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Effects of green algae and napa cabbage on life-history parameters and gut microflora of *Archegozetes longisetosus* (Acari: Oribatida) under laboratory conditions

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Abstract: We compared the effect of green algae (dominated by *Protococcus* sp.) and the earlier studied napa cabbage on the life-history parameters and gut microflora of the oribatid mite *Archegozetes longisetosus* (a chelicerate model organism). Napa cabbage contained more crude ash, protein, and crude fibre than green algae, but *A. longisetosus* developed better on the latter food, displaying higher fertility, lower mortality of offspring and shorter development than on napa cabbage. The gut microflora of *A. longisetosus* depended on the kind of food and developmental stage of this mite. The adults fed with napa cabbage had more abundant and more active microflora than those fed with green algae, whereas in the tritonymphs the microflora was more abundant when they were fed with green algae, and was more active in the group fed with napa cabbage. Irrespective of the treatment, the highest percentage of the isolated bacteria were gram-positive bacilli or gram-negative bacteria, but the mites feeding on *Protococcus* sp. contained no cocci, whereas those fed with cabbage had no gram-positive bacteria.

Keywords: oribatid mites, development, fertility, mortality, microflora, *Archegozetes longisetosus*

INTRODUCTION

Archegozetes longisetosus Aoki, 1965 is a mite relatively easy to rear in laboratory conditions and therefore it has been used as a chelicerate model organism in several laboratories worldwide (Heethoff et al. 2013). It is a panphytophagous species, as demonstrated by the gut content analyses of individuals collected from the field (Haq 1982; Xavier & Haq 2007). In the laboratory experiments, *A. longisetosus* was fed with different types of food, including moss, green algae, lichens, tree bark from bird cherry (*Prunus padus* L.), decomposed leaves and twigs, pollen of lodge-

pole pine (*Pinus contorta* Dougl. ex Loud.), plant debris, corn flour, mould growing on a substrate, filter paper, yeast, and different species of fungi (Haq 1978, 1982; Seniczak 1998; Estrada-Venegas et al. 1999; Smrž & Norton 2004). Most often, however, it was fed with unicellular green algae (mainly *Protococcus* sp.) (Honciuc 1996; Seniczak 1998; Seniczak & Seniczak 2002; Alberti et al. 2003, 2011; Smrž & Norton 2004; Köhler et al. 2005; Heethoff et al. 2007; Remén et al. 2010). Napa cabbage [*Brassica rapa* L. subsp. *pekinensis* (Lour.) Hanelt] was used as food (also known as "Chinese cabbage") in a laboratory experiment on the effect of cadmium on the development, life-history parameters, and gut microflora of *A. longisetosus* (Seniczak et al. 2009).

The type of food affects not only the development of oribatid mites (Haq 1978; Haq & Adolph 1980; Seniczak 1998; Estrada-Venegas et al. 1999; Seniczak & Seniczak 2002), but also the quality and quantity of their gut microflora. For example, the poorest gut microflora was found in lichenophagous species, which eat only the thallus of lichens, boring holes in it, irrespective of what species of lichen they fed on. A rather poor microflora was also found in xylophagous and panphytophagous oribatid mites, whereas a rich microflora was present in microphytophagous species, and in all these groups the gut microflora varied with the type of food that was offered to the mites (Stefaniak & Seniczak 1976, 1983).

The variety of food types offered to *A. longisetosus* in different laboratories raises a question: how food affects these mites, their life history parameters and the gut microflora? While green algae used in many laboratories were collected from the local environment in different parts of the world, it is difficult to compare their nutritive value, so using a popular vegetable, like napa cabbage, could be an alternative "standardised" food for the cultures of *A. longisetosus*. Another advantage of using napa cabbage is that it is easier to contaminate it (e.g. with heavy metals) to obtain the desired concentrations of a substance, so it could be used in this type of experiments as well. The aim of this study was to determine if green algae vs. napa cabbage differ in their composition, in terms of protein, crude ash and crude fibre content and how they affect *A. longisetosus*. We hypothesized that the tested food (1) affects the life-history parameters of *A. longisetosus*; (2) the composition and activity of its gut microflora; and (3) food type affects differently the adults and juveniles.

