

## Is small mammal mycophagy relevant for truffle cultivation?

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The role of mycophagous small mammals as vectors of hypogeous fungi is well established. However, little is known about dispersal of gourmet truffle species by mammal vectors, or about the potential role of mycophagy in truffle plantations. We hypothesize that small mammal mycophagy contributes to the productivity of truffle plantations by providing inoculum for truffle mycelium establishment and mating. Spread of non-desired competitors of gourmet truffles is a potential adverse effect of small mammal mycophagy.

**Keywords:** *Tuber aestivum*, *Tuber melanosporum*, mutualism, symbiosis, mycorrhiza, ectomycorrhiza

### INTRODUCTION

Hypogeous fungi and other macrofungi are part of the diet of small rodents (*Rodentia*) such as voles (*Arvicolinae*, *Cricetidae*, *Myomorpha*), mice (*Murinae*, *Muridae*, *Myomorpha*), dormice (*Gliridae*, *Sciuromorpha*) and squirrels (*Sciuridae*, *Sciuromorpha*) (Maser, Claridge and Trappe 2008). Recently it was found that insectivorous shrews (*Sorex* spp., *Soricidae*, *Eulipotyphla*) frequently feed on hypogeous fungi as well (Kataržytė, Kutorga 2011; Schickmann et al. 2012). The nutritional ecology of most small mammal species seems to be rather flexible, highly adaptive and more diverse than commonly assumed. Spore dispersal in hypogeous fungi including

gourmet truffle species depends entirely on animal vectors, and the mutualistic relationship between mycophagous animals and hypogeous fungi can be considered obligate for the latter. Small mammal mycophagy was extensively studied in North America and Australia, since it was recognized as a process potentially important for the maintenance of fungal and mammal biodiversity and for forest regeneration (Claridge 2002; Maser et al. 2008). In Europe, relatively few studies demonstrated the role of mycophagy in bank vole, (*Myodes glareolus*, Blaschke and Bäumler 1989), red squirrel (*Sciurus vulgaris*, Grönwall, Pehrson 1984; Bertolino et al. 2004) and wood mice (*Apodemus* spp., Blaschke, Bäumler 1989).

During a study conducted in primary and secondary forests in two regions of the eastern alps we found that all mammal species native to Central Europe mountainous forests investigated feed on hypogeous fungi, albeit with different intensity (Schickmann et al. 2012). Based on frequency, quantity and diversity of fungal spores detected in faeces by microscopy and with molecular tools, we concluded that at least one native small mammal species, the bank vole (*Myodes glareolus*) is preferentially mycophagous. All other species captured, including wood mice (*Apodemus flavicollis* and *A. sylvaticus*), other vole species, shrews (*Sorex* spp.) and the fat dormouse (*Glis glis*) were found to be opportunistically mycophagous. All small mammal species were found to feed on a diversity of fungal species. Similar results were reported from hemiboreal forests in Lithuania (Kataržytė, Kutorga 2011).

## METHODS

Here we synthesize available information to infer potential roles of small mammals in truffle plantations. A description of methods used for life trapping of small mammals, fecal pellet sample collection and microscopical analysis is found in (Schickmann et al. 2012). A protocol optimized for DNA extraction from fungal spores in fecal pellets of small mammals was developed (Schickmann et al. 2011).

## RESULTS

### POTENTIAL ROLES OF SMALL MAMMALS IN TRUFFLE PLANTATIONS

**Effects on host trees.** Small mammal communities differ with habitat types. Rodent species are more or less herbivorous, and some species are known to damage host trees, at least at high population densities. Red squirrels are likely to occur in mature plantations, especially if hazelnuts (*Corylus* spp.) or conifer seeds are available. Squirrels are very effective seed predators, hoarding of excess food in caches contributes to seed dispersal. Squirrels can damage trees by bark stripping, however, this behaviour is a major issue in the invasive grey squirrel (*Sciurus carolinensis*) but not in the native red squirrel (Bertolino, Genovesi 2003). Voles can cause severe damage in young truffle plantations. Newly established truffle plantations in

agricultural environments are more likely to be colonized by common voles (*Microtus arvalis*) than by species occurring in more natural habitats such as field vole (*Microtus agrestis*) and bank vole (*Myodes glareolus*). An invasion of voles into an experimental truffle plantation was observed when a neighbouring maize field was harvested and the animals lost shelter (Ronald Vogl, personal communication). Debarking of the stem base killed one *Corylus colurna* tree (4 yrs after outplanting). It appears that some tree species are less preferred by voles, e.g., pine species are avoided (Borowski 2007). Lime tree (*Tilia cordata*) seedlings were reported to be preferentially attacked by bank voles (Pigott 1985).

