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Survey of the Endogonaceae in Minnesota With Synoptic Keys to Genera and Species

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ABSTRACT—Sixteen species in the Endogonaceae (Zygomycotina) were identified from 22 different plant species from a native prairie, an intensively cultivated vegetable field, a reclaimed iron ore tailings basin, an undisturbed site adjacent to the iron ore tailings basin, and from a *Pinus resinosa* plantation. Seven species of Endogonaceae identified in this study are new records for Minnesota. Synoptic keys to genera and species are presented.

Introduction

In 1885, Frank (1) coined the term mycorrhiza, which means "fungus root," to describe the symbiotic association between plant roots and mycorrhizal fungi. He distinguished two types of mycorrhizae—those with fungal hyphae that predominately grow intercellularly and develop outside the root and those that develop predominately within root cells. These fungal-root associations are now known as ectomycorrhizal and endomycorrhiza, respectively. Endomycorrhizal (vesicular-arbusicular mycorrhizae-VAM) and ectomycorrhizal fungi are commonly associated with most plants worldwide.

The Endogonaceae (Endogonales, Zygomycetes) contain only two ectomycorrhizal species in the genus Endogone. These are *E. flamicorona* Trappe & Gerd. and *E. lactiflua* Bk. & Br. The remaining Endogonaceae are endomycorrhizal VAM fungi, consisting of 129 species in six genera.

The importance of vesicular-arbuscular mycorrhizal (VAM) fungi in plant establishment and growth in natural communities is widely recognized (2, 3, 4). These fungi benefit plants in several ways, including improved nutrient uptake (5), resistance to drought stress (6, 7), and protection against soil borne diseases (8, 9). Vesicular-arbuscular mycorrhizal plants are also used in the revegetation of disturbed ecosystems (4, 10, 11). This mutually beneficial association with most land plants continues to attract significant input from the scientific community ranging from basic to applied research in ecology and agriculture. The current interest and future goals of sustainable agriculture in Minnesota must include research on VAM fungi in an environment of reduced inputs of fertilizers, pesticides, and water.

Species of VAM fungi differ in their ability to improve plant growth (12). It is imperative, therefore, that isolates of these fungi be identified correctly. Walker (13) has revealed the widespread confusion regarding the misidentification and species limits of *Glomus fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske. This fungus has been used extensively in plant-fungal interaction studies. The misidentification of VAM fungi casts doubt on the validity of some studies reported in the scientific literature; *Glomus fasciculatum* is an example.

Because of the importance of mycorrhizal fungi to ecology and agriculture, we initiated studies to identify indigenous ecto- and endomycorrhizal fungi in the family Endogonaceae at five locations in Minnesota. These locations were chosen because each area represented a very distinct and different type of ecosystem. The objectives of this study were to survey the occurrence of mycorrhizal fungi collected from soils and to develop a synoptic key to species of Endogonaceae identified.

Materials and Methods

Spores of VAM fungi were extracted from soil samples collected monthly from June through September, from the Ordway Prairie (tall grass native prairie, Pope County), an intensively cultivated vegetable field (Dakota County), a reclaimed iron ore tailings basin (St. Louis County), and an undisturbed vegetated area of *Bromus inermis* Leyss. adjacent to the iron ore tailings basin. Epigeous fruiting bodies (sporocarps) of *Endogone* species were collected in a *Pinus resinosa* Ait. plantation in Chisago County. To extract VAM spores, soil and plant roots were collected from the top 25-cm soil, wet sieved (14), decanted and subjected to sucrose density centrifugation (15). The extracted spores were mounted in polyvinyl alcohol mountant (16) and examined microscopically to identify species. Root pieces were cleared with KOH and stained with a 0.1% trypan blue in lactophenol (17) to determine VAM infection. Pot cultures were established with the various VAM species collected using *Sorghum sudanense* (Piper) Stapf. as the host plant. A synoptic key of the mycorrhizal fungi identified in this study was developed using a computer software program developed by Rhoades (18).

