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Fungal Spore Dispersal by the Eastern Box Turtle (*Terrapene carolina carolina*)

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ABSTRACT.—Although spores from most macrofungi are wind- or water-dispersed, dispersal may also occur via biotic vectors. The Eastern box turtle (*Terrapene carolina carolina*) is a facultative mycophage that may play an important role in fungal spore dispersal although, to date, no information exists on fungi occurring in fecal samples of box turtles or on the ecological significance of box turtles as spore dispersal vectors. Consequently, a study of the potential for Eastern box turtles to act as vectors for spore dispersal was initiated by capturing wild turtles and collecting fecal samples. Serial dilutions from fecal samples were made to enumerate spores, quantify the number of spores per gram of fecal material and to isolate and identify fungi. Fungal spores were found to be extremely abundant throughout all samples. Fecal samples from 36 turtles yielded a total of 23 different fungal taxa in the Zygomycota, Ascomycota and Basidiomycota. Two yeasts that were isolated, *Cryptococcus albidus* and *Rhodotorula mucilaginosa*, are reported to naturally occur on *Trifolium* seeds found in fecal samples. A mold previously unreported from fecal material, *Aspergillus wentii*, was also found in fecal samples. Data collected suggests Eastern box turtles influence fungal spore dispersal by browsing on plant materials and defecating large numbers of fungal spores within their home ranges.

INTRODUCTION

Although most macrofungi disperse their spores through the action of wind or water (Dobbs, 1942; Ingold and Hudson, 1993; Miller *et al.*, 1994; Tuno, 1999), dispersal events may also occur via biotic vectors. Endozoochory, the dispersal of spores by animals, has been documented in a variety of taxa, including flies (Tuno, 1998; Tuno, 1999), rodents (Janos *et al.*, 1995; Reddell *et al.*, 1997; Gordon and Comport, 1998; Mangan and Adler, 2000; Mitchell, 2001; Pyare and Longland, 2001) and Australian marsupials (Johnson, 1994; Reddell *et al.*, 1997).

Advantages of spore dispersal by animals include range expansion, population stabilization and maintenance of genetic variability within populations (Gregory, 1966; Ingold and Hudson, 1993). Additional advantages associated with biological transport may include food sources for soil organisms, increased nutrient availability, inhibition of soil pathogens and bacteria, enhanced primary succession and improvement of soil structure (North *et al.*, 1997; Pyare and Longland, 2001). For example, (Gehring *et al.*, 2002) found that when terrestrial vertebrates were excluded from a rain forest, the number of mycorrhizal spores in the soil decreased significantly. Thus, the lack of adequate spore dispersal can have dramatic effects on community structure and function (Harley, 1989; Moore-Landecker, 1996; Gehring *et al.*, 2002; Frank *et al.*, 2003).

The Eastern box turtle (*Terrapene carolina carolina*) is a facultative mycophage that may play an important role in fungal spore dispersal. The distribution of the Eastern box turtle ranges from Massachusetts to Georgia and west to Michigan, Illinois and Tennessee (Conant and Collins, 1998). Box turtles are opportunistic omnivores that consume a variety of food items including annelids, molluscs, insects, amphibians, small mammals, carrion, plants and

fungi (Ernst *et al.*, 1994; Dodd, 2001; Liu *et al.*, 2004). In addition, turtles actively feed on sporocarps of fungi in the Ascomycota and Basidiomycota and may ingest large numbers of fungal spores. Furthermore, since fungi are ubiquitous, it is highly probable that there is incidental ingestion of fungal spores when turtles feed on other food items. For example, turtles ingest berries and fruit which may contain yeasts as well as fungal spores and hyphae. In addition, turtles may ingest fungal spores from the soil when feeding on vegetation, molluscs and carrion. The phenomenon of incidental fungal spore ingestion and subsequent dispersal has been documented in birds (Cafarchia *et al.*, 2002) and may represent an uncommon, but important dispersal pathway.

To date no information exists on the types of fungi occurring in fecal samples of the Eastern box turtle or on the ecological significance of box turtles as fungal spore dispersal vectors. Thus, a study of the potential for Eastern box turtles to act as fungal spore vectors in central Illinois was initiated by capturing wild turtles and collecting fecal samples to address the following questions: (1) What is the abundance of fungal spores in fecal samples of the Eastern box turtle?; 2) What types of fungi are found in fecal samples of the Eastern box turtle?; and 3) Do seasonal variations occur in fungal spore dispersal by Eastern box turtles?

