Short Communication

Modification of a leaf-washing apparatus for the recovery of mites

Jozefína Jedličková*
Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, SK-842 06 Bratislava, Slovakia
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ABSTRACT

Boller's funnel and sieve apparatus for extracting phytoseiid mites (Acari: Phytoseiidae) from leaves was modified. The modification consisted of including a reservoir dish enabling the placing of the whole sample of leaves into the apparatus, attaching a collecting chamber, simplification of the fixing of the fine lower sieve and adding an upper fine sieve. This modification enables the rapid processing of samples of up to 150 leaves.

Key words: Acari, Phytoseiidae, sampling, leaf-washing apparatus.

INTRODUCTION

Estimates of the number of small arthropods, in particular spider mites (Acari: Tetranychidae) and predatory mites (Acari: Phytoseiidae), from foliage are often necessary in population ecology and pest control. A direct census of mites on leaves using a stereomicroscope is tedious, time-consuming and, therefore, may be subject to inaccuracies. This has led many authors to develop methods that make the whole procedure of extracting small arthropods, in particular mites from foliage samples, easier, quicker and more precise. Different methods have been developed for the extraction of mites from foliage, such as a brushing machine (Henderson and McBurnie, 1954) or shake and wash methods (e.g. Henderson, 1960) in which the mites are washed off foliage using different solutions, for example ethanol (Zacharda *et al*., 1988), sodium hypochloride (Andres, 1957) or detergents (Boller, 1984). The use of shake and wash procedures is associated with the construction of a reasonably complicated apparatus. In the simple cases, the apparatus consists of a funnel and sieves (Boller, 1984) or a separating funnel (Zacharda *et al*., 1988); the more complicated apparatuses are rotation washing machines (Leigh *et al*., 1984).

During research of autochthonous phytoseiids on both wild and cultivated

*To whom correspondence should be addressed at: Tel: 421 7378 3266; Fax: 421 7374 284.

shrubs and trees, the author was faced with the problem of processing samples of 100 leaves; this sample size was necessary because of the low density of phytoseiids, in particular in the first half of the season. The same problem occurred in another study, where samples of 100 apple leaves sampled in the framework of a study of phytoseiids in apple orchards were processed using Boller's apparatus (Boller, 1984). The need to process samples consisting of up to 150 leaves each led to a modification of Boller's apparatus, enabling the processing of numerous larger samples in a short time.

MATERIALS AND METHODS

Description of the apparatus

The equipment (Fig. 1) consisted of the following.

(1) A plastic hemispherical or commercial rinse dish used for fruit washing, with holes (4×4 mm) in the bottom. A rinse dish with an upper diameter of 28 cm was used, but any other suitable dish can be used. The dish serves as a reservoir in which to place the whole sample.

(2) A rough sieve (mesh approximately 2 mm) for catching dirt and bigger leaf fragments and/or bigger arthropods if present. Standard laboratory sieves with an appropriate mesh size may be used.

(3) A middle washing sieve (mesh size 0.3–0.5 mm) for catching smaller leaf fragments, bigger trichomes, dust and/or arthropods.

(4) A funnel with a diameter of approximately 60 cm.

(5) A circular nylon net (mesh size approximately 0.16 mm) with a diameter approximately 4 cm larger than the diameter of the plastic chamber (7). In the middle of the net there is a circular opening with the same diameter as the lower funnel tube. The net prevents the possible escape of mites that could float on the detergent foam during the waste washing.

(6) Rubber rings for fastening the net (5) to the chamber (7).

(7) A plastic collecting chamber made from a wide-necked plastic bottle (200–250 ml) at least 11 cm high, by cutting off the bottom (in the apparatus the top); the neck should be at least 4 cm in diameter and provided with a thread.

(8) A circular net of the same material as the net (5), with a diameter at least 1 cm larger than the neck of the chamber (7). This net serves to catch mites and transfer them to a microscope or to a vial where the mites can be deposited for later study. The preparation of a large number of such nets is recommended.

(9) A plastic pot with the bottom cut off, approximately 5 cm high, with a bottom diameter the same as the outside diameter of the neck (serving as the bottom) of the collecting chamber. If the plastic pot has a thin wall, it is recommended strengthening it with adhesive tape. Instead of a plastic pot, the twist cap (stopper) from the bottle (7) may be used; its centre is carved out. The pot serves to fasten the net (8) to the collecting chamber (7) and as a base for the apparatus if a portable laboratory stand is not used.