MATERIALS AND METHODS

Food analyses

Green algae were collected from tree bark of bird cherry in Scots pine forest in Bydgoszcz, whereas napa cabbage was bought in a local market and dried in the laboratory in the oven at 105°C until all the free water evaporated. Both types of food were analysed at the Department of Cattle Breeding and Animal Nutrition (University of Technology and Sciences in Bydgoszcz) using the Weende analysis method (Naumann & Bassler 1993). The total ash content was determined by ignition of a sample at the temperature 500°C, at which the organic compounds were removed and inorganic constituents remained (i.e. soluble and insoluble minerals, including macro and micro forms). To determine the total protein and crude fibre, the Kjeltec

Auto Distillation, Fibertec System 1010 Het Extraction, and Soxtec System HT 1043 Extraction Unit were used, respectively.

Study animals

Archegozetes longisetosus originated from the laboratory hatchery of the Department of Ecology (University of Technology and Sciences in Bydgoszcz) that started in 1996 from a few individuals obtained from Prof. R.A. Norton (University of Syracuse, USA), and originated from one gravid female from Puerto Rico. They were kept in rearing boxes with the bottom filled with plaster of Paris/charcoal mixture (4:1), at constant conditions: 30°C and 90% relative air humidity.

Mites were fed with green algae and we compared their life-history parameters and the gut and environment microflora with the results obtained in a similar experiment on napa cabbage (Seniczak et al. 2009). The study group exposed to green algae consisted of 10 replicates, similarly like those fed with napa cabbage (Seniczak et al. 2009): each was a young female (shortly after moulting), placed separately in the rearing box, and her offspring. The mites were provided with fresh food every other day and at the same time their development and biology were observed. The experiment continued until all mites from the offspring generation achieved the adult stage. Then we assessed the longevity and fertility of the females, mortality of the offspring and the time of their development.

Statistical analysis

The basic statistical descriptors included the minimum, maximum, mean values and standard deviation. Normality of the distribution was tested with the Kolmogorov-Smirnov test, whereas the equality of variance in different groups, with the Levene test. The assumption of normality or equality of variance was not met, so the non-parametric Kruskal-Wallis test was used, followed by the Mann-Whitney *U* test. Statistical calculations were carried out with STATISTICA 10.0 software.

Microbial analyses

For the microbiological analyses, 10 adult mites and 10 tritonymphs from the offspring generation (i.e. after about 30 days of exposure to food) were selected at random. Both stages were 3 days after the last moult. Material for microbiological determination was taken from the gut of mites after submerging the proterosoma in paraffin. Then the opisthosoma was cut off with a sterile scalpel and gut tracts were taken out with a sterile preparation needle. The material from 10 individuals from each experimental group was pooled together. The environmental samples (similar amount as gut material from 10 mites) were collected from the bottom of the culture boxes where the mites were fed with algae, with faecal pellets and food remains, and processed in the same way as the gut material.

All the samples were transferred to empty Petri dishes and pounded in a small amount of sterile sand; and then poured with the melted and cooled medium agar-standard (Merck, cat. no. 1.07881). Incubation was conducted at 20°C for 7 days. The aim of the inoculation was to estimate quantitatively the bacteria, fungi and actinomycetes. From the bacterial colonies, pure cultures were isolated, in which some

features were determined: morphological (stainability in the Gram method, forming endospores), physiological (the hydrolysis of starch, cellulose, pectin and protein) and biochemical (fermentation of sugars, producing catalase). The detailed methods were described earlier (Seniczak et al. 2009).