Results and Discussion

Sixteen Endogonaceae species were identified during this study (Table 1) from 22 different plant species (Table 2). Eight species of Endogonaceae were identified from the Ordway Prairie (Table 1). Three of the eight species, *Glommus albidum, G. etunicatum,* and *G. mosseae,* appear to be well adapted to a wide range of soil nutrient concentrations, since they occurred in both a native prairie and an

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Table 1. Identification and location of Endogonaceae from Minnesota.

Endomycorrhizal (VAM) fungi:	
Acaulospora levis	Undisturbed area adjacent to iron ore tailings basin
Entrophospora infrequens	Ordway Prairie
	Reclaimed iron ore tailings basin
	Undisturbed area adjacent to iron ore tailings basin
Gigaspora gigantea	Undisturbed area adjacent to iron ore tailings basin
*Glomus albidum**	Ordway Prairie
	Cultivated vegetable field
Glomus caledonicum	Reclaimed iron ore tailings basin
*Glomus etunicatum**	Ordway Prairie
	Cultivated vegetable field
Glomus fasciculatum	Cultivated vegetable field
	Undisturbed area adjacent to iron ore tailings basin
Glomus geosporum	Ordway Prairie
	Undisturbed area adjacent to iron ore tailings basin
Glomus macrocarpum	Ordway Prairie
	Undisturbed area adjacent to iron ore tailings basin
*Glomus microcarpum**	Ordway Prairie
Glomus mosseae	Ordway Prairie
	Cultivated vegetable field
	Undisturbed area adjacent to iron ore tailings basin
*Glomus occultum**	Ordway Prairie
*Scutellospora calospora**	Ordway Prairie
Ectomycorrhizal fungi:	
*Endogone flammicorona**	*Pinus resinosa* plantation
*Endogone lactiflua**	*Pinus resinosa* plantation

*New records of *Endogonaceae* from Minnesota

intensively cultivated vegetable field that had received heavy annual applications of nitrogen, phosphorus, and potassium. In each location reproductive potential was high, and root sections were heavily colonized with VAM hyphae. Such adaptation to a broad range of soil conditions is significant because some species of Endogonaceae are suppressed in the presence of high concentratins of soil nutrients. Studies are needed to determine the influence of the VAM fungi from these habitats on plants of interest, with regard to plant growth and development.

Glomus microcarpum, G. occultum, and *Scutellospora calospora* were identified only from the Ordway Prairie. *Glomus caledonicum* and *Entrophospora infrequens* were identified from the reclaimed iron ore tailings basin (19). However, *E. infrequens* also occurred in the undisturbed area adjacent to the iron ore tailings basin and in the Ordway Prairie. Six Endogonaceae were identified from the undisturbed area adjacent to the iron ore tailings basin.

Endogone flammicorona and *E. lactiflua* form ectomycorrhizae with numerous tree species in the world. They were collected during this study in association with *Pinus resinosa*. These members of the Endogonaceae do not occur in agronomic or prairie ecosystems.

Table 2. Vascular plants and location from which Endogonaceae were collected and identified.

Allium cepa L.	Cultivated vegetable field
Artemisia ludoviciana Nutt.	Ordway Prairie
Aster sericeus Venten & Pers.	Ordway Prairie
Bouteloua curtipendula (Minhx.) Torr.	Ordway Prairie
Bromus kalmii A. Gray	Ordway Prairie
Bromus inermis Leyss.	Ordway Prairie
Compositae	Undisturbed area adjacent to iron ore tailings basin
Cucumis sativus L.	Cultivated vegetable field
Echinacea angustifolia DC.	Ordway Prairie
Fragaria ananassa Duchesne	Cultivated vegetable field
Gramineae	Reclaimed iron ore tailings basin
	Undisturbed area adjacent to iron ore tailings basin
Leguminosae	Reclaimed iron ore tailings basin
	Undisturbed area adjacent to iron ore tailings basin
Liatris pycnostachya Michx.	Ordway Prairie
Pisum sativum L.	Cultivated vegetable field
Pinus resinosa Ait.	*Pinus resinosa* plantation
Poa spp.	Ordway Prairie
Polygonaceae (Rumex crispus L.)	Reclaimed iron ore tailings basin
Andropogon scoparius Michx.	Ordway Prairie
Setaria lutescens (Weigel.) Hubb.	Ordway Prairie
Sorghastrum nutans (L.) Nash.	Ordway Prairie

