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1 Type of contribution: Short communication

2

3 **Avoidance of low doses of naphthalene by Collembola**

4

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6

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12

13 **Capsule**

14

15 PAH avoidance by soil springtails is species-specific and differs among
16 populations of the same species

17

18 **Abstract**

19

20 The introduction of behavioural aspects of soil animals in ecological risk
21 assessment would allow us to better assess soil quality, all the more if a range of
22 animal populations are considered. We compared the avoidance behaviour of several
23 strains of springtails (Arthropoda: Collembola) obtained from different soils.
24 Naphthalene, a polycyclic aromatic hydrocarbon (PAH) widely represented in soils
25 polluted with hydrocarbons, was tested in aqueous solutions on nine springtail species
26 issuing from four sites. Fine quartz sand saturated with an aqueous solution of
27 naphthalene was avoided by most of the tested species, avoidance being however
28 detected down to a concentration of 0.030 mg.L⁻¹. *Folsomia candida* (Isotomidae) was

1 shown to be relatively tolerant to pollutants compared to other Collembola such as
2 *Mesaphorura macrochaeta*, *M. yosii* (Onychiuridae), *Parisotoma notabilis* (Isotomidae)
3 and *Arrhopalites caecus* (Arrhopalitidae). Differences between strains could not be
4 explained by properties of the original soils.

5
6 *Key-words:* Soil pollution; Collembola; Behaviour; Avoidance; Polycyclic Aromatic
7 Hydrocarbon (PAH)

9 **1. Introduction**

10
11 Polycyclic aromatic hydrocarbons (PAHs) are important compounds to be
12 considered in environmental risk assessment (Riser-Roberts, 1998). Naphthalene, one
13 of the smallest (in molecular size) and most common PAHs, is a neurotoxicant (Ritchie
14 et al., 2001) and its carcinogenic effects are now fully recognized (Preuss et al., 2003).
15 This pollutant has been used for decades in homes for deterring insects from clothing
16 and to reduce densities of microarthropods in soils (Best et al., 1978).

17
18 Although still not fully recognized in ecological risk assessment, the avoidance
19 of contaminants by soil animals has proven to be of ecological value at the micro-site
20 level, by allowing sensitive species to move away from the pollution source (Tranvik
21 and Eijsackers, 1989; Filser and Hölischer, 1997). Recent studies in ecotoxicology
22 showed that avoidance tests using Collembola compare well in their outcome with tests
23 of toxicity (Greenslade and Vaughan, 2003; da Luz et al., 2004).

24
25 We may wonder whether naphthalene, known to affect arthropod populations at
26 high concentration (Best et al., 1978; Sverdrup et al., 2002), can be perceived and
27 avoided at low doses, whether avoidance depends on the species and whether several
28 populations of the same species avoid it to the same extent.

1

2 **2. Materials and methods**

3

4 Different species collected from several sites in France were compared (Table
5 1). Soil from four sites was used for starting cultures. NS Brunoy was a neutral soil (pH
6 7.5) in an oak/hornbeam woodlot at Brunoy (Ile-de-France). AS Brunoy was an acid
7 soil (pH 4.5) in an oak/pine woodland at Brunoy. NS Pechelbronn was a neutral soil
8 (pH 7) in an abandoned oil refinery at Pechelbronn (Alsace). AS Pfaffenbronn was an
9 acid soil (pH 4.5) in an oak/beech woodland at Pfaffenbronn (Alsace).

10

11 Previous to each experimental run all specimens used had never been into
12 contact with any kind of pollutant. They stemmed from batch cultures performed from 1
13 to 3 years (according to species) on a standard substrate made of fine quartz sand
14 moistened with tap water, powdered cow dung being added *ad libitum*. The test
15 specimens were selected among fully developed (adult) animals from the same rearing
16 box.

