Ethanol mediates host selection and gallery-associated fungal communities in ambrosia beetles in beech forests

Eleonora Cresta[1,2] *, Rachele Ricci[1], Mario Contarini[1], Stefano Speranza[1,3]
*eleonora.cresta@unitus.it
1 Department of Agriculture and Forest Sciences, University of Tuscia, Viterbo, Italy
2 National Biodiversity Future Center (NBFC), Palermo, Italy
3 Centro de Estudios Parasitológicos y de Vectores (CEPAVE, CONICET-UNLP), La Plata, Argentina

Ethanol is a key volatile compound mediating host location and colonisation in ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). Beyond its role as an olfactory attractant, ethanol within host tissues may promote mutualistic fungi while suppressing antagonistic taxa, potentially shaping both colonisation success and gallery-associated fungal communities. However, how ethanol concentration influences these processes under natural forest conditions remains poorly understood.

This study investigated the response of ambrosia beetles to different ethanol concentrations in protected European beech (*Fagus sylvatica*) forests in central Italy (Viterbo province), using ethanol-baited logs (10x30 cm) deployed in the field. Three ethanol treatments (0%, 5%, and 90%) were tested in a randomised block design across two sites over two years (2024-2025). We focused on two species, *Xyleborinus saxesenii* and *Xylosandrus germanus*. Beetle colonisation, gallery development, and offspring production were assessed, and fungal communities associated with their galleries were characterised through culturing and ITS sequencing.

Both species showed significantly higher colonisation rates on ethanol-treated logs compared to controls (0%), confirming ethanol as a strong attractant under natural conditions. However, no differences were detected between 5% and 90% ethanol, suggesting that host selection is primarily driven by ethanol presence rather than concentration. Offspring were recorded only in ethanol-treated logs; while *X. saxesenii* showed no differences between concentrations, *X. germanus* produced more offspring at 5% than at 90%.

Fungal analyses revealed no significant differences in alpha diversity across treatments or beetle species. However, beta diversity analyses indicated that fungal community composition was significantly influenced by ethanol concentration. Gallery-associated communities also differed from control wood samples, showing higher fungal diversity in the presence of ambrosia beetle galleries.

Overall, our results indicate that ethanol acts as a primary colonisation cue, while ambrosia beetle activity contributes to structuring and increasing fungal diversity within woody substrates.