RESULTS AND DISCUSSION

Food quality and life history parameters

The food offered to *A. longisetosus* differed in concentrations of crude ash, protein and crude fibre, which were 70%, 60%, and 30% higher in napa cabbage, respectively, than in green algae (Table 1). Despite that, green algae turned out to be better food for *A. longisetosus* than napa cabbage. Feeding on green algae resulted in distinctly higher fertility, lower mortality, and shorter time of development of mites, compared to those fed with napa cabbage. Only the longevity of adults fed with napa cabbage was slightly higher than of those feeding on green algae (Table 2).

Table 1. Selected components of food (% of dry matter, d.m.) served to *Archegozetes longisetosus*

Component	Green algae	Napa cabbage
Crude ash (% d.m.)	9.59	12.72
Crude fibre (% d.m.)	8.52	13.54
Total protein (% d.m.)	15.51	26.26

Table 2. Life-history parameters (mean ± SD; range) of *Archegozetes longisetosus*; SD = standard deviation. Asterisks denote significant differences between food types ($n = 10$, $P < 0.05$; [1] after Seniczak et al. 2009)

Life-history parameter	Green algae	Napa cabbage[1]
Longevity of initial adults (days)	56.7 ± 6.4 (48.0–64.0)	64.2 ± 12.3 (44.0–78.0)
Fertility per initial female (number of individuals)	119.3 ± 29.0 (89.0–184.0)	61.9* ± 13.8 (41.0–89.0)
Mortality of juveniles (%)	1.1 ± 0.8 (0.1–2.7)	23.6* ± 8.6 (9.4–34.0)
Egg-to-adult development (days)	30.0 ± 1.6 (28.0–33.0)	37.0* ± 1.7 (34.0–39.0)

Gut microflora

The gut microflora of *A. longisetosus* depended on the kind of food and developmental stage of mites. The adults fed with napa cabbage contained 2.5-fold more bacterial colonies, 1.7-fold more fungal colonies and 5-fold more actinomycetes than those fed with green algae (Table 3). In contrast, the gut microflora of the tritonymphs fed with green algae was more abundant than of those fed with napa cabbage: the former contained 1.5-fold more bacterial colonies and 3-fold more fungal colonies, but actinomycetes were absent here. The gut microflora of the tritonymphs fed with green algae was nearly 4-fold richer in bacteria and 3-fold richer in fungi than in the adults, whereas in those fed with napa cabbage the number of bacterial colonies was similar as in the adults, but the number of colonies of fungi and actinomycetes was lower than in the adults.

Table 3. Numbers of microbial colonies isolated from the alimentary canal of *Archegozetes longisetosus* adults and tritonymphs fed with different types of food for 7 days at 30°C (n = 10), and contributions of different groups of microorganisms (% of colonies) to the gut microflora

Groups of microorganisms	Developmental stage of mites	Green algae	Napa cabbage[1]
Bacteria	adults	10 (72%)	37 (90%)
	tritonymphs	39 (81%)	22 (92%)
Fungi	adults	3 (21%)	2 (5%)
	tritonymphs	9 (19%)	1 (4%)
Actinomycetes	adults	1 (7%)	2 (5%)
	tritonymphs	0	1 (4%)

[1] after Seniczak et al. (2009)

In the mites fed with algae, the same groups of bacteria were found both in the adults and tritonymphs, but in different proportions (Fig. 1). In the adults, gram-positive bacilli reached the highest percentage, whereas in the tritonymphs, gram-negative bacteria predominated. In the adults fed with napa cabbage, only 2 groups of bacteria were found, whereas in the tritonymphs, 3 groups were isolated and the most abundant were cocci, which were not found in any other experimental group.