17

18 Avoidance experiments were performed in sterile polystyrene Petri dishes. The
19 diameter of the dish was 35 mm (*Mesaphorura macrochaeta*, *M. floriae*, *M. yosii*,
20 *Proisotoma minima*), 52 mm (*Folsomia candida*, *Isotomiella minor*, *Parisotoma*
21 *notabilis*) or 85 mm (*Arrhopalites caecus*, *Heteromurus nitidus*), depending on the size
22 and activity of the species. Previous observations showed that tiny, short-legged
23 springtails such as *Mesaphorura* spp. could not perform a choice within the duration of
24 the experiment if they were not allowed to explore the whole dish in a first step.
25 Conversely, bigger, very motile species such as *Heteromurus nitidus* were unable to
26 make a choice if they lacked space for their movements. Two half-disks of glass fiber
27 paper were moistened with deionised water then deposited at the the bottom of the
28 Petri dish. The entire surface of each half-disk was covered with a thin layer (1 mm) of

1 Fontainebleau sand saturated with different concentrations of the test solution (test half
2 disk) or deionised water (control half disk). The two half-disks were separated by a 2
3 mm space, at the centre of which one naive specimen was deposited. The position of
4 the animal was recorded every 20 minutes for 100 minutes. This time interval was
5 selected on the base of similar experiments where the position of animals was
6 recorded every 10' (Salmon and Ponge, 2001). During the first 20' the animals
7 wandered continuously, then when a difference between both sides of the Petri dish
8 was perceived, most of them shifted to one or the other side. Twenty replicates were
9 used for each treatment, in two successive batches of ten. During the experiment, Petri
10 dishes were placed under a fluorescent illuminator in a temperature-controlled chamber
11 at 20°C. Care was taken that the animals were not disturbed during observations, done
12 by transparency through the cover lid. Blank experiments checked for the absence of
13 effects of any light gradient which could bias the results (Salmon and Ponge, 1998).
14 For each of the four concentrations of naphthalene, differences between test and
15 control sides of Petri dishes were tested by paired t test after check for the normal
16 distribution of count data (Sokal and Rohlf, 1995). Statistical tests were done on the
17 number of times (from 0 to 5) an animal was counted on each side of the Petri dish.
18 Results are presented as mean percent presence of animals on the test side (total of
19 5x20 counts divided by 100).

20

21 The test pollutant was naphthalene (C₁₀H₈). Its water solubility is 30 mg.L⁻¹ at
22 20°C (Verschueren, 2001). A new solution of naphthalene was prepared before each
23 test run to prevent the appearance of degradation products. Before use, the solution
24 was put on a magnetic blender for one night at 20°C. Three dilution rates (0.3, 0.03,
25 0.003 mg.L⁻¹) and the mother solution (30 mg.L⁻¹) were tested.

26

27 **3. Results and discussion**

28

1 Table 2 shows the results of all behavioural tests. For *H. nitidus*, only the
2 saturated concentration (30 mg.L⁻¹) was tested because of a lack of individuals in the
3 rearing box. At the highest concentration of naphthalene (30 mg.L⁻¹), specimens from
4 all but three strains avoided the polluted side (mean percent presence ≤32% on the
5 test side) but no mortality was found. Avoidance was still displayed by most strains at
6 0.3 mg.L⁻¹. Naphthalene was not avoided by *F. candida* (AS Pfaffenbronn), *H. nitidus*
7 (NS Pechelbronn) and *P. minima* (AS Brunoy). Only *M. yosii* (AS Brunoy) and *P.*
8 *notabilis* (NS Pechelbronn) avoided the naphthalene at 0.03 mg.L⁻¹. No species was
9 sensitive at 0.003 mg.L⁻¹.

