INTRODUCTION

Several methods have been devised for the determination of numbers of eelworms in soil samples: direct microscopy (Stöckli, 1944), the Baermann funnel method and variations of it (Overgaard Nielsen, 1949), Cobb's decanting and sieving method (Cobb, 1918, Goodey, 1949), a combination of the latter and the Baermann funnel method (Christie and Perry, 1951), methods replacing the decanting by a separation by means of an upward current of water (Cobb 1924, Spereiter 1953, Oostenbrink 1954) and centrifugal flotation of soil suspensions in a sugar solution (Caveness and Jensen, 1955). The first two methods are for different reasons unsatisfactory. They were discussed by the writer in an earlier paper (Seinhorst, 1950). According to Lownsberry, Lownsberry and Mai (1951) only 30% to 50% of larvae of Heterodera rostochiensis added to soil samples could be recovered by Cobb's method. When applied to Dutch soils this method was also found to be inadequate, as a considerable number of eelworms were not recovered from the samples and the fine organic particles in these soils made the separation of the eelworms from the soil particularly difficult. Very good results may be obtained by centrifugal flotation of soil suspensions in a sugar solution (Caveness and Jensen, 1955) but the quantities of soil that can be investigated are generally too small for work on plant parasitic nematodes. Total numbers of eelworms recovered per unit of soil by means of the other methods appear to be generally much lower than those found in similar soils by direct microscopy, centrifugal flotation or the methods described below.

The separation of eelworms from soil by Cobb's method is based on the fact that soil particles, with both a lower and slightly higher settling speed in water than eelworms of a certain size, have a smaller
diameter than the meshes of sieves through which the eelworms do not easily pass (diameter of meshes about 0.1 to 0.05 of length of eelworms), and that soil particles, which have a larger diameter than the meshes of these sieves, have a distinctly higher settling speed in water than eelworms with a length up to 20 times the diameter of these meshes.

A homogeneous suspension of the soil sample to be investigated is made in water. When this suspension is left standing until all soil particles larger than a certain size have reached the bottom of the vessel, a large proportion of the eelworms shorter than 20 times this diameter is still in the suspension together with a proportion of the smaller soil particles. This suspension of the eelworms and small soil particles can be separated from the deposit by decanting carefully. The eelworms are then separated from the small soil particles by sieving the decanted suspension through a sieve with meshes of diameter slightly larger than those of the largest soil particles decanted. The majority of the eelworms remain on the sieve whilst all soil particles pass through. To separate eelworms of various sizes from soil, different settling times and sieves with meshes of appropriate size can be applied successively.

As the eelworms in the suspension do not all have to travel the same distance to the bottom of the vessel, a number settle between the soil particles and remain there whilst the supernatant suspension is being decanted. The separation can be improved by bringing the deposit into suspension again and repeating the process but this tends to become time consuming. Theoretically, therefore, if the settling speed of the nematodes could be reduced to zero or if they could be moved slowly upwards the separation would be much better. With small samples this can be done by using salt or sugar solutions of a high specific gravity.

For large samples a better method is to use an upward current of water. Two types of apparatus based on this principle have been constructed. They both consist of three parts:

1. An erlenmeyer flask (contents 1-2 litre) with a funnel shaped mouth piece;

2. A U tube with a narrow cylindrical leg, inner diameter 5 mm, and a wider partly cylindrical partly conical leg.

3. An apparatus producing a constant flow of water.

The separation of the eelworms from the soil is done in two stages.