Mycorrhizal Sedge:

Composition and Abundance of Mycorrhizal Fungi in *Carex pensylvanica* from Wisconsin

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Preface: When I entered grad school, I had been doing environmental contract work on Fort McCoy military base in Wisconsin. My work was all vegetation work—invasive control, rare plant mapping, and vegetation monitoring. During my vegetation monitoring, *Carex pensylvanica* (common name: Pennsylvania sedge), an inconspicuous sedge, was in the vast majority (91%) of the quadrats we studied. As I figured out my thesis project, I read many previous students' theses and found mycorrhizal studies and endophytes studies interesting. I wanted to do a mycorrhizal and/or a dark septate endophyte research project. Because sedges were typically considered to be one of the few groups of non-mycorrhizal plants, I thought it would be interesting to investigate the fungi associated with the roots of *Carex pensylvanica*.

Tom Volk passed away in November of 2022, but he was very much a part of this research. He (and others) thought our findings were interesting and worth sharing with the scientific community. This article is very much due to Tom's teachings and the encouragement that he gave all his students. He is missed and this article demonstrates that his impact will live on.

Introduction

Sand prairies are a rare habitat type found in Wisconsin. They're similar to a tallgrass prairie in terms of plant communities, but are more sparse due to the sandy soils. Fort McCoy has some of Wisconsin's most intact native sand prairies (and other habitats, for that matter), so it was an ideal location for our study. *Carex pensylvanica* ranges from Iowa to Nova Scotia in a wide array of niches including cliff edges, shaded woodlands, open prairies, and so on. It has a very developed underground network of roots and rhizomes that connect upper portions of the plant. It also happens to be one of the most widespread plant species on Fort McCoy and was found in 91% of our vegetation monitoring quadrats that were randomly spaced across several prairies/oak savannas. Oak savanna (another rare habitat) is basically a prairie with some oaks scattered throughout it. To gain more insight of what is going on in the roots, we did:

- above-ground plant diversity
- soil testing
- fungal colonization/morphology within the root (microscopy)
- culture based fungal identification
- and molecular mass spectrometer fungal identification.

Research Synopsis and Results

We collected 30 plants, with soil, from 15 sand prairie/oak barrens on Fort McCoy in Wisconsin. Soils were carefully removed from the roots, and the roots were cut, randomized, and separated for further analysis.

Roots for culturing were surface sterilized (Carver, 2013) then plated onto Petri dishes and monitored for six weeks. Fungi that grew out of the roots were isolated, monitored, and tallied for frequency. The most frequent species were further identified with genetic testing. Seventy morphotypes were sorted out of the 366 fungal isolates from direct culturing. Of those 70, 18 were found in more than one plant. The 17 morphotypes that were found in 10% (3 plants) or more were identified (Figure 1). *Fusarium oxysporum* was the species most commonly found in both the most plants and the most root segments.

The root segments, that were to be visually inspected under a compound microscope, were cleared and stained (modified from Barrow and Aaltonen, 2003) to show various fungal structures