10
11 The absence of mortality within the duration of the experiment can be explained
12 by the low concentration of the contaminant in aqueous solution, even when saturated
13 (30 mg.L⁻¹). One-month test cultures on the same substrate (pure fine quartz sand
14 moistened with naphthalene solution, powdered cow dung added ad libitum) did not
15 show any mortality of the introduced specimens, nor any definite trend of cessation of
16 reproduction. Other studies showing the toxicity of naphthalene to soil Collembola used
17 naphthalene as a powder (Best et al., 1978) or diluted in lipophilic solvents (Sverdrup
18 et al., 2002), which added a vapour phase in the soil atmosphere. In our experiment,
19 the odour of naphthalene was nil after the introduction of fine quartz sand. Previous
20 assays without the addition of sand revealed a definite odour at the highest
21 concentration, which did not allow the animals to perceive a difference between both
22 sides of petri dishes. The presence of highly diffusible olfactory compounds should be
23 considered as a severe limitation to the use of Petri dishes for testing avoidance,
24 olfactometers, being recommended, although they are unfortunately not commercially
25 available (Bengtsson et al., 1988).

26
27 The avoidance test discriminated between species and strains and no attraction
28 was shown even at the lowest concentration. Most species avoided naphthalene at the

1 highest concentration (30 mg.L⁻¹) but still avoided it at 0.3 mg.L⁻¹. Only three species
2 were not sensitive at all to the tested PAH, including *F. candida*. This species does not
3 seem to be representative of the Isotomidae family, if not of the Collembola group,
4 despite its standard use in ecotoxicology (ISO 11267). *Mesaphorura macrochaeta*,
5 *Mesaphorura yosii*, *Parisotoma notabilis* and *Arrhopalites caecus* seem to be more
6 sensitive indicators of soil toxicity.

7

8 Differences in the sensitivity of collembola to the studied PAH cannot be
9 explained by ecological requirements of the species. Soil acidity (Senart, Pfaffenbronn)
10 and PAH pollution (Pechelbronn) did not seem to select for more tolerant species and
11 different strains of the same species did not avoid naphthalene at the same
12 concentration threshold (Table 2). To the present state of our studies, acidophilic
13 collembola do not seem to be more resistant to pollution by PAHs or heavy metals.
14 Garnier and Ponge (2004) showed that despite similarities between the chemical
15 environment of acid soils and that of metal-polluted soils, differences in pH and
16 microbial communities prevented acid-tolerant populations from surviving and
17 reproducing when introduced in a polluted site. In the present experiments, we
18 demonstrated that, when placed in the same environmental conditions (for culture and
19 experiment), acid-tolerant animals were not less sensitive to naphthalene than acid-
20 intolerant species. Two hypotheses can be proposed, i) an acclimatization time is
21 required to reveal the tolerance of a given population, ii) populations which withstand
22 low pHs and associated chemicals such as heavy metals, terpenes and phenolics are
23 not necessarily tolerant to every contaminant.

24

25 Naphthalene is the commonest agent of soil and water pollution by
26 hydrocarbons (Riser-Roberts, 1998; Preuss et al., 2003). Among PAHs, its higher
27 solubility in water and higher diffusibility in the air make it mostly responsible for the
28 immediate sensitivity of organisms to hydrocarbons (Ritchie et al., 2001; Sverdrup et

1 al., 2002). Measuring the avoidance of naphthalene by soil animals can thus be
2 considered as a relevant tool to assess the immediate sensitivity of these organisms to
3 hydrocarbon pollution.

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6
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10 New York, NY.

1 **Table 1.** List of collembolan species used in eco-ethological tests, and their origin and
 2 taxonomic affiliation. NS = neutral soil (pH_{water} 7 or 7.5), AS = acid soil (pH_{water} 4.5)

