Title: INFLUENCE OF HOLM OAK LEAF DECOMPOSITION STAGE ON THE BIOLOGY OF *Onychiurus sinensis* Stach (COLLEMBOLA: ONYCHIURIDAE)

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INFLUENCE OF HOLM OAK LEAF DECOMPOSITION STAGE ON THE BIOLOGY OF *Onychiurus sinensis* Stach (COLLEMBOLA: ONYCHIURIDAE)

INFLUENCE DU STADE DE DECOMPOSITION DES FEUILLES DE CHENE VERT SUR LA BIOLOGIE DE *Onychiurus sinensis* Stach (COLLEMBOLA: ONYCHIURIDAE)

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Abstract: The trophic attractiveness of different stages of development and decomposition of holm oak leaves and the influence of their nutritional quality on the biology of the springtail *Onychiurus sinensis* were studied. Collembola fed preferentially on leaves at an advanced stage of decomposition. Biological parameters such as moulting, fecundity, survival rate and linear growth, indicated the influence of the quality of food upon the biology of these microarthropods. Whole parameters were maximized when the leaves were more decayed and when the nitrogen content was the highest.

Keywords: Collembola/ litter quality/ palatability/ holm oak leaves/ growth/ fecundity

Résumé: Des feuilles de chêne vert à différents stades de leur évolution et de leur décomposition ont été étudiées pour leur attrait trophique vis-à-vis d'*Onychiurus sinensis* et pour l'influence de leur qualité nutritive sur la biologie de ces Collembolles. Les Collembolles consomment préférentiellement les feuilles à un stade de décomposition avancé. Les paramètres biologiques considérés, la mue, la fécondité, le taux de survie et la croissance linéaire, mettent en évidence l'impact de la qualité de la nourriture sur la biologie de ces microarthropodes. L'ensemble des paramètres est optimisé en présence des feuilles les plus dégradées et les plus riches en azote.

Mots-clés: Collemboles/ qualité de la litière/ appétence/ feuilles de chêne vert/ croissance/ fécondité.

1. INTRODUCTION

Feeding habits of Collembola cover a wide range. Most species are fungivores or detritivores, but many of these exhibit an indiscriminate feeding habit, exploiting the food resources that are abundant in their habitat at a particular time. However, feeding preferences are common among Collembola [3, 8, 10, 13, 16, 18, 21, 22, 26, 32, 34, 35, 36, 43] and animals may select the most beneficial resources available to them, taking profit from high nutritional values or low toxicant content [1, 5, 7, 12, 30, 32, 35, 40, 44].

Numerous investigations have been carried out on the selection of leaves from different plant species by soil fauna [4, 14, 33, 39] but only few studies considered their degree of decomposition [9, 20, 38].

A simplified experimental model was used to study the feeding behaviour of *Onychiurus sinensis* Stach (Collembola, Onychiuridae) according to changes occurring during the development and decomposition of holm oak (*Quercus rotundifolia* Lam.) leaves. Different stages of the plant substrate were identified according to some morphological criteria (colour, thickness, rigidity and pilosity of the lower side of the leaves). They represent physical and chemical differences that can have an influence not only on the choice of food by Collembola, but also on their biology as revealed by some biological parameters (moulting, fecundity, survival rate and linear growth).

2. MATERIAL AND METHODS

Two experimental series were conducted under laboratory conditions, in which Collembola were placed in the presence of holm oak

leaves at different stages of development and decomposition. The first experiment (palatability tests), carried out over a short period of time (3 hours), aimed to measure the trophic attractiveness of a given stage for *Onychiurus sinensis*. The second experimental series (rearing and nutritional value experiments) was performed for a longer period (9 weeks) to estimate the nutritional value of each leaf stage, measured by several biological parameters of the animals.