METHODS

This study was conducted from April to September 2003 in Clark, Clay, Coles, Moultrie and Shelby counties of Illinois. A total of 38 Eastern box turtles were collected by visually scanning forest floors and roadways. Once captured, the turtles were placed in five gallon plastic buckets and transported to the laboratory. Each turtle remained in the lab for up to three days to collect fecal samples. Turtles were released at the point of capture after obtaining at least one fecal sample per turtle or until three days had passed without a sample. All fecal samples were removed from the buckets and dried at 23 C for 48 h. Dried samples were then removed and stored in sterile containers at 5 C.

To isolate fungi in fecal samples, sterile mortars and pestles were used to gently break up the samples. Spore enumeration techniques were adapted from (Malloch, 1997). Although it is common practice to use one gram of a sample for serial dilution plating, several individual fecal samples weighed less than one gram. As a result, a homogeneous 0.25 g subsample of each fecal sample was diluted to concentrations of 1:20, 1:1000 and 1:10,000. All dilutions were vortexed for 30 s to evenly distribute the homogenate before fungal spore enumeration. Aniline blue was added to help differentiate fungal spores from debris in the samples. Samples from each dilution were examined using a Neubauer Haemocytometer to quantify fungal spore density in fecal material. Subsequently, a Kruskal-Wallis test was used to determine if there was any variation in spore density over time.

To culture fungal spores, one mL from each dilution was pipetted onto sterile disposable 100 × 15 mm Petri plates containing 20 mL of Rose Bengal Agar (RBA) with 30 mg/L of ampicillin and tetracycline and spread with a sterile glass rod. All plates were placed in the dark at 25 C. Each plate was examined in 24-h increments for the first 72 h. After 72 h most of the plates were overgrown with a variety of fungi and bacteria making it difficult to isolate individual fungal colonies.

To determine the types of fungi present in fecal samples, filamentous colonies were removed from the RBA plates with a sterile wire loop and transferred to 100 × 15 mm Petri plates containing 20 mL of Potato Dextrose Agar (PDA). Wax-like yeast colonies were transferred to 100 × 15 mm Petri plates containing 20 mL of Sabouraud Dextrose Agar (SDA). All plates were individually placed in Ziploc[®] plastic bags, sealed and incubated in the dark at 25 C for 3–5 d. Conidial development was initiated by removing the filamentous

isolates from the dark and storing them in clear plastic containers where they were exposed to ambient light conditions. A small portion of each isolated colony was removed and placed on a glass slide with 1–2 drops of deionized water or lactophenol cotton blue stain for identification. Identification of yeasts was made using bioMerieux api 20 C AUX yeast identification strips following manufacturer's guidelines. Dilutions were made from each yeast isolate and loaded into test strip wells. The test strips were placed in a 25 C incubator without light and examined in 24-hour increments for 72 h. The bioMerieux yeast database was subsequently used to identify each yeast isolate.

RESULTS

Fungal spores were found in all 36 fecal samples analyzed. The 1:1000 dilutions were most appropriate for counting fungal spores. While the 1:20 dilutions contained too much debris for accurate enumeration, the 1:10,000 dilutions contained too few fungal spores to yield reliable counts. In the 1:1000 dilutions, a mean number of 8.07×10^{10} fungal spores per gram of fecal material was recorded and there was no significant variation in spore density over time (Kruskal-Wallis test, $\chi^2 = 6.921$, $df = 4$, $p = 0.140$).

Fungal isolates were obtained from 21 of the 36 fecal samples. The other 15 samples either failed to produce colonies or did not generate diagnostic characters that could be used for identification. A total of 23 different fungal taxa were found on Eastern box turtle fecal samples (Table 1) representing the Zygomycota (2 taxa), Ascomycota (20 taxa) and Basidiomycota (1 taxon). Many of the fungi found on turtle fecal materials also occur naturally on organic debris, soil, fruits and nuts (Jordan, 2004).