3

Species	Family	Acidity	Humus form	Origin	Code
<i>Arrhopalites caecus</i>	Arrhopalitidae	Neutral	Mull	Brunoy	NS Brunoy
<i>Folsomia candida</i>	Isotomidae	Acid	Moder	Pfaffenbronn	AS Pfaffenbronn
<i>Isotomiella minor</i>	Isotomidae	Acid	Moder	Brunoy	AS Brunoy
<i>Isotomiella minor</i>	Isotomidae	Acid	Moder	Pfaffenbronn	AS Pfaffenbronn
<i>Mesaphorura florae</i>	Onychiuridae	Neutral	Mull	Pechelbronn	NS Pechelbronn
<i>Mesaphorura macrochaeta</i>	Onychiuridae	Acid	Moder	Pfaffenbronn	AS Pfaffenbronn
<i>Mesaphorura yosii</i>	Onychiuridae	Acid	Moder	Brunoy	AS Brunoy
<i>Mesaphorura yosii</i>	Onychiuridae	Acid	Moder	Pfaffenbronn	AS Pfaffenbronn
<i>Parisotoma notabilis</i>	Isotomidae	Neutral	Mull	Pechelbronn	NS Pechelbronn
<i>Parisotoma nitabilis</i>	Isotomidae	Acid	Moder	Pfaffenbronn	AS Pfaffenbronn
<i>Proisotoma minima</i>	Isotomidae	Acid	Moder	Brunoy	AS Brunoy
<i>Proisotoma minima</i>	Isotomidae	Acid	Moder	Pfaffenbronn	AS Pfaffenbronn

4

1 **Table 2.** Avoidance tests. Mean (\pm standard error) percent presence of collembola on
 2 test side of Petri dishes (5 counts at 20 minute intervals, 20 replicates) at 4 dilution
 3 rates of the naphthalene solution. Differences between control and test side were
 4 tested by t test. Significant departure from no effect values (50% presence on test side)
 5 is indicated in bold underlined type ($P < 0.05$).

6

Species	Strain origin	0.003 mg.L ⁻¹	0.03 mg.L ⁻¹	0.3 mg.L ⁻¹	30 mg.L ⁻¹
<i>H. nitidus</i>	NS Pechelbronn				48.00 \pm 10.60
<i>F. candida</i>	AS Pfaffenbronn	49.00 \pm 8.64	40.00 \pm 7.68	44.00 \pm 8.16	52.00 \pm 7.73
<i>I. minor</i>	AS Pfaffenbronn	42.00 \pm 8.07	44.00 \pm 9.47	<u>31.00\pm7.88</u>	<u>32.00\pm7.46</u>
<i>P. notabilis</i>	AS Pfaffenbronn	49.00 \pm 7.88	46.00 \pm 9.30	40.00 \pm 7.11	<u>30.00\pm8.27</u>
<i>P. notabilis</i>	NS Pechelbronn	37.00 \pm 9.87	<u>30.00\pm7.33</u>	<u>26.00\pm8.96</u>	<u>28.00\pm8.00</u>
<i>P. minima</i>	AS Brunoy	49.00 \pm 7.47	48.00 \pm 8.75	<u>32.00\pm8.13</u>	<u>33.00\pm7.15</u>
<i>P. minima</i>	AS Pfaffenbronn	58.00 \pm 7.80	53.00 \pm 8.86	51.00 \pm 8.88	37.00 \pm 8.62
<i>M. floriae</i>	NS Pechelbronn	50.00 \pm 7.75	42.00 \pm 7.66	<u>20.00\pm6.96</u>	<u>31.00\pm7.18</u>
<i>M. macrochaeta</i>	AS Pfaffenbronn	51.00 \pm 7.61	51.00 \pm 9.78	<u>31.00\pm6.72</u>	<u>29.00\pm5.52</u>
<i>M. yosii</i>	AS Brunoy	52.00 \pm 8.51	<u>18.00\pm5.96</u>	<u>28.00\pm8.87</u>	<u>24.00\pm8.16</u>
<i>M. yosii</i>	AS Pfaffenbronn	36.00 \pm 8.03	43.00 \pm 9.21	<u>32.00\pm8.00</u>	<u>13.00\pm5.48</u>
<i>A. caecus</i>	NS Brunoy	53.00 \pm 8.62	41.25 \pm 8.37	<u>25.56\pm7.64</u>	<u>22.00\pm6.94</u>

7