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## A method for observing and rearing the root-feeding larvae of *Longitarsus albineus* (Foudras) (Col. Chrysomelidae)

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A method is described for observing and rearing the immature stages of *Longitarsus albineus* (FOUDRAS). Mated females, kept in a petri dish, laid eggs in the folds of a moist filter paper strip wrapped around the petiole of a *Heliotropium europaeum* leaf. Four heliotrope seedlings were transferred into an 800ml beaker between the glass and a dark coloured blotting paper cylinder filled with coarse sand. After sufficient, further root growth, eggs or larvae were placed on the rootlets where they developed. A brown paper sheath placed around the beaker kept the rootlets in darkness and could be removed to observe larval development.

This method was also used successfully to rear *Sitona* spp. (Col. Curculionidae) and may be used to observe and rear other small, root-feeding invertebrates.

Current methods for observing or rearing root-feeding insects (PETERSON, 1964) were found to be unsuitable for the rigorous testing of the host specificity of the larvae of the flea beetle, *Longitarsus albineus* (FOUDRAS) (Col. Chrysomelidae), that is necessary to clear it for introduction into Australia for biological control of the weed, *Heliotropium europaeum* L. A new method was developed which enabled the beetle to be reared from the egg while allowing observations to be made on the immature stages without undue disturbance to them. The larvae of *L. albineus* had not previously been found in nature (WILSON, 1960) and previous attempts at rearing the beetle failed (V.L. DELUCCHI, pers. comm. to J.P. AESCHLI-MANN; BALCELLS, 1952).

Mating pairs of *L. albineus* collected from the host plant in the field were kept in 5 cm diameter plastic petri dishes. Food was provided in the form of a fresh *Heliotropium* leaf with its petiole rolled in a strip of white blotting paper moistened with water. Females readily laid eggs between the folds of the paper at the junction with the petiole. The blotting paper was unrolled daily to expose the eggs which were transferred with a moistened brush to a moist filter paper in a petri dish for incubation. Old leaves were replaced with fresh ones every few days.

A broad rimmed, lipless, unmarked, 800ml beaker was lined on the inside with a close fitting cylinder of green blotting paper glued at the join and ending 5mm below the beaker rim. A disk of blotting paper slightly larger than the beaker diameter was then placed at the bottom of the beaker with its upturned edges pressing against the inside of the paper cylinder. Seedlings of host or test plant to be used were carefully removed from the trays in which they had been grown and their roots washed free of soil. From 4-6 plants (depending on size) were arranged around the beaker between the glass and blotting paper with their root collars 10mm below the top edge of the paper. Once the seedlings had been positioned the blotting paper was kept in place by filling the beaker with clean, coarse, sand subsequently moistened with water until the blotting paper was completely wetted. An opaque paper sheath glued at the join was placed around the beaker to keep

