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TECHNICAL NOTE

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Taphonomic Mycota: Fungi with Forensic Potential

ABSTRACT: Forensic archaeologists and criminal investigators employ many different techniques for the location, recovery, and analysis of clandestine graves. Many of these techniques are based upon the premise that a grave is an anomaly and therefore differs physically, biologically, or chemically from its surroundings. The work reviewed in this communication demonstrates how and why field mycology might provide a further tool towards the investigation of scenes of crime concealed in forest ecosystems. The fruiting structures of certain fungi, the ammonia and the postputrefaction fungi, have been recorded repeatedly in association with decomposed mammalian cadavers in disparate regions of the world. The ecology and physiology of these fungi are reviewed briefly with a view to their potential as a forensic tool. This application of mycology is at an interface with forensic archaeology and forensic taphonomy and may provide a means to detect graves and has the potential to estimate postburial interval.

KEYWORDS: forensic science, forensic taphonomy, forensic archaeology, ectomycorrhizal fungi, ammonia fungi, postputrefaction fungi, cadaver decomposition, clandestine graves, post-burial interval, fungal fruiting succession

The science of taphonomy was based on the premise that information concerning buried remains may be gathered through field and laboratory-based analyses of the conditions in which they are preserved (1). Forensic taphonomy aims to understand the processes of decomposition and the factors influencing them while estimating postmortem interval (PMI) as well as cause and manner of death (2). This must be carried out within the constraints imposed by the legal system. To accomplish this, forensic taphonomy has incorporated many techniques from a wide range of disciplines. Fields such as archaeology (3,4), entomology (5,6), soil chemistry (7), soil microbiology (8) and botany (9,10) have been used to locate, recover, and analyze clandestine graves.

In recent decades, a number of field experiments and case studies in mycology have amassed a substantial body of data relevant to cadaver decomposition. These data have demonstrated that certain chemoecological groups of fungi can act as above-ground grave markers in forest ecosystems (11–17). These fungi are known as ammonia fungi (18) and postputrefaction fungi (19). In this communication we briefly review the potential for these fungi to act as clandestine grave markers as well as a tool for the estimation of post-burial interval (PBI).

Grave Markers

Ammonia fungi form fruiting structures (i.e., mushrooms) on forest soils experimentally treated with urea, ammonium (NH_4^+), or other nitrogenous compounds that release ammonia (NH_3) upon decomposition (18). When these fungi occur naturally (in sites that have not undergone experimental chemical treatment) they are termed postputrefaction fungi (19). To date, postputrefaction fungi have been found in association with decomposed human (14,19), cat (13,16,17), dog (11,16), crow (20), rabbit (21), snake (12), and kangaroo (15) cadavers. These cadavers have been in the late stages of decomposition, when remains include bone and hair. Sagara (17) also notes adipocere in association with postputrefaction fungi. It is important to note that these fungi have also been found fruiting in close proximity to excrement (19,22,23) mouse/mole middens (19,22,24–26) and decomposed wasp nests (27,28). These reports have come from forests in Australia (12,15), England (26), Japan (11,13,16,20), North America (14), and Switzerland (30).

Timely survey of fungal fruiting structures on forest floors could be used to designate potential graves, thereby reducing the amount of time required to examine a large area. Typically autumnal or wet seasons are the most productive for fungal fruiting and hence surveys. These surveys would be appropriate where burial over months or years is suspected as cadaver-related fruiting would not occur immediately after burial.

Estimating Post-burial Interval

In order to utilize the fruiting ammonia fungi and postputrefaction fungi as a tool for the estimation of PBI, it is necessary to understand their fruiting behavior and physiology. Ammonia and postputrefaction fungi undergo a "succession" of fruiting where one set of fungi is later replaced by another. This succession has been divided into early and late stages (18). Early stage fungi comprise ascomycetes, deuteromycetes, and saprotrophic basidiomycetes (Table 1). These fungi can fruit from one to ten months

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after fertilization of forest floors with urea, NH_4^+, or other nitrogenous materials that release NH_3 upon decomposition (23,31). Late stage fungi comprise ectomycorrhizal basidiomycetes that can fruit from one to four years after fertilization (Table 1) (23,31). This may be due, in part, to nitrogen (N) utilization. Experimental work suggests that NH_3 is the key compound responsible for the fruiting of these fungi (23,32,33). Most early stage fungi fruit on soils with high concentrations of NH_3 but do not apparently utilize N from nitrate (NO_3^-) in large amounts (34,35). Late stage fungi utilize organic N, NO_3^-, and NH_4^+ (34,35). This shift in N utilization might be anticipated as the formation of NO_3^- takes place after NH_4^+ in the N cycle (36). The fungal succession associated with cadavers may be similar to the succession of ectomycorrhizal fungi during forest development (e.g., 37) and would provide the basis for estimating PBI. At present, these fruiting stages could be employed only crudely to estimate if a grave has existed for up to one year or from one to four years based on the current understanding of early and late stages.