2.1. Collembola

The study focused on *Onychiurus sinensis* Stach (Collembola Onychiuridae), a frequent and abundant inhabitant of the holm oak litter (*Quercus rotundifolia* Lam.) in Toufliht (High Atlas of Marrakech, Morocco). Animals were extracted in Berlese funnels, then cultured on a mixture of plaster of Paris and charcoal on an algal diet (*Desmococcus sp.*).

2.2. Leaves

Leaves were collected directly from the tree and in litter horizons. Still attached leaves were separated into fresh (green) and senescent (yellow) leaves, irrespective of their age. The mean duration of life of holm oak leaves has been estimated to two years, but leaf fall may occur during their first as well as during their third year of life [28].

Litter leaves were divided into 6 stages of decomposition (*Table I*). They were washed and the distal part of leaf blades was stamped once with a 6mm diameter copper punch, avoiding the main nerve.

The total nitrogen content of each stage of leaves was measured by the Kjeldahl method on an aliquot of the same material used for the experiments.

2.3. Methods

Individuals of *Onychiurus sinensis* were starved for two days before each experimental run. Only young adults were used because at this stage, which is the start of sexual maturity, animals still have a high growth rate and a higher feeding activity than younger or older individuals [11]. The mean overall length ($\pm$ SE), measured with a reticle eye-piece on a batch of a hundred individuals, was 1.36 ± 0.01 mm. After the starvation period, 20 individuals were transferred to a bottomless plastic cylinder (1cm diameter) placed at the center of a Petri dish (5.6cm diameter) which had been partly filled with a mixture of plaster of Paris and charcoal.

A diameter line drawn on the cover lid was used to divide the Petri dish into two sectors of equal size. A leaf disk (6 mm Ø) was placed at the center of one of both sectors. Once all boxes were thus prepared (five replicates for each leaf decomposition stage and for control without leaf disk), the plastic cylinder was removed, then observations were made every 10min for 3 hours. Animals were counted in both sectors and on the test substrate. When significantly more or less than 50% of the animals were present in a given sector, this indicated that something in this sector was attractive or repulsive for the animals, respectively. During each experimental run, dishes were kept in darkness, because it had been observed that the studied animals avoided light. Attractiveness of the different leaf stages was compared by one-way ANOVA [37] using mean numbers of animals present on the leaf disk over the whole experimental period. Comparisons between the two sectors as well as between the leaf disk and the rest of the corresponding sector were done by *t*-tests [37]. In order to avoid artifacts due to negative correlations between the two sectors (when an animal moved to one sector, the other sector was affected negatively), differences between sectors were compared to a theoretical

null value indicating the absence of attractiveness or repulsion by the considered leaf stage.

Boxes used for the first experimental series (except stage VI which was not used in further experiments) were kept for culturing animals over 9 weeks, at 22°C and in darkness. Animals, exuviae and eggs were counted daily. The body length of each surviving individual was measured at the end of the experiment, using a reticle eye-piece. Data were analysed by one-way ANOVA [37]. Means were compared between leaf categories using Newman-Keuls test [37]. The capability of the different leaf stages to ensure both moulting, reproduction, survival and growth of *O. sinensis*, was estimated by ranking the leaves for each biological parameter. Ranks were summed up in order to classify the leaf stages into three groups (described later).

3. RESULTS

3.1. Palatability tests

The foraging behaviour of *Onychiurus sinensis* is estimated by the mean number of individuals present at a given time on a given leaf stage. The results (*Table II*) show a significant increase in the number of individuals from 0.2 (fresh leaves) to 1.7 (litter III) and then to 10.9 (litter VI).

In the course of this study, it was noted that there was an absence of aggregative behaviour. The control experiment showed that animals moved from one sector to another independently of each other (*Figure 1*). *Figures 2 and 3* show that fresh and senescent leaves exhibited the lowest attractiveness, some animals being present on the substrate only at the end of the experiment. The substrate became more palatable from litter stage I on (*Figures 4 to 6*), a higher number of animals being present on the test food from 40 min onwards. However numbers of animals present on the

substrate remained low in respect to stages IV (*Figure 7*), V (*Figure 8*), and VI (*Figure 9*). The maximum number of individuals was observed on stage VI, i.e. litter with rhizomorphs of the white rot fungus *Marasmius quercophilus* where most Collembola were attracted to the leaf disk from the start of the experiment (*Figure 9*).