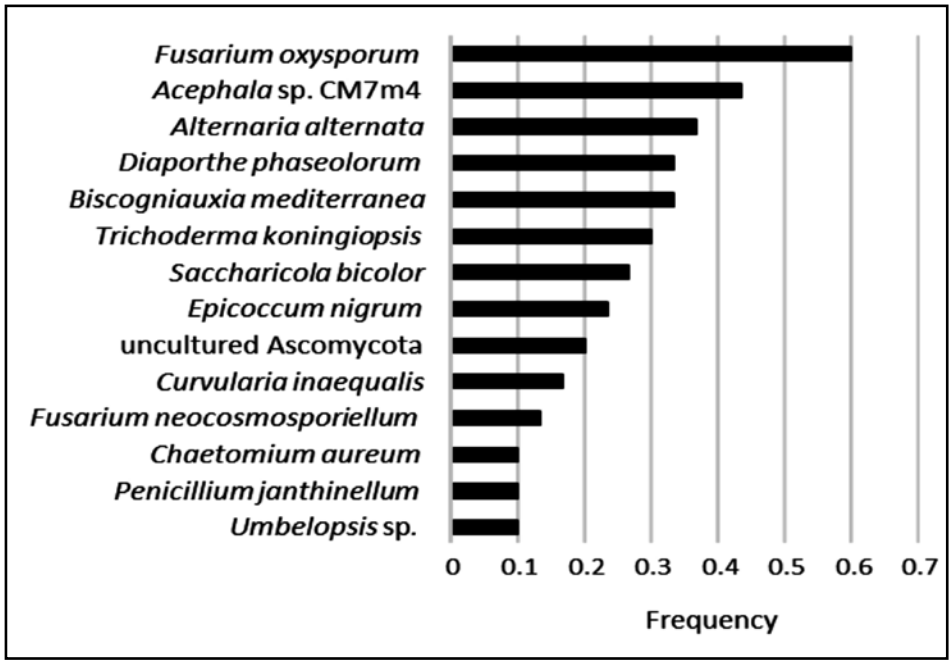

Figure 1. Frequency of most commonly isolated fungal root endophytes from *Carex pensylvanica* via direct culturing. Measured by number of plants found in/number of plants samples. Plants were collected from Fort McCoy, Wisconsin; (n=30).

