



Tullgren extraction of soil mites (Acarina): Effect of refrigeration time on extraction efficiency

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Introduction

Soil microarthropods constitute one of the most species rich communities in forest ecosystems (Crossley & Blair, 1991). The effects of soil fauna in these systems on decomposition rates, nutrient regeneration and soil structure have been well documented; however, dependable estimates of population size and community structure largely depend upon adequate sampling (Anderson, 1988; Seastedt & Crossley, 1988). Problems inherent in sampling microarthropods include their patchy distribution, large abundance and minute size. In practice, microarthropod sampling involves collection of leaf litter or soil which is returned to the laboratory for extraction by one of two methods: flotation or Tullgren extraction.

Flotation extraction does not depend on the mobility of arthropods and thus, from the organisms perspective, it can be considered a passive procedure. This type of extraction recovers both active and inactive microarthropods by suspending them in an immiscible fluid (Walter *et al.*, 1987). Conversely, the Tullgren extraction method is an active procedure that relies on the migration of arthropods from the sample. Mobile microarthropods move down through the soil sample in response to changes in heat and humidity gradients (Merchant & Crossley, 1970).

For forest ecosystems or others with highly organic soils, Tullgren extraction is the method of choice based on its simplicity and quick processing time (Crossley & Blair, 1991). However, no method is completely efficient. Tullgren extraction efficiency may be more variable than the flotation methods due to its failure in extracting inactive stages, or differential efficiencies for some groups of microarthropods.

We examined another variable affecting Tullgren extraction efficiency: storage time. Traditionally, when soil microarthropod samples are collected in the field, they are refrigerated until they can be extracted. Where field sites are remote from the laboratory, long refrigeration times may be necessary. The objective of this research was to examine the effects of five different refrigeration times on the extraction efficiency of microarthropods in modified Tullgren extractions.

Materials and Methods

Samples for this study were collected from a relatively undisturbed pine plantation on the Savannah River Site, near Aiken, South Carolina. The area is categorized as Upper Coastal Plain, and the dominant vegetation is ca. 50-year-old loblolly pines (*Pinus taeda*), with a developed understory. The study site is characterized by excessively drained soils with loamy subsoil, and a slope ranging from 0 to 10% (Brooks & Crass, 1991). The average litterfall inputs for October 1993 were 79.6 g/m² (Bailey-Lakly, 1994).

The coring tool and extractor described by Crossley and Blair (1991) were used in this study. Twenty samples were collected on October 31, 1993 with a round 5 cm diameter by 5 cm deep soil corer. All samples were taken from a single 0.5 m by 0.5 m plot, with care taken to avoid compaction of the samples. These were then wrapped in aluminum foil and placed in a Styrofoam cooler with ice for transport to the laboratory.

The twenty samples were then randomly assigned to five refrigeration periods resulting in four replicates of each. Immediately upon return to the laboratory, one set ($N = 4$) was set up for extraction using the modified Tullgren extraction method described by Crossley and Blair (1991). This method relies on a constant light source (7-W, 110-V Christmas tree lights) fitted inside beverage cans which are placed above one end of the sample. These are fitted into baffles and suspended over collection funnels. A temperature gradient of 20°C develops between the top and bottom of the sample within 6 h and may reach 30°C after 4 days of extraction (Crossley & Blair, 1991). The four remaining sets were stored in a 6°C refrigerator for 48, 96, 144, and 192 h, respectively before extraction.

Sample extraction preparation included spraying the top portion of the sample with 2–3 ml of water to create a greater moisture gradient within the core. Samples were covered with a double layer of cheesecloth and a layer of 0.5 mm mesh. They were then inverted and placed in the extractor for a seven day period. The collections of microarthropods were stored in 95% alcohol until they could be sorted.

Microarthropods were sorted to order, and the Acarina (mites) were further sorted to suborder. Oribatid mites were separated into immature and adult stadia.

Results and Discussion

The number of Acarina recovered from samples decreased linearly with longer storage times ($R^2 = 0.92$, Figure 1). An average of 547 mites per sample was recovered after 9 h of storage; after 192 h, the average number recovered had dropped to 268 per sample.

Among the groups of Acarina, the numerically dominant immature oribatid mites showed the greatest decline with increasing storage (Figure 2). Adult oribatids and prostigmatic mites exhibited less of a decrease with storage times, but recoveries were clearly depressed after 96 h of storage under refrigeration.

During refrigeration, soil cores doubtless experience a temperature drop that would induce changes in moisture conditions within the samples. We would expect that these changes in microclimate could alter the mobility of small, soft-bodied organisms including most prostigmatic mites and immature oribatids. We would further expect that the mobility of larger, more heavily sclerotized mites would not be as greatly affected.