There are two species of postputrefaction fungi, *Rhopalomyces strangulatus* and *Hebeloma radicosum*, not recognized as ammonia fungi because they have not been found fruiting following chemical N treatment (19). From this one might hypothesize that *Rhopalomyces strangulatus* and *Hebeloma radicosum* take an active role in decomposition rather than relying upon mineralized by-products (NO_3^-, NH_4^+) as sources of N. These fungi may utilize organic N from protein (38) and/or amino acids (39,40). Other ectomycorrhizal fungi (*Hebeloma* spp.) have taken an active role in the apparent decomposition and nutrient utilization of seeds in axenic culture and symbiosis (40,41). *Hebeloma* spp. also displayed a preference for organic N (glutamic acid) over mineral N (NH_4^+) in pure culture (40,42). Of these, arctic strains displayed a greater bias for organic N than temperate strains. This may have implications for the taphonomic use of postputrefaction fungi in cold climates as low temperature can regulate soft tissue decomposition (43,44) and N mineralization (45).

Conclusion

It is generally accepted that fungi are "agents" of decomposition (46), possibly appearing on the surface of a cadaver (47,48). The majority of work referring to fungi in the burial environment is concerned with the modification of hair (49–51), bone (52,53), and associated materials such as clothing (47,54,55). Ammonia and postputrefaction fungi represent naturally occurring phenomena that can act as visible grave markers in forest ecosystems. The succession of fruiting and N utilization provides the basis for the estimation of PBI. Current evidence suggests that early and late stage fungi can be viewed roughly as occurring up to one year or from

TABLE 1—*Description of ammonia fungi (AF) and postputrefaction fungi (PPF) including nutrient source, associated vegetation, and location.*

Fungal Species	AF	PPF	Nutrient Source	Vegetation	Location	Reference
			EARLY FRUITING STAGE			
Zygomycetes						
Rhopalomyces strangulatus	−	+	Mammalian cadaver	Not stated	Not stated	18,19
Deuteromycetes						
Amblyosporium botrytis	+	+	Midden	Not stated	Not stated	18,19,23
Ascomycetes						
Ascobolus denudatus	+	+	Mammalian cadaver, urine, faeces	*P*	Japan	18,19
Ascobolus hansenii	+	+	Mammalian cadaver, faeces	*P-C*	Japan	19
Tephrocybe tesquorum	+	+	Mammalian cadaver, urine, faeces	*P*	Japan	19
Peziza (?) sp.*	+	+	Mammalian cadaver, urine, faeces	*P-C*	Japan	18,19
Peziza morovecii†	+	+	Mammalian cadaver, urine, faeces	Not stated	Not stated	18,19
Coprinus neolagopus	+	+	Mammalian cadaver	Not stated	Not stated	19
Coprinus phlyctidosporus	+	+	Mammalian cadaver	Not stated	Not stated	19
Coprinus stercorarius	+	+	Faeces	*P-Q*	Japan	19
Crucispora rhombisperma	+	+	Urine, faeces	Not stated	Not stated	19
Humaria velenovskyi	+	+	Urine, faeces	*P-C*	Japan	18,19
			LATE FRUITING STAGE			
Basidiomycetes						
Hebeloma vinosophyllum	+	+	Mammalian cadaver, avian cadaver	*C, P, Q*	Japan	11,16,20
Hebeloma aminophilum	+	+	Mammalian cadaver	*E*	Australia	12,15
Hebeloma spoliatum	+	+	Mammalian cadaver, wasp nest, midden	*P, C, Q, P-Q*	Japan	17,19,22,28
Hebeloma syrjense	?	+	Mammalian cadaver	NA	North America	14
Hebeloma radicosoides‡	+	+	Mammalian cadaver, wasp nest, midden	*P, C, Q, P-Q*	England, Japan	17,19,22,28
Hebeloma radicosum	-	+	Mammalian cadaver, urine, faeces, midden	*P, F-Q, Q-C*	Japan	19,24,25
Lactarius chrysorrheus	+	+	Mammalian cadaver, urine, faeces, midden	*P*	Japan	19
Laccaria bicolor§	+	+	Mammalian cadaver	*P*	Japan	17
Laccaria amethystine	+	+	Mammalian cadaver	Not stated	Not stated	19
Laccaria spp.	+	+	Mammalian cadaver, midden	Not stated	Not stated	19
Lepista nuda	+	+	Urine, faeces	Not stated	Not stated	19
Suillus luteus	+	+	Midden	Not stated	Not stated	19
Suillus bovinus	+	+	Midden	Not stated	Not stated	19
Mitrula sp.	+	+	Mammalian cadaver, faeces	Not stated	Not stated	19

NOTE: C = *Castanopsis cuspidata*; E = *Eucalyptus* spp.; P = *Pinus densiflora*; Q = *Quercus serrata*; P-C = *Pinus-Chamaecyparis*; P-Q = *Pinus-Quercus*; F-Q = *Fagus-Quercus*.
* *Gelatinodiscus* sp. in Refs 29
† *Peziza* sp. no. 1 in Refs 19,29.
‡ *Hebeloma radicosum* in Refs 13,19,29,26,28.
§ *Laccaria proxima* in Refs 17,18,29.

Years 1 to 4 following fertilization, respectively (23,31). A defined relationship between decomposition and fruiting stages near cadavers is not clear, and much more detailed experimental work is required to develop this concept as a forensic tool. Molecular approaches such as denaturing gradient gel electrophoresis (56) and fatty acid methyl ester (57) analysis may also have a role in allowing wider microbiological succession to contribute to PBI estimates. It is important to note that ammonia and postputrefaction fungi do not fruit upon cadaver burial, but the subsequent release of N during its decomposition. How this relates to cadaver decomposition is unknown. Thus, it is necessary to determine the quantity and form of N released from a cadaver during decomposition in order to increase the accuracy of ammonia and postputrefaction fungi as tools to estimate PBI.

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