Planting a diversity of tree species may be the best strategy to limit overall damage, given the diversity of potential pest species. According to our experience, voles are rarely a cause of excessive tree mortality in truffle plantations, but pressure can vary considerably. If the risk of losing tree seedlings due to damage by voles is high, the roots and stem bases can be protected with a non-galvanized wire-basket which will decompose by corrosion. Mulching can provide shelter for small mammals depending on the material used and should be avoided or adapted if pressure is critical.

Small mammal species are an important source of prey for predators such as various species of birds of prey, owls, red fox (*Vulpes vulpes*), wildcat (*Felis silvestris*), and various mustelids such as weasel (*Mustela nivalis*), European polecat (*Mustela putorius*) and European badger (*Meles meles*). Providing habitat for predators and protecting them from being hunted is an effective way to keep small mammal populations at levels compatible with plantation management objectives.

According to our experience, game species, like European hare (*Lepus europaeus*) and roe deer (*Capreolus capreolus*) usually impose much higher pressure on young truffle plantations and need to be excluded.

**Effects on truffle populations.** Little is known about effects of mycophagy on truffle populations in managed plantations. The small rodent species common in habitats like truffle plantations, e.g., voles, wood mice (*Apodemus sylvaticus* and *A. flavicollis*) and squirrels are well known to be mycophagous (Maser et al. 2008). Squirrels even cache hypogeous fungi (Vernes, Poirier 2007). Recently it was shown that also insectivores such as shrews (*Sorex* spp.) frequently feed on hypogeous fungi (Kataržytė, Kutorga 2011; Schickmann et al. 2012). This result is of interest for two reasons: 1) Shrews are not herbivorous and they are very unlikely to harm host trees. 2) Shrews are insectivorous and ground-dwelling, and may contribute to controlling insects parasitic on truffles or host tree roots.

From the truffle growers perspective, mycophagy may be regarded as a waste of valuable crop. Small mammals are important dispersal agents for truffle spores, but no data are available which prove that there is a role for mycophagist in plantations established with mycorrhizal trees. Once established, genets of truffle mycelium can grow and extend, without apparent need of additional spores. However, three lines of evidence suggest that mycophagy may be essential for the long term fertility and productivity of truffle plantations: 1) Truffle spores in faeces of small mammals are a viable source of inoculum (Schickmann et al. 2012). 2) Artificial inoculation of soil with truffle spores is reported to increase yields of *Tuber aestivum* (P. Sourzat, unpublished). 3) Sexual reproduction and outcrossing has been proven in some gourmet truffle species (Paolucci et al. 2006; Riccioni et al. 2008), and ascospore or

conidiospore (Urban et al. 2004) dispersal is likely to play a role in fertilisation of opposing mating types.

Truffle spore dispersal by small mammal vectors has one potential drawback: mycophagists use to feed on a variety of species of hypogeous fungi. Thereby, they likely vector non-marketable or low-value species which may compete with the target gourmet truffle within the plantation.

## CONCLUSIONS

Currently, information on the role of small mammal mycophagists in truffle plantations is scarce, despite the significance of mycophagy in the truffle life cycle. Small mammals, at least if present in excess, are typically regarded as pests, by foresters and truffle-growers. Some truffle growers use to combat small mammals by different means of pest control, but systematic experimentation is still lacking. At present we do not know whether the contribution of spore dispersal by small mammals to productivity is comparable to the relevance of pollination in fruit orchards. The impact of biotic interactions on productivity is more difficult to assess than the influence of abiotic factors, such as climate. Experimental work on mycophagy in truffle plantation and natural truffle sites is needed to obtain reliable data on the role of small mammals in the dispersal and reproduction of truffle species.

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