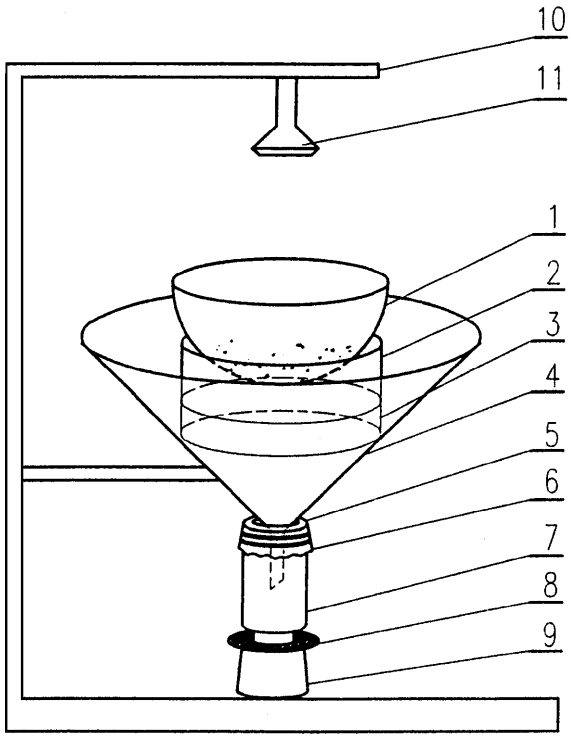

Fig. 1. The design of the leaf-washing apparatus. (1) Plastic hemispherical rinse dish, (2) rough sieve, (3) middle washing sieve, (4) funnel, (5) circular nylon net, (6) rubber rings, (7) plastic collecting chamber, (8) circular net, (9) plastic pot with cut off bottom, (10) portable laboratory stand and (11) laboratory hand-controlled shower.

(10) A portable laboratory stand for fastening the apparatus; if the pot (9) is used then a portable laboratory stand is not required.

(11) A laboratory hand-controlled shower.

Construction

The nylon net (5) is placed on top of the collection chamber (7) (originally the bottom of the bottle) and fixed by the rubber rings (6). The net (8) is fixed on the lower part of the collecting chamber (7) using the plastic pot (9) with the cut off bottom upwards. The funnel outflow tube is inserted into the circular opening of the nylon net (5). Then both washing sieves (2 and 3) are put into the funnel (4) (the rough sieve over the middle one). The rinse dish (1) is placed over the rough washing sieve (2). The apparatus is then place into the laboratory sink on the plastic pot (9) in such a way that water outflow is guaranteed. The equipment can be stabilized by the laboratory stand (10).

Sample processing

The sample, consisting of up to 150 leaves, is preferably placed into a plastic bag, sealed and transported to the laboratory. In the laboratory, the sample is transferred into a dish containing water to which approximately $1\,ml^{-1}$ of a commercial detergent (for example, Pur^{TM} produced by Henkel Palma, Bratislava) is added. Laboratory bath tanks with a volume of approximately 1.5 l or wide-necked bottles were found to be suitable. The sample is kept in the bath for 1.5–2 h during which time the leaves are shaken or stirred from time to time. After the bath, the whole sample is transferred into the rinse dish (1) and the detergent solution is poured over the batch. Most of the mites, loosened from the leaves in the bath, are now flushed into the collecting chamber (7). Then, under a fine stream from a laboratory shower (11) the leaves are washed on both sides over the dish (1). During this procedure all the remaining mites on the leaves are washed into the apparatus.

In the following order, the rinse dish (1), sieves (2) and (3) and funnel (4) are removed from the apparatus. Each of the removed parts is washed with a fine stream of water from a laboratory shower (11) to flush off any mites that may remain on the walls of the equipment.

At this stage of the sample processing, all of the mites are in the collection chamber (7); most of them can be found on the circular net (8), but some may rest on the circular nylon net (5) having floated there by the detergent foam during the waste washing. After being released, the inner surface of the circular nylon net (5) is washed with water from a sprinkling bottle into the collecting chamber (7). The inner walls of the chamber are washed in the same way, so that all mites are found on the circular net (8). After removing the collecting chamber (7) and the plastic pot or twist cap (9), the mites can be transferred with the circular net (8) to the stereomicroscope for direct examination or can be fixed and deposited in a suitable vessel (e.g. a vial).

The apparatus was used for processing 120 samples (100 leaves each) of wild and cultivated fruit species with different leaf sizes and shapes. The number of phytoseiids per sample varied from one to several hundred. All the samples were processed by the method described and afterwards they were checked for phytoseiids; no residual phytoseiids and spider mites were found on the leaves after processing. The high accuracy of the method may be due to the fact that each leaf is washed on both sides ensuring that all the mites on the leaves are washed off.

DISCUSSION

The modification of both the apparatus and the technique emerged as a consequence of processing a great number of samples of 100–150 leaves each. Most of the methods developed by Boller (1984), Leigh *et al*. (1984) and Zacharda *et al*. (1988) allow the processing of samples of 25 leaves or less. If

bigger samples had to be processed, they had to be divided into smaller subsamples which is time-consuming. The use of a rinse dish in the modified apparatus enables the processing of the whole sample without decreasing the effectiveness and introduces the possibility of use with other plant parts, e.g. fruits. Flushing the mites into a rinse dish accelerates the processing and the washing of the leaves with a laboratory shower decreases the possibility of mites remaining among the trichoma or near leaf veins.

Rapid washing with a detergent may cause foaming. This may slow the processing and, if the foam overflows, may cause some loss of floating mites. This problem is avoided by using the collecting chamber which can contain a larger quantity of washing solution and the wider bottom opening (compared with the original apparatus) enables a higher washing speed. The circular nylon net (5) prevents the escape of mites on the foam and enables a higher washing speed as well.

The construction of the apparatus, handling and sample processing is simple and rapid. After some experience, the processing of one sample of 100–150 leaves takes no more than 10 min, including the assembly and dismantling of the apparatus. The processing of the samples is continuous. The apparatus is very economical and may be constructed in every standard equipped laboratory.

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