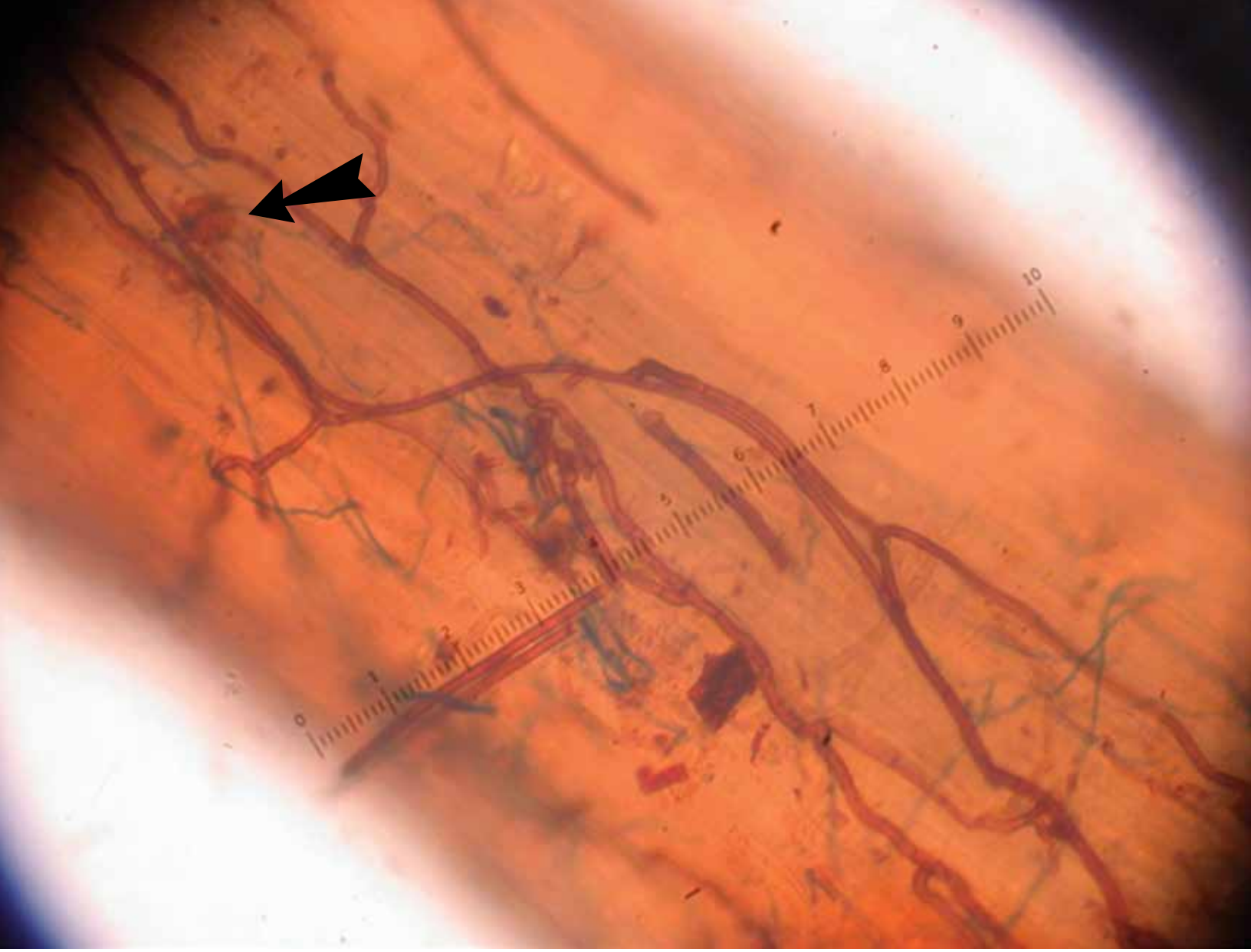

Figure 2. Root section of *Carex pensylvanica* with abundant fungal colonization. Both dark septate endophytes (brown hyphae) and arbuscular mycorrhizae (blue hyphae) are present. Arrow pointing at a vesicle. Photographed under 400x magnification (1 micrometer tic mark=2.5µm).

(Figure 2). After staining, the root segments were made into permanent microscope slides. Following a modified Magnified Intersection Method (McGonigle et al., 1990), root segments were pseudo-randomly examined 3 times each for 60 observations per plant, and 1,800 total (Figure 3). Slides were positioned so that a root section would be in the field of view. Once in place, observations were made through the eyepiece where the cross hair intersected the root and recorded what the cross hair touched (no fungi, hyaline hyphae, dark septate hyphae, clamped hyphae, microsclerotia, or other fungal structures). Observations were made with a compound light microscope at 400x and 1000x magnification.

Roots for Illumina Sequencing were DNA extracted and amplified (Ivors et al., 2004; Smith and Peay, 2014). Ten of the 30 samples were lost due to breaking tubes and poor amplification. The remaining 20 were sequenced using Illumina 2 x 250 TruSeq Nano at the UW Biotechnology Center (Madison, WI). In the 18 samples we got data back for, there were a total of 363 Operational Taxonomic Unit (OTUs are basically genetic species). Fourteen OTUs were found in all plants, including DSE *Acephala harenae* nom. prov. and ectomycorrhizal *Tomentella ferruginea*. Glomeromycota were found in all samples, but no individual AMF species across the board.

Total fungal colonization levels did not significantly correlate with any soil factors. Water holding capacity had a borderline significant positive relationship with fungal colonization, but the R 2 was low (y = 77.811x + 27.718, t = 1.761, n = 30, P = 0.089, R 2 = 0.0998). Although they only accounted for about 14% of the variability each, pH increased and organic matter decreased hyaline hyphae colonization. (pH: y = -0.865x + 0.6762, t =-2.166, n = 30, P = 0.039, R 2 = 0.1435) (OM: y = 0.0282x + 0.138, t = 2.089, n = 30, P = 0.046, R 2 = 0.1349). Compared to hyaline hyphae, dark septate endophyte colonization showed an opposing, weakly negative trend with pH (y = 0.0966x – 0.1512, t = 1.775, n = 30, P = 0.087, R 2 = 0.1012) and was unaffected by organic matter (y = 0.0022x + 0.3633, t = -0.116, n = 30, P= 0.908, R 2 = 0.0005). None of the measured types of fungal colonization

