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Haris Rana  
*Southside High School*

Shayla Smithson  
*University of Arkansas–Fort Smith*

Jack Jackson  
*University of Arkansas–Fort Smith, Jack.Jackson@uafs.edu*

Ragupathy Kannan  
*University of Arkansas–Fort Smith, ragupathy.kannan@uafs.edu*

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Bird Usage of Black *Marasmius* Fibers as Nest Material

H. Rana$^3$, S. Smithson$^1$, J.L. Jackson II$^2$, and R. Kannan$^1$

$^1$Department of Biology, University of Arkansas - Fort Smith, Fort Smith, AR 72913
$^2$Department of Mathematics, University of Arkansas - Fort Smith, Fort Smith, AR 72913
$^3$Southside High School, Fort Smith, AR 72913

*Correspondence: Ragupathy.Kannan@UAFS.edu

Running Title: Bird Usage of *Marasmius* Fibers as Nest Material

Fungal fibers are used for nest construction by 176 species of birds (Elliott *et al*. 2019). At least 98 bird species use the black rhizomorphs of *Marasmioid Basidiomycetes* fungi as nest material (Hansell 2000; Aubrecht *et al*. 2013; Caballero 2020). The Yellow-olive Flycatcher (*Tolmomyias sulphurescens*), a common bird of forests and forest edges in Central and South America, principally uses these black *Marasmius* fibers for nesting (Fig. 1; Anciães *et al*. 2012; Menezes *et al*. 2014). Several hypotheses have been proposed to explain this phenomenon. There are conflicting reports on whether the fibers have antibiotic properties (Melin *et al*. 1947; Meng *et al*. 2011; Ramesh and Pattar 2010; Seupaul 2021). These slender fungal filaments have been shown to have anti-carcinogenic properties (Rosa *et al*. 2009), but whether this is a factor in nest material selection by birds has not been examined. An additional selective advantage proposed of these so-called horse-hair fungi, owing to their resemblance to long black horse hairs, is that they are longer than locally available grass fibers and hence afford flexibility and convenience in the construction of long pendulous nests (Freymann 2008). This too remains to be addressed empirically. Another possible advantage of choosing horse-hair fungi may be that they are more water-resistant than grass material. This has been tested and supported by data (Freymann 2008).

Two more hypotheses have either been incompletely tested or untested thus far. The first is that these fungal filaments are physically stronger than grass fibers, and thus they are preferred for their durability (Freymann 2008; Aubrecht *et al*. 2013). To test this, Freymann (2008) conducted experimental trials comparing tensile strengths of *Marasmioid* filaments used by Streak-backed Orioles (*Icterus pustulatus*) in Costa Rica, with grass fibers extracted from nest linings from the same nests. He demonstrated that the fungal filaments were stronger than the grass lining material. An obvious drawback of his study was that he compared the physical performance of fungal filaments with grass linings of nests, and not to grass fibers used in the main nest structure. Such a comparison would be necessary to establish if fungal filaments afford greater durability in terms of higher load bearing than alternate grass material used in similar ways by coexisting bird species that also construct pendent fiber nests.

The second hypothesis is that using *Marasmius* fibers in nest provides some advantage in the control of temperature exchange between the interior and exterior of the nest. Until now, this hypothesis has not been tested empirically.

In this study, we tested these two hypotheses. We compared tensile strengths of *Marasmius* fibers obtained from six nests of Yellow-olive Flycatchers in Belize, Central America, with grass fibers extracted from a Yellow-tailed Oriole (*Icterus mesomelas*) nest from the same general area. Both species build pendent nests made of fibers. We also compared nest interior temperatures of the five *Marasmius* nests with that of the control grass nest and concurrent ambient temperatures.

All nests were inactive and empty at the time of collection in June 2019. We could not determine exactly when they were in use, but the intact condition of the nests indicated that they were in use that summer. The flycatcher nests were predominantly or exclusively made of black fungal fibers. The oriole nest was comprised of grass material only. We provisionally identified the flycatcher nest fibers as *Marasmius* in the field based on their black color (Fig. 1) and their thin and wiry form (Hedger 1990; Hedger *et al*. 1993; Koch *et al*. 2020) resembling horse hairs.

To confirm that the black fibers were indeed aerial rhizomorphs of *Marasmius* fungi, a sample of fibers was sequenced with NS1 [5’(GTA GTC ATA TGC TTG TCT C)3’] and NS8 [5’(TCC GCA GGT TCA CCT ACG GA)3’] primers used for higher fungi. Examination of 1659 base pairs (bp) of the full 18S rRNA sequence
of the sample showed 99% similarity to Marasmius sp. (Bit-score=3020, E-Value= 0.0). The sequenced region (bp 54-1712) covered approximately 93% of the total 18S rRNA gene sequence. The Maximum Likelihood Phylogeny Tree as reconstructed from this data revealed that the sample was closely allied to other marasmoid fungi (Fig. 2). Partial sequencing of small subunit rRNA gene of five samples (bases 1-949) indicated that one was M. oreades (GenBank Accession No. OK103912.1) and the rest Marasmius sp. (GenBank Accession Nos. OK103913.1, 103916.1, 103918.1, 103919.1).

To test the tensile strength hypothesis, six fibers were selected from each of the six flycatcher nests. They were compared to six grass fibers from the oriole nest. Each fiber was cut into a standardized length of 15 cm. An eXpert 7600 Series Universal Tester by ADMET was used to measure the tensile strength of the fibers. Each fiber was attached on each end to the hydraulic clamps of the universal tester and stretched to its breaking point. The force gauge transmitted the load at the time it broke to an online database (in pounds), which we converted to a force in Newtons.