In the mites fed with green algae, the activity of the gut microflora was higher in the adults than in the tritonymphs, whereas in those fed with napa cabbage it was similar in both the developmental stages. All the bacteria isolated from the guts showed hydrolytic activity in relation to starch, cellulose, pectins and gelatine, and they fermented sugars (Table 4). The microorganisms isolated from the adults that

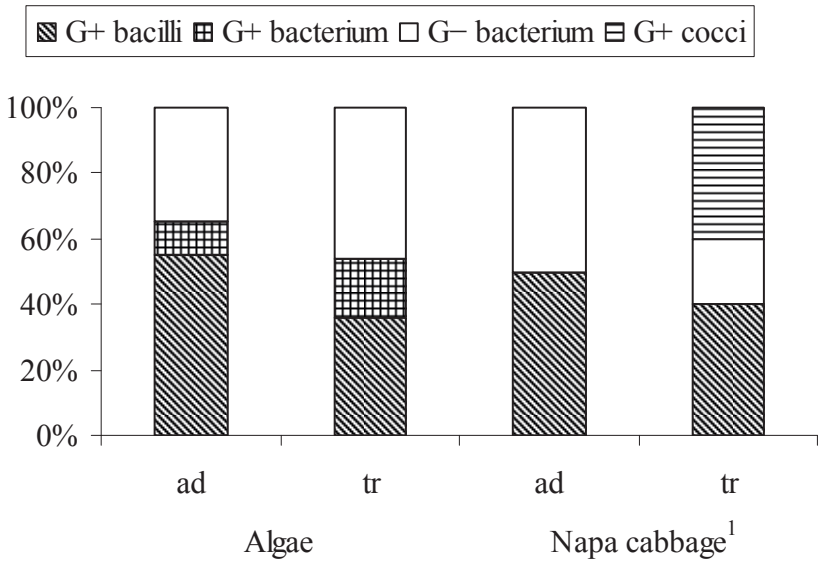

Fig. 1. Morphology and reaction of bacteria in the alimentary canal of *Archegozetes longisetosus* adults (ad) and tritonymphs (tr) to the Gram method (% of colonies); G+ = gram-positive; G− = gram-negative; [1] after Seniczak et al. (2009)

Table 4. Activity of substrate-based physiological groups of bacteria in the guts of *Archegozetes longisetosus* adults and tritonymphs and in the culture environment

Substrate	From guts				From culture environment	
	adults		tritonymphs			
	green algae	napa cabbage[1]	green algae	napa cabbage[1]	green algae	napa cabbage[1]
Starch	++	+++	++	+++	+++	+++
Cellulose	+	++	+	++	no	no
Pectin	++	+++	++	+	no	no
Protein	++	+++	+	++	no	no
Lactose	+	+	+	+	+	+
Sucrose	++	++	+	++	+++	+++
Maltose	+++	+++	++	++	+++	++
Glucose	++	++	+	++	++	++
Glycerol	+	+	+/-	+	+	+
Catalase production	+	no	++	++	+++	+++
Mean	++	++	+	++	++	+

[1] after Seniczak et al. (2009). Activity: +++ very strong; ++ strong; + weak; +/- very weak

fed on cabbage did not produce catalase, and the bacteria from the mites' environment did not hydrolyse cellulose, pectins, and proteins.

General remarks

The type of food is one of the most important ecological factors that affect the development of mites (Haq & Adolph 1980; Seniczak 1998). This was confirmed in the present study, where feeding with green algae (*Protococcus* sp.) resulted in distinctly higher fertility, lower mortality, and shorter time of development of *A. longisetosus* in comparison to the mites fed with napa cabbage (Seniczak et al. 2009). Although napa cabbage contains a higher percentage of protein as well as crude ash (which contributes to mineral content, including metal salts), it also has a higher crude fibre content, which reflects indigestible components of food, like cellulose, lignin, and other components of this type, as opposed to amyloid carbohydrates.

Also in Hubert et al.'s (2001) study, green algae were better food for oribatid mites than plant material. Green algae were used successfully as food for *A. longisetosus* in many other experiments (Honciuc 1996; Seniczak 1998; Seniczak & Seniczak 2002; Alberti et al. 2003, 2011; Smrž & Norton 2004; Heethoff et al. 2007, 2013; Remén et al. 2010). The positive effect of green algae on this mite was explained by the presence of easily assimilable polysaccharides or other growth factors that are stored in algal cells (Honciuc 1996). Smrž & Norton (2004) considered green algae *Protococcus* sp. to be nutritious food for *A. longisetosus*, but since no cellulose activity was detected, those authors suggested that the mites used only simple food components that are present in green algae. However, in our study the activity of microflora towards cellulose was detected in both feeding groups. This indicates that *A. longisetosus* is able to digest the cell walls and also utilize the cell contents.

