A METHOD FOR EXTRACTING PEAR THRIPS FROM FOREST SOILS

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Introduction

A standardized method of extracting pear thrips from forest soils, modified from a procedure of Walter et al. (1987) was developed. Several factors were considered of prime importance when developing this extraction method:

1. Accuracy of determining number of thrips per sample,
2. Minimization of extraction processing time,
3. Simplicity of methodology,
4. Consistency of extraction efficiency,
5. Cost.

Soil Sampling

Samples are taken with a hand-held tulip bulb planter (5.72 cm diameter, 10.16 cm length), giving a volume of about 261.09 cm^3 of soil and bagged individually (Fig. 1). The leaf litter is removed prior to taking soil samples. Our research has shown that thrips are not found in this litter layer in September and October, when soil samples are collected. Removal of this light organic matter increases the rate of extraction by reducing the amount of material that floats with the thrips.

Figure 1. Pear thrips soil sampling using a hand-held bulb planter.

Sample Rinsing and Sieving

Thrips are separated from the homogenized soil using USA Standard full height testing sieves (ASTME-11 specification) arranged in three tiers with a top sieve (205-mm diam) with 2.0-mm mesh, a middle sieve (305-mm diam) with 850-micron mesh and a bottom sieve (305-mm diam) with a 250-micron mesh. The sample is placed in the top sieve and washed with water using a 100-cm wide water breaker fan attached to a hose (Fig. 2). Remaining clumps of soil are broken apart by hand. Disposable surgical gloves are worn to protect the hands. The top sieve is lifted to allow the underside to be lightly rinsed over the second sieve, thereby removing any thrips that may adhere to the bottom. This rinsing procedure is repeated with the middle sieve. The residues from the first two sieves are then discarded.

Figure 2. Rinsing of soil sample to remove large pieces of organic matter prior to flotation.

The residue from the bottom sieve is rinsed with cold water into a 1-litre Nalgene bottle, sealed with a tight-fitting screw cap and stored at about 5°C for 8-12 hours or processed immediately as described below. Samples containing a large amount of organic matter are generally stored for about 8 hours to allow some of the unwanted organic matter to become waterlogged and sink during the flotation process.

Heptane Extraction/Flotation Process

Protective goggles should be worn during the following procedures. The contents of the Nalgene bottle are poured through a short stem funnel into a 2000 ml separatory funnel which is positioned on a ring stand in a fume hood. The Nalgene bottle and sides of the funnel are rinsed with water from a plastic squeeze bottle to ensure all contents reach the separatory funnel.

Additional water is added until the total volume in the separatory funnel is 1,215 ml. To facilitate this a line is drawn on the separatory funnel indicating the approximate level desired. Next, using an automatic dispensing pipette, 50 ml of technical grade heptane is added (Fig. 3). This forms a 3-mm layer above the water and organic matter (Fig. 4).

The separatory funnel is removed from the ring stand and held in a horizontal position. The contents is gently shaken back and forth to remove the organic matter that might be lodged in the funnel stem or neck and then the funnel contents is rotated 10 times to mix the heptane with the water - organic matter layer (Fig. 5). Each rotation should be done in the same deliberate manner, pausing between each swirl to allow separation within and taking care not to use excessive vigor (force). To avoid organic matter becoming lodged in the neck, the funnel is tipped slightly during the rotation process. The contents is then shaken back and forth 10 times. This completes the mixing and the separatory funnel should be returned to the ring stand.

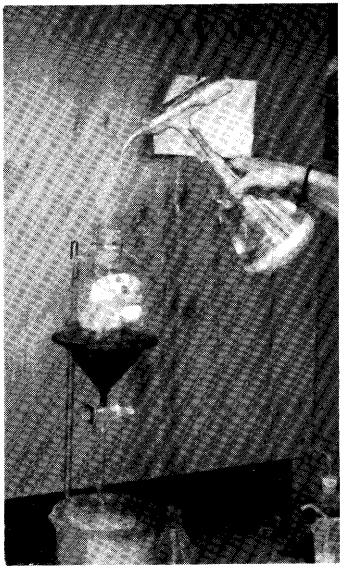

Figure 3. Addition of 50 ml of heptane to separatory funnel for flotation of thrips from soil residue.

Figure 4. Separatory funnel containing water, soil residue with thrips and a thin layer of heptane floating on the surface. Thrips float at the water-heptane interface.

Figure 5. Agitation of material in the funnel to stir heptane into the sample residue solution.

The glass stopper is removed to relieve internal pressure and the sides are rinsed into the funnel with a small amount of water from a squeeze bottle. The separatory funnel is returned to the ring stand and left to stand for a minimum of two minutes to allow the heptane to partition from the water, the organic matter to sink and the thrips to move to the water-heptane interface. The stop-cock is opened carefully and the water and debris are allowed to drain slowly until approximately 60 ml of water and the heptane remains in the funnel. Care is taken not to open the stop-cock to the point where a whirl-pool motion occurs during the draining. To remove fine debris if it adheres to the side of the separatory funnel, the drainage process is stopped and the funnel side is tapped or the funnel is shaken near the stem. Care is taken not to overly agitate the entire contents of the funnel as thrips may be forced down into the portion being drained.

The layer of heptane and remaining water is drained onto a disposable prefolded coffee filter over a Buchner funnel attached to a suction pump (Fig. 6). The sides of the separatory funnel are rinsed to

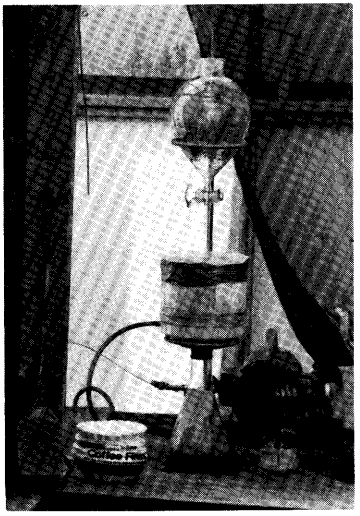

Figure 6. Drainage of thrips and light organic matter onto paper filter over Buchner funnel.

remove any organic material or thrips that may remain. More than one filter are used if a large amount of organic matter residue is obtained in the flotation process.

Thrips Counting Procedure

The paper filter is spread smoothly onto a 20 x 20 cm glass plate marked with a 1-cm^2 grid. This is placed on a light table and thrips are counted using a binocular microscope with 8x magnification. For species identification, slide mounts are made for viewing under high power. Testing is underway at the Entomology Research Laboratory to test the efficiency of this extraction method for recovering pear thrips from the soil.

Figure 7. Inspection of filter containing organic matter and thrips over an illuminated light table.

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References Cited

Walter, D. E., J. Kethley & J. C. Moore. 1987. A heptane flotation method for recovering microarthropods from semiarid soils, with comparison to the Merchant-Crossley high-gradient extraction method and estimates of microarthropod biomass. Pedobiologia 30: 221-232.