

## Spatial distribution of diseased workers in honeybee winter clusters revealed by diagnostic radioentomology

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Honeybee winter clusters are 3D and non-transparent, rendering it impossible to study the spatial distribution of workers within them using conventional methods. Here we use non-invasive Diagnostic Radioentomology (DR), emerging as a new method for 3D computerised X-ray tomographic imaging of insects, to trace workers within natural clusters. Sealed worker brood combs from 4 colonies heavily infested with Varroa mite were incubated until adults emerged. Then, newly emerged workers (N=160) from cells containing 2 or more Varroa were cohort-specific labelled using Barium sulfate and introduced into 4 nucleus colonies on 3 combs. We excluded bees showing clinical symptoms of Deformed Wing Virus (DWV). A Siemens human body CT scanner was used for 3D computerised X-ray tomographic imaging to assess the winter cluster structure and the distribution of the labelled workers three times per week for three weeks. The data (N= 2 observations, preliminary analyses) show that younger bees (1 to 2 days old) congregate towards the centre of the winter cluster (N= 37 observations, 2.7 % away from the central core) and become more mobile as they age (15 days old, N=16 observations, 12.7 % away from the central core). This study showed that DR has particular advantages for non-invasively and non-destructively observing bees in winter clusters. We were able to visualize bee behaviour and general bee distributions inside clusters under normal winter conditions and the results suggest that young bees congregate towards the centre of the winter cluster and become more mobile as they age.

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