3.2. Rearing and nutritional value experiments

Moulting, fecundity, linear growth and survival rate of the animals in response to food quality are represented in *Table III*. Although differences among means were insignificant for moulting, it was noted that the total number of moults was greater for individuals feeding on litter leaves than on fresh and senescent leaves.

The effect of food quality on fecundity varied markedly between the different stages. The number of eggs increased steadily from fresh leaves to stage V where a maximum was registered. Newman-Keuls tests identified two homogeneous groups of leaf stages. The egg laying rate of *Onychiurus sinensis* (*Table III*) increased together with the leaf nitrogen content of leaves (*Table II*), as indicated by a significant rank correlation coefficient ($r_s = 0.79$, $P < 0.05$).

Newman-Keuls test showed 3 homogeneous groups for linear growth. In the first group, represented by stage V only, individuals had a maximum rate of growth since they reached 1.57 ± 0.01 mm at the end of the rearing experiment. Individuals of the second group had a final size varying from 1.47 to 1.54 mm, while those of the third group (fresh leaves) showed the lowest linear growth (1.42 mm).

The survival rate varied according to the ingested food, increasing from fresh leaves (79%) to litter V (92%), but differences between stages were insignificant.

The sum of ranks occupied by each leaf stage for each parameter may discriminate the nutritional properties of each food substrate for the Collembola. This ranking displays three groups distinguishable for their stimulatory or inhibitory effects on one or the bulk of biological parameters. A first group (A), very favourable for the four parameters considered, is composed of stages V and IV, whereas the second group (B) acts in a qualified manner, either favouring moulting (stages II and III), growth (stage I) or fecundity (stage III). Leaves taken on the tree corresponded to the third group (C) that was not favourable to these Collembola.

4. DISCUSSION

Results of this study indicated that food quality can influence the attractiveness of a given leaf stage for *Onychiurus sinensis* and also its biology. Despite this was the only nutrient measured, the nitrogen content of leaves was probably at least partly responsible for the noted differences. Thus, leaves at stages IV and V were most attractive as their nitrogen content was almost twice that of most other stages. This is in agreement with ANDERSON [2], KING & HEATH [15], PETER & LUXTON [23] and REICHLE [29] who showed that feeding preferences of some animals for different species of leaf litter were linked to the chemical composition of the leaves and principally to their nitrogen content.

The low trophic attractiveness of leaves taken on the tree can be explained by the presence of tannins which are absent from decomposed leaves. RACON et al. [27] showed that functional holm oak leaves (*Quercus ilex*) and those just falling down had a very high tannin content (2.1% dry weight), this content decreasing to 0.4% in the L2 horizon (corresponding to stages III and IV of the present study). Among litter

leaves, the test substrate is much preferred as it is more decomposed, which corresponds to maximum nitrogen and amino-acid content [30], resulting mainly from the development of microflora. In parallel to the increase in nitrogen content two other processes may explain the increase in attractiveness in the course of decomposition. In previous studies on holm oak leaf litter it was demonstrated that there was detoxication of the plant material [25, 27] and attraction by colonizing fungi [32]. In the present experiment, it was noticed that the rhizomorphs of *Marasmius quercophilus* were actively consumed by animals attracted to stage VI leaves. All these factors, with the addition of water requirements [41], may explain the deep distribution of onychiurids in thick holm oak litter layers [24, 31].

