A NEW EFFECTIVE FLOTATION METHOD FOR THE COLLECTION OF NIDICOLOUS PARASITES AND OTHER NEST INHABITANTS

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Abstract. The paper is based on experiments carried out with the aim of finding a new effective method and a suitable solution for flotation. 23 different solutions were used and 2 techniques were tried, using Balogh's vessel and modified Erlenmeyer's flasks. A total of 60 nests (50 nests of Delichon urbica, 8 nests of Passer domesticus, 2 nests of Micromys minutus) were used in the experiments. The results were evaluated according to the number of extracted arthropods and their quality in preparations and were confirmed by a control flotation. On the basis of all facts obtained the following solutions can be recommended for the flotation of nidicolous arthropods: a) flotation solution of technical tetrachloride (CCl₄) and 96% denaturated alcohol (205:65) of specific gravity 1.400 (a newly used combination), b) technical tetrachloride of specific gravity 1.615, c) solution of potassium carbonate (K₂CO₃) in water (1:1) of specific gravity 1.525.

In experimental work with nidicolous arthropods their separation from the material examined is very exacting. The method of direct sorrning by hand under a binocular microscope is very tedious and time-consuming. Experiments were therefore carried out with the aim of finding a more effective technique based on the principle of concentration of arthropods during flotation.

Material and Methods

60 nests were used in the flotation experiments: 50 nests of Delichon urbica (Linné, 1758), 8 nests of Passer domesticus (Linné, 1758) and 2 nests of Micromys minutus (Pallas, 1771). In all experiments a total of 19 groups of nidicolous arthropods were extracted: Aphaniptera, Diptera, Coleoptera, Collembola, Cimicidae, Hymenoptera, Pentatomidae, Thysanoptera, Aranea, Pseudoscorpionidae, Analgoidea, Oribatoidae, Tyroglyphoidae, Trombidiformes, Parasitiformes, Ixodidae, various eggs, larvae and puppae. Apart from the groups obtained from nests also Oribatids from the feces of the bat Myotis myotis (Borkhausen, 1797), larvae and imagos of fleas Hystrichopsylla talpae (Curtis, 1826) from the colonies cultivated at the Institute of Parasitology, Czechoslovak Academy of Sciences, were used in the control flotations.
The experiments were carried out in Balogh's cylinder (Balogh 1938) and in modified Erlenmeyer's flasks, fitted with a vent tube at the side and a stopper at the end of a rod. As flotation media a total of 23 different solutions were used, of which some had already been employed by other authors for flotation in soil zoology or helminthology, some were modified with different specific gravity and some have never been tried in flotation of arthropods before. Each of these 23 solutions was used in 2 experiments respectively and for each of them material was taken from one nest. The original procedure consisted of the following processes: sedimentation I, filtration I, flotation, washing, sedimentation II, filtration II and control of sediment.

In order to know whether the solutions tend to damage the floating arthropods from each experiment 10 smaller specimens from all groups were mounted in the Swan liquid and the preparations were checked twice, at first immediately after mounting, the second time after a 2-month drying process in thermostat at the temperature of 37 °C. The preparations were examined for the presence of air bubbles or crystallized media in the mounted arthropods. The solutions which had proved best were used in control flotation of 7 selected groups of arthropods, each time taking one hundred specimens mixed with 40–50 cubic cm of soil.

Results

From all solutions as most suitable was proved the solution of technical tetrachloride (CCl₄) and 96 % denaturized alcohol in ratio 205 : 65, of specific gravity 1.400 and temperature of 20 °C. In this solution all groups of nest inhabitants were floated with no negative influence upon their mounting. This combination of chemical substances has never before been used in the flotation of arthropods. A control flotation with this solution also yielded very good results. 100 % of all specimens mixed with soil were extracted.

Good results were also obtained with the solution of technical tetrachloride (CCl₄), of specific gravity 1.615 (Sgolina 1935), and with the solution K₂CO₃ + water (1 : 1), of specific gravity 1.525 (after Hovorka 1954). Both solutions, however, tend to drive up a large quantity of various debris together with the liberated arthropods and make further procedure more difficult. With all three solutions the total period necessary for the flotation proper lasts about half an hour.

The Balogh’s cylinder used in first experiments had some faults (a high column of sediment, a narrow space between the stopper and the walls of the cylinder). On the other hand, the modified procedure after Ladell (1936) (using two vessels with a side tube for the flow of the floating material) proved quite good.

The experiments have shown that the processes sedimentation II, filtration I and II can be omitted in the procedure, so that the practical method of obtaining nidicolous animals from the treated material is as follows:

1. Preparation of the flotation solution.

2. Sedimentation. The nest material preserved in 70% alcohol (about 100 ccm) is placed into a flotation vessel (about 750 ccm) and left to settle, the vessel being in a horizontal position.

   Afterwards the fluid fixative is entirely removed.
3. Flotation. The flotation vessel is returned to its vertical position and flotation solution is added into it up to about 5 cm below the draining tube to prevent the arthropods from being carried away eventually during next manipulation. A stream of air is bubbled into the suspension by means of a rubber blowball. When another portion of flotation solution is added, the suspension level rises almost to the opening of the tube and the liberated animals float to the surface, forming an upper layer, which is separated from the flotation solution by lifting a glass rod provided with a cork stopper. The flotation solution is added to the separated layer by means of a washbottle and the animals flow to a cleaning flask filled nearly to its brim with clean solution. The separation of the floating layer is repeated 1—3 times as long as there are any arthropods in it. The second separation of animals from the cleaning flask is done in the same way. Clean arthropods are collected on a filter in a funnel placed in a third flask.

4) Washing and preservation of extracted nidicolous animals. The washing process is done immediately after the animals are collected, while they are still wet; they are washed in water and then in 70% alcohol if the flotation solution comprises water, or in 70% alcohol, if CCl₄ solution is used for flotation. Small arthropods are then preserved in 70% alcohol. The device used for flotation is shown in Fig. 1.

Fig. 1. 1 — a reservoir bottle (about 750 ccm) for storage of flotation solution, 2 — rubber hose, 3 — glass tube, 4 — network of air distribution, 5 — rubber hose, 6 — glass tube, 7 — glass rod with a cork stopper at the end, 8 — flotation (glass) flask, 9 — glass rod with a cork stopper at the end, 10 — (glass) cleaning flask, 11 — funnel (glass or plastic) with filter paper, 12 — flask for the used flotation solution, 13 — laboratory rubber blowball.
Conclusion

On the basis of all facts obtained in the experiments the following solution may be recommended for the flotation of nidicolous arthropods:

a) flotation solution of technical tetrachloride (CCl₄) and 96% denaturized alcohol (205 : 65) of specific gravity 1.400 (a newly used combination)
b) technical tetrachloride of specific gravity 1.615
c) K₂CO₃ solution in water (1 : 1) of specific gravity 1.525.

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