The decision to develop synoptic keys in this study was based on the ease of construction, use, and reliability of such keys. The development, advantages, and use of synoptic keys are discussed in detail by Leenhouts (20), Korf (21), and Stewart and Pfleger (22). The characters and character states used in our synoptic key provide a compact description of the taxa included in this study. Two synoptic keys are presented. The first key consists of a mixture of genera and species and is suitable for keying out genera represented by a single species. The second key allows identification of the nine species within the genus *Glomus*. Number(s)in parentheses following a fungus name in the synoptic keys' taxa lists refer to the cited literature. We are reporting seven new records of Endogonaceae from Minnesota.

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SYNOPTIC KEY TO GENERA AND SPECIES OF *ENDOGONE* AND VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI IDENTIFIED IN MINNESOTA

1. *Acaulospora levis* Gerd. & Trappe (23, 24)
2. *Endogone flammicorona* Trappe & Gerd. (24)
3. *Endogone lactiflua* Berk. & Broome (24)
4. *Entrophospora infrequens* Ames & Schneider (25)
5. *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe (24)
6. *Glomus* Tul. & Tul.(26)
7. *Scutellospora calospora* (Walker & Gerd.) Walker & Sanders (27).

1. POSITION OF SPORE ON HYPHAL ATTACHMENT
   - Spore formed laterally on hypha below a swollen saccule [1]
   - Spore formed at union of gametangia or at tip of a gametangium [2, 3]
   - Spore formed within a hypha below a swollen saccule [4]
   - Spore formed on bulbous hyphal attachment [5, 7]
   - Spore terminally on a simple hypha [6]
   - Spores lacking hyphal attachment [1, 2, 3, 4, 5, 6, 7,]

2. SPORE WALLS
   - Spores with one wall group [1, 5, 6]
   - Spores with two or more wall groups [1, 2, 3, 4, 6, 7]
   - Spores with outer wall pitted [1, 5, 6]
   - Spores that have coriaceous walls [7]
   - Spores that have amorphous walls [1, 7]
   - Spores that have membranous walls [1, 4, 7]
   - Spore wall with flame-shaped projections in cross section [2]
   - Spore wall appearing netted in cross section [3]

3. SPORE ORGANIZATION
   - Sporocarpic: spores produced from gametangia [2, 3]
   - Sporocarpic: spores produced on terminal hyphae [6]
   - Sporocarpic: spores produced below a swollen hyphal saccule [1]
   - Spores occur singly in soil, nonsporocarpic [1, 4, 5, 6, 7]

4. TYPE OF MYCORRHIZAE
   - Ectomycorrhizae [2, 3]
   - Vesicular-arbuscular mycorrhizae [1, 4, 5, 6, 7]

5. MODE OF SPORE GERMINATION
   - Direct through spore wall [5]
   - Through germination shield [7]
   - Through basal hyphal attachment [6]

6. LATEX PRODUCTION
   - Observed following tissue damage [3]
   - Absent [1, 2, 4, 5, 6, 7]

SYNOPTIC KEY TO GENUS *Glomus*

1. *Glomus albidum* Walker & Rhoades (28)
2. *Glomus* caledonicum (Nicol. & Gerd.) Trappe 7 Gerd. (24)
3. *Glomus etunicatum* Becker & Gerd. (29)
4. *Glomus fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske (13)
5. *Glomus geosporum* (Nicol. & Gerd.) Walker (30)
6. *Glomus macrocarpum* Tul. & