A Centrifuging Method for Root Sterilization

In isolating mycorrhizal fungi present in root tissues, the external surfaces must be sterilized without damaging the hyphæ within. Existing techniques involving chemical sterilization or removal of the epidermis under aseptic conditions were found to be unsatisfactory in *Calluna vulgaris* and *Vaccinium myrtillus*, as they involved loss of hyphæ in the epidermis where the hyphæ were concentrated. Accordingly, a centrifuging technique was devised which obviated these difficulties.

The apparatus consists of a small glass tube, fitted into a 'Pyrex' centrifuge tube. A piece of fine muslin is stretched across the top of the inner tube and secured by waterproof adhesive tape. The base of the inner tube is wrapped in cotton wool. This is essential for even distribution of pressure during centrifuging. Sufficient sterile water is added to cover the muslin.

The roots to be surface-sterilized are thoroughly washed in detergent before being placed on the muslin of the inner tube. They are then centrifuged for three to four minutes at 7,500 rev./min., removed and shaken vigorously in two tubes of sterile water to dislodge contaminants still attached to the root surface. Prior to each centrifuging, 50 per cent alcohol is used to sterilize the apparatus. Three times in the centrifuge, each time followed by agitation in two tubes of sterile water, except after the last, when four tubes are used, results in a high proportion of surface-sterile root sections 3–5 mm. in length.

John C. Hopkins

Department of Botany,
Royal Botanic Garden,
Edinburgh 4.
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Environmental Determination of the Sex Ratio of a Plant Parasitic Nematode

The sex ratio of the potato root eelworm *Heterodera rostochiensis* Wollenweber has been under investigation in this laboratory since 1950. From the first it appeared that the ratio is very variable; it is now clear (*a*) that the ratio can be different on primary and lateral roots, and (*b*) that it varies with the intensity of infection.

Plants were raised in pots and grown either on a laboratory window bench or in the field: either they were grown in soil containing eelworm cysts or in sterile sand to which larvæ were added afterwards. As earlier stages are difficult to identify with reasonable ease and certainty, all plants were lifted when the males were adult but still 'encysted'; at this stage females are swollen but still within the root tissues. But as the males leave the roots soon after, care was taken to lift the plants in time. The roots were then carefully washed, treated with lactophenol-acid fuchsin in the standard way[1], and the number of adult males and females counted with the aid of a dissecting microscope; no attempt was made to sex early stages, but the total number of worms was noted. Although most of the worms are readily visible, it was nevertheless necessary more or less to dissect the root system to ensure that all worms were found; and, in the case of the field-grown plants where the root system was particularly extensive, sometimes only a sample of the root system was examined. I am most grateful to Mrs. E. Capstick for the care with which she carried out these examinations.

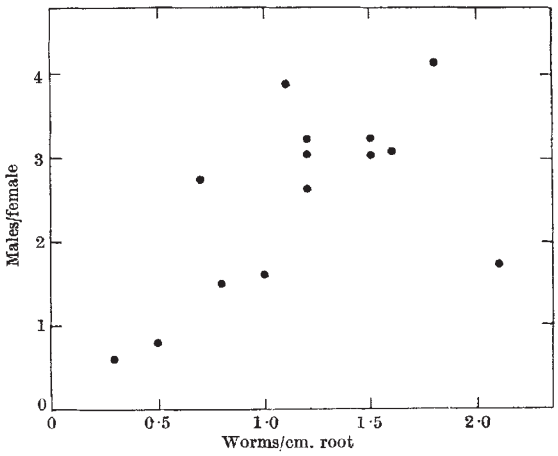

Fig. 1. Increasing proportion of males with increasing intensity of attack

The results for fourteen plants, part of a typical experiment with a number of potato varieties, are shown in Fig. 1; the different plants are compared on the basis of the length of primary roots of the root system examined. The sex ratio (males per female) is plotted against intensity of attack; it will be seen that, as the intensity increases, a higher proportion of males is found, the sex ratio changing from about 1 : 2, at the lower levels, to about four males to one female at the upper end; and higher values have frequently been found. 'Intensity of attack' was estimated from the number of worms of all stages per cm. of primary root, although, of course, worms in both primary and lateral roots are included. The method is not beyond criticism; indeed, an investigation of alternatives, such as the exclusion of juveniles, may itself help to clarify the situation. But other standards do not alter the general trend: the higher the intensity of attack, the higher the proportion of males, or the lower the reproductive rate.

Clearly, the phenomenon may help to explain the puzzling manner in which soil populations of the potato[2,3] and the closely related beet eelworm (*H. schachtii*)[4] increase; for the rate of multiplication decreases with the number of eelworm eggs in the soil.

A fully developed female with her many hundreds of eggs must be a considerable drain on the host plant. Whatever the mechanism, therefore, a reduction in the relative number of females with intensity of attack is a remarkable adaptation. There appear to be two possibilities: either the change in sex ratio is achieved as a result of a differential death-rate; or it is due to environmental influence on the process of sex determination.

A differential death-rate could operate by eliminating a progressively higher proportion of females before they reach the host plant; alternatively, progressively more females might fail to reach maturity within the plant. Careful examination of a number

Table 1. Distribution of Sexes on Primary and Lateral Roots. Figures are mean values per plant for young plants grown in pots to each of which 900 larvæ were added

	Adult male	Adult female	Male/female	Unclassified
Primary	38·3	23·8	1·61	24·9
Lateral	172·9	20·7	8·35	45·1