The number of individuals in contact with the food substrate was always low at the beginning of the experiment (except for stages V and VI), then increased steadily (*Figures 4 to 7*). This increase could be due either to a perception of the substrate odour by each individual, as has been observed with fungi [32] or to an aggregative behaviour using pheromones as an attractant, as has been demonstrated for several members of the family Entomobryidae [17, 42]. In none of the experiments was such aggregative behaviour observed for *O. sinensis* (*Figure 1*), thus the second hypothesis can be excluded for the studied species.

The importance of food quality for growth and regulation of animal populations is widely recognized. SNIDER [36], using three experimental food substrates, characterized by different protein contents, showed that they had different effects on growth, instar duration, survival, fecundity and size of *Onychiurus justus*. BOOTH & ANDERSON [7] found that the isotomid *Folsomia candida* had a higher moulting rate, layed more eggs and had a shorter life time when the fungus offered as food was grown on a medium richer in nitrogen, except at very high nitrogen concentrations.

LAVY & VERHOEF [19] confirmed on the entomobryid *Orchesella cincta* that the nutritional quality of the fungus was related to its nitrogen content.

On the other hand another factor of food quality, such as the repellence or toxicity of phenolic compounds, can be taken into account. Thus, TOUCHOT et al. [39] showed that populations of *Folsomia candida* feeding on hornbeam leaf litter grew at a higher rate than on common oak. Post-embryonic development was optimum for individuals cultured on hornbeam while it was blocked up at the first instar stage on oak, probably owing to higher content of phenolic substances present in oak leaves compared to hornbeam [33]. A previous study on *Folsomia candida* and *Onychiurus zschokkei* demonstrated the toxicity of tannin compounds present in fresh holm oak leaves [25].

Rearing experiments showed similar results as palatability. They underlined a significant impact of leaf nutritional quality, estimated by its nitrogen content, on the biological parameters considered (*Tables II and III*). A food substrate can be termed high quality when the cultured animals show not only the highest reproductive rate and the lowest mortality but also a good linear growth and frequent moulting. All these parameters are optimized with the softer and more decayed leaves (stages IV and V) characterized by their high nitrogen content, low tannin content and high fungal development [6].

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Table I : Morphological features of the six decomposition stages from holm oak leaf litter.

Stages	Morphological features
Litter I	Yellow, externally intact, thick, hard, lower side densely covered with green hairs.
Litter II	Yellow, slightly decayed but still thick and hard, hairs covering the lower side invaded by fungi.
Litter III	Brown, thin, soft, decayed, a few hairs still present.
Litter IV	Brown, thin, very soft, strongly decayed but still recognizable, no hair present.
Litter V	Bleached, very thin, brittle and more or less packed with fungal mycelium.
Litter VI	Bleached, with the presence of rhizomorphs of <i>Marasmius quercophilus</i> (Basidiomycetes)

Table II : Mean ($\pm$ standard error) numbers of individuals of *Onychiurus sinensis* present on each leaf stage over the whole experimental period. Twenty individuals introduced at the start of the experiment (five replicates). Duration of the experiment 3 hours. Means followed by letters indicating homogeneous groups of leaf stages (Newman-Keuls test). Results of t-tests were indicated in bold (significantly higher), italic (significantly lower) or normal (non significant difference) character types for comparisons between sectors or by symbols ($>$, $<$, $=$) for comparisons between the substrate itself and the corresponding sector out of the substrate. Significance thresholds were fixed at $P < 0.05$. ND = not determined.