Since the oriole nest grass fibers were significantly thicker, and thus presumably stronger, than the Marasmius fibers, we controlled for this variable by measuring the diameter of all fibers (control and experimental) with a micrometer. This enabled us to present results as force per cross-sectional area in N/mm² (Fig. 3). We assumed that the cross-sectional areas may be reasonably modeled by a circular cross-section with the diameter measured at an arbitrary position on the fiber.

The mean load per cross-sectional area at breaking point for the 6 grass fibers (896 N/mm²) was higher than the mean for the 36 black fibers (456 N/mm²). The p-value from a 2-sample, 2-tailed t-test for difference in means was 0.0074, strongly indicating that this difference was significant. On average, the grass fibers withstood an additional 440 N/mm² strain more than the Marasmius fibers. A 95% confidence interval for the difference (Grass – Marasmius) in mean load per cross-sectional area is [162.75, 717.37] N/mm².

Our results indicate that the brown grass fiber nest material used by the oriole had a significantly stronger tensile strength than the black Marasmius fiber nest material used by the flycatcher. Tests indicated that the grass fibers are stronger than Marasmius fibers, even after correcting for the greater thickness of the grass fibers. Therefore, we rejected the hypothesis that Marasmius fibers are chosen for their superior strength.
Our tensile strengths data suggests that *Marasmius* fibers do not have a tensile strength advantage to at least one stronger nesting material that was readily available in the area. This seems to contradict the results of Freymann (2008), but it should be reiterated that he compared tensile strength of *Marasmius* fibers used as the main structural component of nests to the grass fibers used to line the same nests. In contrast, our study compares the *Marasmius* fibers used exclusively in a nest of one species to brown grass fibers used exclusively in the nest of a different local species.

We tested the temperature buffering hypothesis by suspending the black fiber nests and control nest from small trees at 4.5m above ground and 1.8m from one another. This study was conducted at the Crystal Paradise Resort, Cayo District, Belize. HOBO MX2201 data loggers were placed inside the empty nests and set to record the temperature every 30 minutes for 24-hour time increments. Temperatures were recorded for the ambient environment, the interior of the grassy control nest and one *Marasmius* nest. In addition, 2 of the other 4 *Marasmius* nest temperatures were recorded on a rotating basis. The mean of the temperatures in the *Marasmius* nests was computed. The absolute value of the differences in the ambient temperature from the oriole nest and from the average flycatcher nest temperatures was computed (Fig. 4). This produced ordered pairs of absolute temperature difference for each time for each type of nest. The mean of these differences was -0.021°C with the oriole sample producing the higher degree of temperature buffering. A one-sample *t*-test was performed on the difference of these pairs, producing a *p*-value of 0.47. While both types of nests provided some degree of temperature moderation, there is no statistical difference in the temperature moderation ability of the two types of nests.

Therefore, we reject both the hypothesis that *Marasmius* nests provide greater tensile strength and that they provide greater temperature moderation than that provided by other readily available nesting material. In fact, we tested one such material and found it provided the same temperature moderation and greater tensile strength.

Nest microclimate is crucial for successful incubation and brooding, and it directly impacts daily energy requirements of adults (Gill 2007). Birds adopt a range of strategies to promote thermal inertia, from nesting communally (Lowney et al. 2020) to placement of nests in cavities and burrows (Ar and Piontkewitz 1994). The choice of nest materials plays a role in buffering external temperatures (Mainwaring et al. 2014). Within species, nests in colder climates have better heat retaining features, aided in part by the nest materials chosen by parents (Kern and van Ripper 1984; Briskie 1995; Rohwer and Law 2010). Given the importance of nest materials in thermoregulation, and the widespread usage of *Marasmius* in tropical birds, it is surprising that this is the first time this nest material has been examined for possible regulation of nest microclimate.

There are two limitations in our study. First was the small sample size, particularly of the grass control material. Using a larger sample size, particularly for the control material, from multiple nests examining different nesting materials, would have strengthened this study. The second was the availability of only five data loggers, requiring us to take data over three days for the five different available black nests and the single nest from the grassy material. Ideally, we would have preferred taking the data concurrently from more nests of both types. Despite these limitations, our study should spur future inquiries examining these and other hypotheses, using and building on the technologies and methods we used.

Our study suggests that neither tensile strength nor temperature moderation is a factor in the frequent usage of black fungal fibers in Yellow-olive Flycatcher nests. Perhaps these fibers are chosen because of their water resistance (Freymann 2008) or simply due to their easy availability in forested environments, compared to grassy material that may be more common in open habitats. Also, *Marasmius* fibers may be better suited for cup nests compared to hanging nests, with a possible tradeoff between physical strength and other properties. Nestlings may be
benefited by these fiber linings in some way (Aubrecht et al. 2013). There is some evidence that uneven distribution of Marasmius may be involved in selective use differences by birds across geographical areas and habitats (Aubrecht et al. 2013).

The complex web of interactions between Marasmius, birds, ant-acacia trees, acacia-ants, and social hymenopterans (Fig. 1) make teasing out cause-and-effect challenging (Young et al. 1990; Flaspohler and Laska 1994; Menezes et al. 2014). Also, there is evidence that many species of Marasmioid fungi form aerial rhizomorphs, and birds selectively use them for different roles (lining, support) in nest (Koch et al. 2020). Given these complexities, we encourage more research to solve the mystery of why these black Marasmius fibers are preferred by the Yellow-olive Flycatcher and other tropical birds.

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Literature Cited