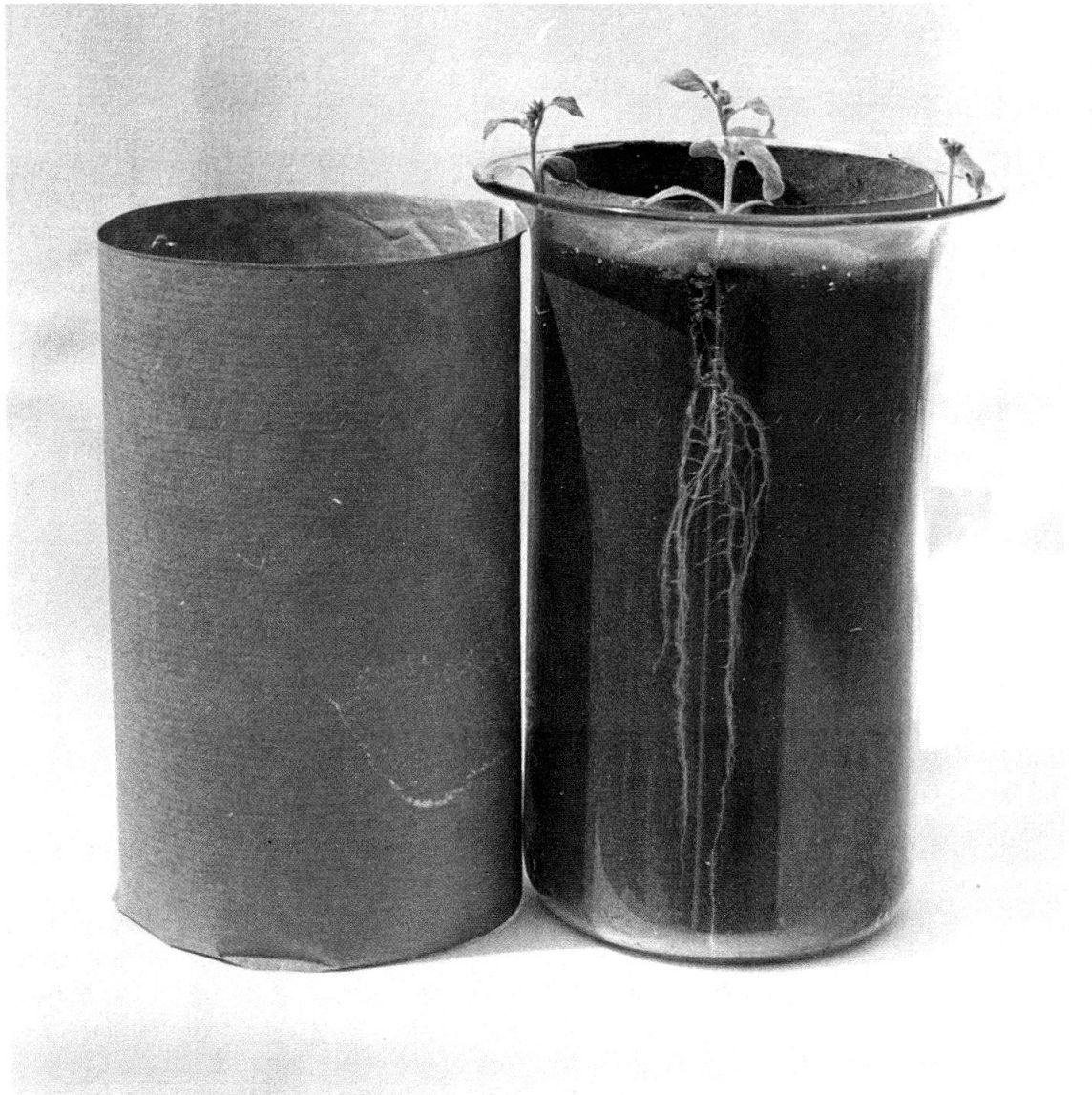


Fig. 1: Beaker with growing *Heliotropium europaeum* plants, set up to receive root-feeding larvae. Opaque paper sheath removed and placed beside beaker.

the roots in darkness but this could be slipped off temporarily to observe the roots and larvae. Fig. 1 shows the plants in the beaker ready for receiving the larvae. The beaker containing the plants was either kept in a fine screen cage, or covered with a transparent plastic cylinder ventilated with gauze windows and resting on the beaker rim, to prevent contamination by unwanted root-feeding insects. Sciarids were a particular nuisance in this regard. Thereafter, the sand was moistened with water as required, care being taken not to overwater as there is no drainage.

When, after one to several weeks depending on plant species, an adequate amount of new root had been produced, eggs of *L. albineus* on the point of hatching (larval head clearly visible through the chorion), or neonatal larvae, were transferred with a fine, moistened brush onto, or near, a plant rootlet by gently separating the blotting paper from the glass with a knife to allow entry of the brush to root level. Gentle compression of the sand closed the gap again. The number of eggs or larvae placed on a plant can be varied depending on the size and vigour of

the host or test plant species. Two larvae per plant were found to be satisfactory for *L. albineus* on its host, *H. europaeum*.

The position of the larvae can be marked on the glass with a red grease pencil and, thereafter, daily records of larval movements and development made. As the white roots contrasted well with the green blotting paper background, any feeding damage was clearly visible under a hand lens. More detailed observations of small larvae were made with a stereoscopic microscope set up for horizontal viewing. If the plants became unthrifty or were killed by feeding larvae before the latter completed their development, the larvae were transferred with a moistened brush to another beaker containing new plants.

Large larvae usually followed roots that had penetrated the blotting paper and had entered the sand. Occasionally, however, mature larvae pupated between the blotting paper and glass, and when this occurred the duration of the pupal as well as the larval stage could be determined accurately. Once pupation occurred, particularly if pupae were formed against the glass, watering was reduced as the pupae were very prone to fungal attack under excess humidity.

If, before adding the sand during preparation of the beaker, crushed, plastic drinking straws were intercalated vertically between the glass and the blotting paper such that the individual plants were separated from one another, more space was provided for pupation and larger larvae were prevented from wandering from plant to plant. This modification, although advantageous, is not, however, essential for successful rearing.

When this method was used, the average times taken for the development of eggs, larvae, and pupae of *L. albineus* were 12, 32, and 12 days respectively at approximately 20–22 degrees centigrade. The combined larval and pupal development was the same as when the insects were reared on potted *Heliotropium* plants kept at approximately the same temperature.

The main advantage of the method is that the larvae are only briefly disturbed, and then only by light, when observations are made on their feeding and development. This simple and direct method is extremely useful where the major aim is to see whether a particular test plant will be attacked by root-feeders over a short time period as in host specificity or host preference studies.

The method was successfully used to rear *Sitona* spp. on annual *Medicago* (J.P. AESCHLIMANN, pers comm.). Mites and nematodes as well as larvae of sciarids appeared to thrive under the conditions provided in the beaker. The method can therefore be used to observe and rear a wide variety of root-feeding invertebrates under relatively natural conditions.

#### *Acknowledgement*

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