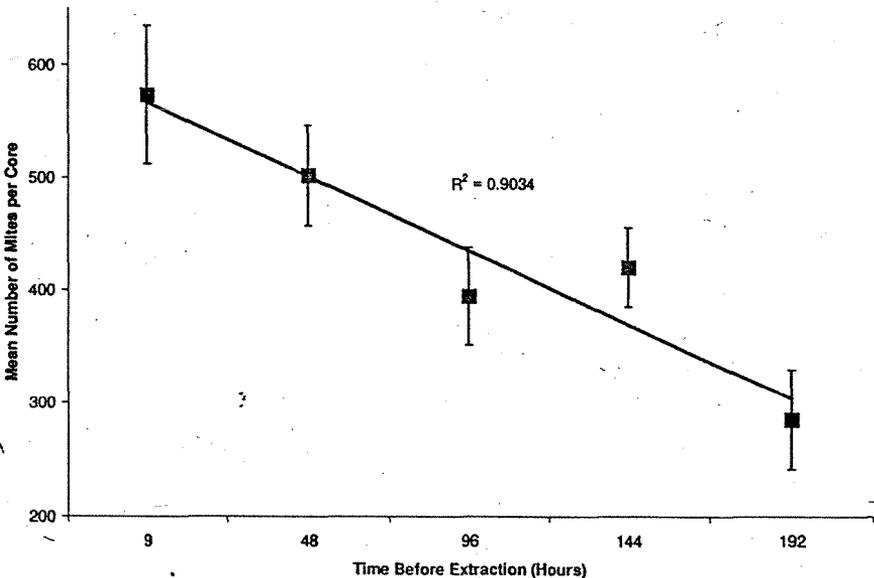


Figure 1. Acarina extracted from soil cores following refrigeration. Points shown are mean \pm standard errors ($N = 4$).

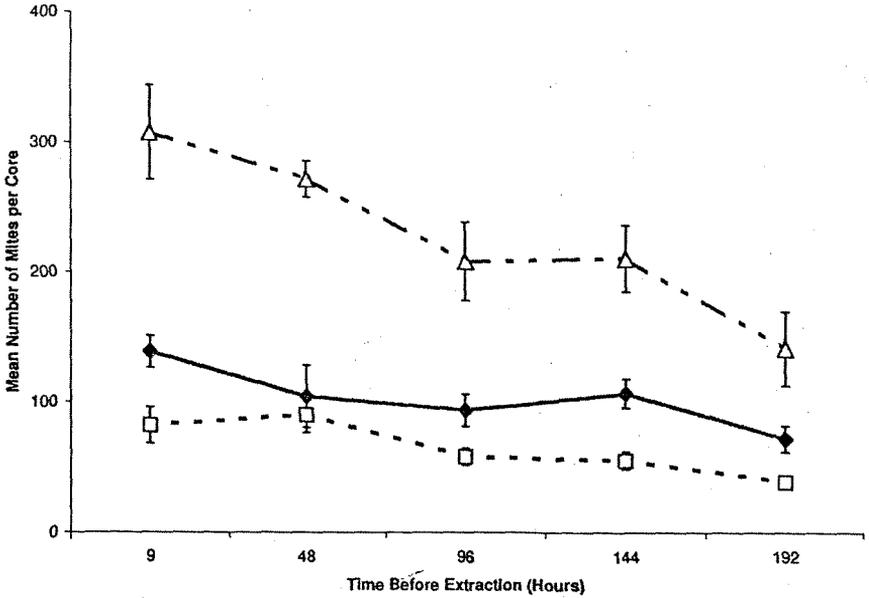


Figure 2. Numbers of adult Oribatei, immature Oribatei and Prostigmata (Acarina) extracted from soil cores following refrigeration. Points shown are mean \pm standard errors ($N = 4$). - \square , Prostigmata; \blacklozenge , Oribatei; \blacktriangle ; Immature Oribatei.

Our results are consistent with these expectations. Adult oribatids in general are larger, more heavily sclerotized mites, and they showed the least effect of storage under refrigeration. Conversely, immature oribatids are unable to move as quickly as adults, and this combined with a rapidly changing microclimate under refrigeration may have hindered their extraction efficiency (Usher, 1975). In fact, these immature forms were most negatively affected by refrigeration ($p = 0.001$, $R^2 = 0.94$) (Figure 2).

The Prostigmata includes a variety of forms, some susceptible to desiccation (i.e., Eupodidae) and some resistant (i.e., Tarsonemidae). They may not respond as well to Tullgren extraction as other mite groups (Price, 1973). In our samples, prostigmatic mite numbers declined with increasing refrigeration, although not as much as the immature oribatids (Figure 2).

This preliminary data suggest that refrigeration time affects the efficiency of Tullgren extractions from highly organic soils such as those in coniferous forests. The numbers collected after only 9 h of storage are not different from those obtained when extracting fresh, unrefrigerated samples (Merchant & Crossley, 1970; Crossley & Blair, 1991). We were surprised to find that refrigerated storage for only 48 h induced some reductions in numbers of mites recovered.

The limited scope of this study did not allow determination of potential mechanisms responsible for the decline in extraction efficiency with increased storage time. These could include direct mortality due to temperature and moisture changes, dormancy, or changes in soil structure that inhibit microarthropod movement. We would suggest that a more comprehensive study including different temperature regimes, a larger sample size and direct comparison with a flotation extraction method in soils of lower organic content could address these mechanisms. However, based on these initial results, we suggest that soil cores should be stored for as little time as possible before extraction. If a refrigeration period is necessary, the authors suggest an alternate method of soil microarthropod extraction, such as the flotation method of McSorly and Walter (1991), be employed.

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