In contrast, Haq & Prabhoo (1977) and Haq & Adolph (1980) observed that both the adults and juveniles of *A. longisetosus* rejected green algae. Haq & Prabhoo (1977) fed the adults of *A. longisetosus* with decomposed leaves, moss, and *Trichoderma* sp., whereas the nymphs preferred decomposed leaves, moss, and *Alternaria* sp., but both groups rejected, except for algae, also other food types: yeast, lichens, decomposed twigs and stems, *Penicillium* sp., *Pythium* sp., *Colletotrichum* sp., *Agaricus* sp. partly decomposed cladodes, seeds of *Casuarina* sp., bark of higher plants, faecal pellets of oribatid mites, minced meat, and small soil animals. However, in another experiment, Haq & Adolph (1980) observed broader food preferences in the juveniles of *A. longisetosus* than in the adults: the nymphs fed on fungi (*Trichoderma* sp. and *Alternaria* sp.), moss as well as on decomposed leaves of *Artocarpus* sp. and giant bamboo (*Bambusa gigantea* Wall.), whereas the adults fed only on the leafy material. Studies of the gut content of *A. longisetosus* collected from the field showed the presence of fungal hyphae and spores, algal spores (Haq 1978), and high amounts of leaves (Xavier & Haq 2007), as was noted also in the laboratory experiments. Some differences between field and laboratory studies can be explained by the amount of food available to the mites. In the laboratory, food is plentiful, so many mite species feed on the components that are the easiest to digest, but in the field, when food becomes scarce and efficiency is needed, the same species

will use its enzymatic tools to digest other types of food that are available (Siepel & de Ruiter-Dijkman 1993).

In the experiment conducted by Seniczak et al. (2009), the adults of *A. longisetosus* utilized napa cabbage better than the tritonymphs. Their longevity was slightly higher than in the group fed with green algae in the present study and the former had more colonies of bacteria, fungi and actinomycetes in their gut systems than the mites feeding on green algae. However, the fertility of mites fed with napa cabbage was reduced to 61.9 in comparison to the mites feeding on green algae (119.3) and was similar as in mites that were fed with tree bark (54.4) or lichens (48.4) (Seniczak 1998). The food type affects also clearly the time of development of *A. longisetosus* (Table 5). Depending on food, the time of development of this species ranged from 30 to 50 days at 30ºC, and 48-88 days at 26ºC. The shortest development was observed when the mites were fed with green algae, indicating that this food is very valuable for *A. longisetosus*.

Feeding on leafy material, *A. longisetosus* plays an important role in decomposition of soil organic matter, contributing much to the mechanical breakdown of leaf litter (Haq & Prabhoo 1977). Mites can digest plant material that contains β-glycosidic cellulose and hemicellulose only with the contribution of the symbiotic microorganisms that live within the mites' guts (Hartenstein 1962). The microorganisms hydrolyse also protein, starch, and chitin (Prusinkiewicz et al. 1975). The gut microflora of oribatid mites is more numerous and diverse than soil microflora and is symbiotic (Prusinkiewicz et al. 1975; Seniczak & Stefaniak 1978; Stefaniak & Seniczak 1981). This was reflected in the present study, since cellulolytic, pectinolytic and proteolytic bacteria were isolated from the gut tracts of *A. longisetosus* but were absent on the bottom of the rearing boxes. Although Zinkler (1971) reported that in gut systems of *Phthiracarus piger* (Scopoli, 1763) and *Northus silvestris* (Nicolet, 1855) the enzymes carboxymethylcellulase, xylanase, and pectinase were present, they were probably produced by the gut microflora. This is supported by Luxton (1972), who observed that most of the chitinase activity measured in the oribatid mites disappeared after the application of a bactericide. Similarly, when oribatid species fed on sterilised food, their feeding rate clearly decreased (Haq 1994).