TABLE 1.—List of 23 fungal taxa found growing on *Terrapene carolina carolina* fecal samples

Order	Taxon	Sample No.
Hypocreales	<i>Acremonium</i> sp.	11
Pleosporales	<i>Alternaria</i> sp.	17A
Eurotiales	<i>Aspergillus fumigatus</i> Fresenius	26
Eurotiales	<i>Aspergillus glaucus</i> (Mich: Fr.) Link	32
Eurotiales	<i>Aspergillus</i> sp.	37
Eurotiales	<i>Aspergillus wentii</i> Wehmer	32
Saccharomycetales	<i>Candida stellata</i> (Kroener & Krumble) Meyer & Yarrow	12A
Sodariales	<i>Chaetomium bostrychodes</i> Zopf	2, 9
Mycosphaerellales	<i>Cladosporium</i> sp.	32
Agaricales	<i>Coprinus</i> sp.	7, 24
Filobasidiales	<i>Cryptococcus albidus</i> (Saito) Skinner	37
Eurotiales	<i>Eupenicillium</i> sp.	32
Eurotiales	<i>Fennellia nivea</i> (Wiley & Simmons) Samson	11
Hypocreales	<i>Myrothecium</i> sp.	37
Eurotiales	<i>Paecilomyces</i> sp.	38A
Eurotiales	<i>Penicillium</i> sp.	3, 6, 12, 15, 38
Sporidiales	<i>Rhodotorula mucilaginosa</i> (Jorgensen) Harrison	5, 7, 14, 37
Sordariales	<i>Sordaria fimicola</i> (Roberse ex Desm.) Ces. & De Not.	17B
Eurotiales	<i>Talaromyces</i> sp.	12B
Eurotiales	<i>Talaromyces wortmanii</i> (Klocker) C.R. Benj.	6
Hypocreales	<i>Trichoderma</i> sp.	6
Mucorales	<i>Mucor</i> sp.	21, 28
Mucorales	<i>Circinella</i> sp.	20, 26, 30

DISCUSSION

Fungal spores were found to be extremely abundant throughout all fecal samples and the fungi isolated represented a wide diversity of taxa. These data suggest that fungal spore dispersal by Eastern box turtles may operate in a very different manner than typical vectors. Spatial patterns of turtle dispersal would tend to concentrate fungal spores together rather than random dispersal via abiotic factors such as wind and water. Since fungal spore dispersal based on edaphic factors such as wind and water is highly variable, dispersal distances can vary from several meters to thousands of meters (Ingold and Hudson, 1993) and fungal spores dispersed by wind and water may often land in nutrient poor habitats. As such, fecal material from Eastern box turtles may provide an immediate and available supply of moisture and nutrients, and the concentration of fungal spores in nutrient rich habitats may significantly increase the chances for fungal reproductive success. In fact, Cork and Kenagy (1989) and Claridge *et al.* (1992) demonstrated that spore germination can be enhanced by passage through the digestive system of an animal. This may also facilitate an increase in reproductive success of these fungi.

Spore dispersal is linked inherently with the movement and behavior of vectors. Movement determines the spatial and temporal distribution of spores across a landscape and should be considered when determining the dispersal importance of a vector. Box turtles have been shown to move widely within their home ranges (Stickel, 1950; Dodd, 2001). The average distance a box turtle moves in one day is approximately 50 m, but they often return to their form (sleeping site) at the end of the day (Stickel, 1950; Strang, 1983). The paths turtles traverse vary greatly with moisture, temperature and humidity (Stickel, 1950; Claussen *et al.*, 2002; Dodd, 2001), leading to variation in habitat utilization. Because the distances box turtles move are appreciable, and their gut retention times may span several days, they are potentially important dispersal vectors (Braun and Brooks, 1986).

The two yeasts isolated, *Cryptococcus albidus* (Saito) Skinner and *Rhodotorula mucilaginosa* (Jorgensen) Harrison, are reported to naturally occur on *Trifolium* (Farr *et al.*, 1989) which coincides with *Trifolium* seeds found in the fecal samples. *Aspergillus wentii* Wehmer was the only fungus found on the fecal samples that has been reported as naturally occurring on *Prunus serotina*. The remainder of the fungi found occurring on Eastern box turtle fecal samples may either be species not previously reported from *Rubus* spp or be coprophilous fungi. Overall, Eastern box turtles function as effective dispersal agents of fungal spores. The data presented conclusively demonstrates the potential for fungal spore dispersal via Eastern box turtles.

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