' of the isopod *Porcellio*. *Crustaceana* 29:186-192.

KUITERS, A.T., and SARINK, H.M. 1986. Leaching of phenolic compounds from leaf and needle litter of several deciduous and coniferous trees. *Soil Biol. Biochem.* 18:475-480.

KUKOR, J.J., and MARTIN, M.M. 1986. The effect of acquired microbial enzymes on assimilation efficiency in the common woodlouse, *Tracheoniscus rathkei*. *Oecologia* 69:360-366.

LANE, R. L. 1988. The digestive system of *Porcellio scaber* Latreille, 1804 (Isopoda, Oniscoidea): histology and histochemistry. *Crustaceana* 55:113-128.

LJUNGDAHL, L.G., and ERIKSSON, K.-E. 1985. Ecology of microbial cellulose degradation. *Adv. Microb. Ecol.* 8:237-299.

MARTIN, J.S.,and MARTIN, M.M. 1983. Tannin assays in ecological studies. *J. Chem. Ecol.* 9:285-294.

MARTIN, J.S., MARTIN, M.M., and BERNAYS, E.A. 1987. Failure of tannic acid to inhibit digestion or reduce digestibility of a plant protein in the gut fluids of insect herbivores: Implications for theories of plant defense. *J. Chem. Ecol.* 13:605-621.

MOLE, S., and WATERMAN, P.G. 1987. Tannins as antifeedants to mammalian herbivores–still an open question?, pp. 572-587, *in* Waller, G.C. (ed). Allelochemicals Role in Agriculture and Forestry. American Chemical Society, Washington, D.C.

NEUHAUSER, E., and HARTENSTEIN, R. 1976. Degradation of phenol, cinnamic acid and quinic acid in the terrestrial crustacean, *Oniscus asellus*. *Soil Biol. Biochem.* 8:95-98.

POINSOT-BALAGUER, N., RACON, L., SADAKA, N., and LE PETIT, J. 1993. Effects of tannin compounds on two species of Collembola. *Eur. J. Soil Biol.* 29:13-16.

RETNAKARAN, A., and BECK, S.D. 1968. Amino acid requirements and sulfuramino acid metabolism in the pea aphid, *Acyrthosiphon Pisum* (Harris). *Comp. Biochem. Physiol.* 24A:611-619.

REYES, V.G., and TIEDJE, J.M. 1976. Ecology of the gut microbiota of *Tracheoniscus rathkei* (Crustacea, Isopoda). *Pedobiologia* 16:67-74

SANTOS, C.D., and TERRA, W.R. 1986. Distribution and characterization of oligomeric digestive enzymes from *Erinnyisello* larvae and inferences concerning secretory mechanisms and the permeability of the peritrophic membrane. *Insect Biochem.* 16:691-700.

SINSABAUGH, R.L., and LINKINS, A.E. 1987. Inhibition of the *Trichoderma viride* cellulase complex by leaf litter extracts. *Soil Biol. Biochem.* 19:719-725.

STAMM, B., D'HAESE, J., and PETERS, W. 1978. SDS gel electrophoresis of proteins and glycoproteins from peritrophic membranes of some Diptera. *J. Insect Physiol.* 24:1-8.

TERRA, W.R., FERREIRRA, C., and DE BRANCHI, A.G. 1979. Distribution of digestive enzymes among the endo- and ectoperitrophic spaces and the midgut cells of *Rhynchosciara* and its physiological significance. *J. Insect Physiol.* 25:487-494.

TREVES, D.S., and MARTIN, M.M. 1994. Cellulose digestion in primitive hexapods: effect of ingested antibiotics on gut microbial populations and gut cellulase levels in the firebrat, *Thermobia domestica* (Zygentoma, Lepismatidae). *J. Chem. Ecol.* 20:2003-2020.

ULLRICH, B., STORCH, V., and SCHAIRER, H. 1991. Bacteria on the food, in the intestine and on the feces of the woodlouse *Oniscus asellus* (Crustacea, Isopoda). *Pedobiologia* 35:41-51.

WALDBAUER, G.P. 1968. The consumption and utilization of food by insects. *Adv. Insect Physiol.* 5:229-289.

WIESER, W. 1965. Untersuchungen über die Ernährung und den Gesamtstoffwechsel von *Porcellio scaber* (Crustacea: Isopoda). *Pedobiologia* 5:304-331.

WOOD, S., and GRIFFITHS, B.S. 1988. Bacteria associated with the hepatopancreas of the woodlice *Oniscus asellus* and *Porcellio scaber* (Crustacea, Isopoda). *Pedobiologia* 31:89-94.

WRIGHT, J. C., O'DONNELL, M. J., and SAZGAR, S. 1997. Haemolymph osmoregulation and the fate of sodium anc chloride during dehydration in terrestrial isopods. *J. Insect Physiol.* 43:795-807.

ZIEGLER, A., and SCHOLZ, F. H. E. 1997. The ionic hemolymph composition of the terrestrial isopod *Porcellio scaber* Latr. during molt. *J. Insect Physiol.* 43:536-542.

ZIMMER, M. 1997. Surfactants in the gut fluids of *Porcellio scaber* (Isopoda: Oniscidea), and their interactions with phenolics. *J. Insect Physiol.* 43:1009-1014.

ZIMMER, M. 1998. Wechselwirkungen zwischen Vertretern der saprophagen Bodenmakrofauna und saprotrophen Mikroorganismen. Cuvillier, Göttingen.

ZIMMER, M., and TOPP, W. 1997a. Does leaf litter quality influence population parameters of the common woodlouse, *Porcellio scaber* (Crustacea: Isopoda)?. *Biol. Fertil. Soils* 24:435-441.

ZIMMER, M., and TOPP, W. 1997b. Homeostatic responses in the gut of *Porcellio scaber* (Isopoda: Oniscidea) otimize litter degradation. *J. Comp. Physiol.* 167:582-585.

ZIMMER, M., and TOPP, W. 1998a. Do woodlice (Isopoda: Oniscidea) produce endogenous cellulases? *Biol. Fertil. Soils* 26:155-156.

ZIMMER, M., and TOPP, W. 1998b. Microorganisms and cellulose digestion in the gut of *Porcellio scaber* (Isopoda: Oniscidea). *J. Chem. Ecol.* 24:1397-1408.

ZIMMER, M., and TOPP, W. 1998c. Nutritional biology of terrestrial isopods (Isopoda: Oniscidea): Copper revisited. *Isr. J. Zool*. 44:453-462.