In the group of *A. longisetosus* fed with green algae, the tritonymphs had more abundant microflora than the adults, while in the group fed with napa cabbage, the abundance of microflora in both developmental stages was similar. The juvenile Oribatida are usually considered more active than the adults and they have a richer, more diverse and more active gut microflora than the adults (Stefaniak & Seniczak 1976). As reported in *Heptacarus hirsutus* Wallwork, 1964, from all the compared developmental stages, the most abundant and most active microflora was found in the tritonymphs, indicating their most active involvement in decomposition of organic matter (Haq 1987).

In our studies, the highest percentage of bacteria isolated from the gut system of *A. longisetosus* were gram-positive bacilli or gram-negative bacteria, but the mites that were fed with napa cabbage had no gram-positive bacteria. Interestingly, the latter bacteria were found in *A. longisetosus* fed with napa cabbage that was contaminated with a low concentration of cadmium (25 μg $Cd \cdot g^{-1}$), while gram-positive

Table 5. Life-history parameters and morphological traits of *Archegozetes longisetosus* fed with different types of food in laboratory conditions

Life-history parameter	Temperature	Food type						References
		green algae	lichens	tree bark	plant debris, different fungi	leafy material	pine pollen, yeast, algae, corn flour, mould	
Fecundity (eggs per female during 31 days)	30°C	120.0						Honciuc (1996)
Fertility (offspring per female during lifetime)		101.8	48.4	54.4				Seniczak (1998)
		137.6						Seniczak & Seniczak (2002)
						61.9		Seniczak et al. (2009)
Mortality (%)		4.1	9.3	37.9				Seniczak (1998)
		1.8						Seniczak & Seniczak (2002)
						23.6		Seniczak et al. (2009)
Time of development from egg to adult (days)		32.3	39.9	44.6				Seniczak (1998)
		30.0						Seniczak & Seniczak (2002)
					50.0			Haq (1978)
						34.4		Haq & Adolph (1980)
						37.0		Seniczak et al. (2009)
Longevity (days)		51.2						Seniczak & Seniczak (2002)
						64.2		Seniczak et al. (2009)
Body length (µm)		1119.0	980.0	884.0				Seniczak (1998)
Fecundity (eggs per female during 31 days)	26°C	112.0						Honciuc (1996)
Time of development from egg to adult (days)		48.0-50.0						Honciuc (1996)
							78.0-88.0	Estrada-Venegas et al. (1999)
Fertility (offspring per female during 51 days)	23°C	55.0						Heethoff et al. (2007)
Fecundity (eggs per female during 31 days)	22°C	156.0						Honciuc (1996)

bacteria accounted for 22% of the total microflora in the adults and 8% in the tritonymphs (Seniczak et al. 2009). However, the life-history parameters of the mites exposed to cadmium were worse than in the control group, so the presence of gram-positive bacteria does not improve the life-history parameters of *A. longisetosus*. In other mite species, as well as in other soil invertebrates, like termites, earthworms, and springtails, the species of the genus *Bacillus* were the most abundant bacteria of the gut, and that is consistent with our results. *Bacillus* species are known to contain efficient enzymes that are useful for digestion of a wide spectrum of substrates (Smrž & Trelova 1995) and play a major role in the first and second steps of the degradation of polymer material under oxygen limitation (König 2006, 2011).

CONCLUSIONS

Algae were better food for *A. longisetosus*, resulting in a higher fertility, lower mortality of the offspring, and faster development of mites. The adults fed with napa cabbage had more abundant and more active microflora than those fed with green algae, whereas in the tritonymphs the microflora was more abundant when they were fed with green algae, and was more active in the group fed with napa cabbage.

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