Stages	Total nitrogen content (%)	On the leaf disk	In sector with leaf disk	In sector without leaf disk
Fresh leaves	0.92	$0.2_f \pm 0.0$ <	13.9 ± 0.6	6.1 ± 0.6
Senescent leaves	0.70	$0.8_e \pm 0.2$ <	11.6 ± 1.7	8.4 ± 1.7
Litter I	0.89	$1.3_{de} \pm 0.2$ <	14.0 ± 0.9	6.0 ± 0.9
Litter II	0.91	$1.3_{de} \pm 0.3$ <	13.8 ± 1.4	6.2 ± 1.4
Litter III	1.17	$1.7_{cd} \pm 0.3$ <	12.6 ± 1.9	7.4 ± 1.9
Litter IV	1.65	$2.4_{bc} \pm 0.2$ <	11.4 ± 1.2	8.6 ± 1.2
Litter V	2.04	$3.1_b \pm 0.1$ <	12.1 ± 1.0	7.9 ± 1.0
Litter VI	ND	$10.9_a \pm 1.4$ =	15.9 ± 1.0	4.1 ± 1.0

Table III : Biological parameters (mean $\pm$ standard error) of *Onychiurus sinensis* cultured at 22°C for 9 weeks on different leaf stages. Mean body length of individuals at the beginning of the experiment 1.36 ± 0.01 mm. Twenty individuals introduced (five replicates). Means followed by letter indicating homogeneous groups at the 0.05 threshold (Newman-Keuls test). Leaves ranked for each biological parameter (ranks indicated in bold type). Sums of ranks classify leaves into three food quality groups (left column). Newman-Keuls test insignificant for moulting and survival rates.

Groups	Leaf stages	Total number of moults	Total number of eggs	Number of surviving individuals	Final body length (mm)	Sum of ranks
A	Litter V	32.2 ± 1.9 2	$41.6_a \pm 5.2$ 1	18.4 ± 1.1 2	$1.57_a \pm 0.01$ 1	6
A	Litter IV	35.0 ± 3.8 1	$21.4_b \pm 4.5$ 2	18.6 ± 0.5 1	$1.54_{ab} \pm 0.02$ 2	6
B	Litter III	31.6 ± 5.2 3	$14.6_b \pm 1.4$ 3	17.8 ± 0.5 3	$1.51_{ab} \pm 0.01$ 4	13
B	Litter II	30.6 ± 1.4 4	$12.4_b \pm 3.3$ 4	17.8 ± 1.0 3	$1.51_{ab} \pm 0.01$ 4	15
B	Litter I	26.8 ± 2.1 5	$12.0_b \pm 5.0$ 5	17.0 ± 0.8 5	$1.52_{ab} \pm 0.02$ 3	18
C	Senescent leaves	23.0 ± 3.4 6	$9.0_b \pm 2.2$ 6	16.2 ± 1.2 6	$1.47_{ab} \pm 0.01$ 6	24
C	Fresh leaves	22.8 ± 3.1 7	$6.6_b \pm 2.8$ 7	15.8 ± 0.6 7	$1.42_b \pm 0.02$ 7	28

LEGENDS OF FIGURES

Figure 1. Control: Distribution of 20 individuals of *Onychiurus sinensis* in a Petri dish divided into 2 sectors and without food substrate. At time 0 the animals were allowed to move freely in the Petri dish. Each value is the mean of five replicates $\pm$ standard error.

Figure 2. *Onychiurus sinensis*/ fresh leaves: Distribution of 20 individuals in a Petri dish divided into 2 sectors, the one with a leaf disk and the other without food. At time 0 the animals were allowed to move freely in the Petri dish. Each value is the mean of five replicates $\pm$ standard error.

Figure 3. *Onychiurus sinensis*/ senescent leaves: otherwise as for *Figure 2*.

Figure 4. *Onychiurus sinensis*/ litter I: otherwise as for *Figure 2*.

Figure 5. *Onychiurus sinensis*/ litter II: otherwise as for *Figure 2*.

Figure 6. *Onychiurus sinensis*/ litter III: otherwise as for *Figure 2*.

Figure 7. *Onychiurus sinensis*/ litter IV: otherwise as for *Figure 2*.

Figure 8. *Onychiurus sinensis*/ litter V: otherwise as for *Figure 2*.

Figure 9. *Onychiurus sinensis*/ litter VI: otherwise as for *Figure 2*.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9