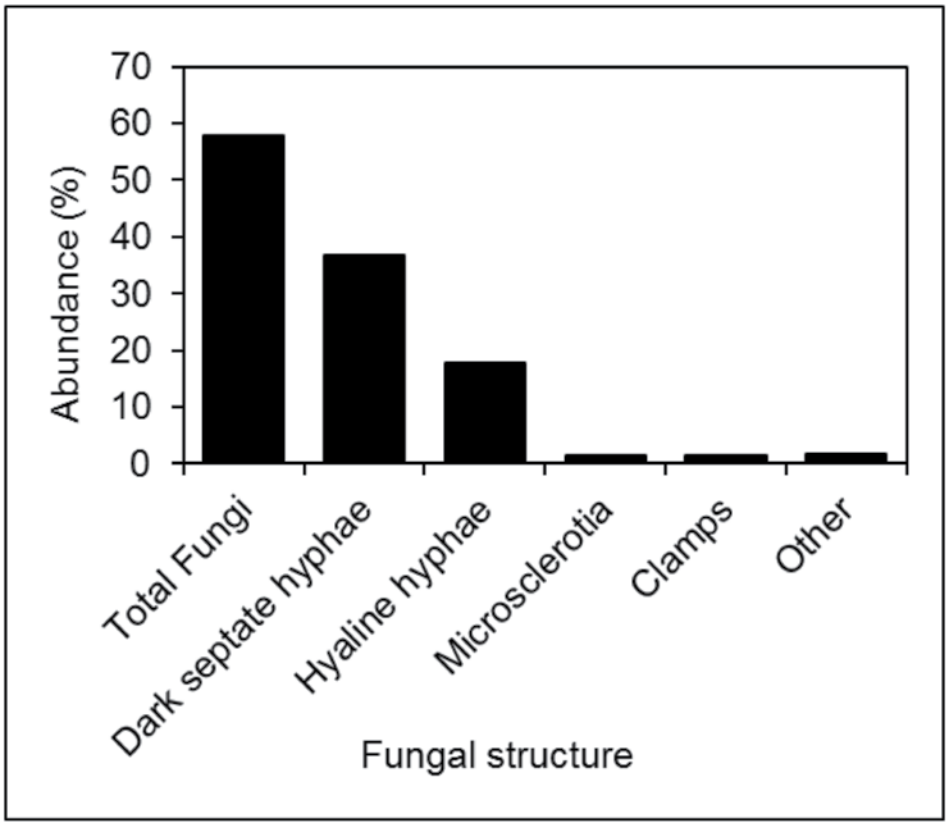

Figure 3. Abundance of various fungal structures in *Carex pensylvanica* roots collected from Fort McCoy, Wisconsin. Thirty plants were observed 60 times each, totaling 1800 observations. Abundance measured as total number of times observed over total number of observations, then converted to percentage.

were shown to be significantly influenced by phosphorus, total nitrogen or water holding capacity.

Discussion

We found lots of dark septate endophytes (DSE), but more interestingly we found cohabitation with arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (ECM), both visually and with sequencing. Because sedges were typically considered to be one of the few groups of non-mycorrhizal plants, these findings are very significant.

We potentially helped tease out a new species of DSE, *Acephela harinae nom. prov.* Sand prairies turned out to foster high fungal colonization with 58% of cross sections containing fungal structures of some kind.

We did not see much correlation between the soil conditions we tested for and fungal colonization or composition.

Carex pensylvanica, an upland sedge is mycorrhizal with AMF, ECM, and harbors DSE, sometimes all at once. This may mean other sedges are mycorrhizal as well.

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See also: Miller, R.M., C.I. Smith, J.D. Jastrow, and J.D. Bever. 1999. Mycorrhizal status of genus *Carex* (Cyperaceae). *American Journal of Botany* 86: 547–553.

Including Metaphor

The ground has been erupting
for days. little tremors
only a flea could feel.

Camera in hand. I walk
a mile through the woods
documenting the aftermath.

Boletus. Chanterelle. Slippery
Jack. And bursting out of a log
some thready white stems

that might be psilocybin – the little
gods – the ones telling us
that everything is metaphor.

Tony Alcantara
Colorado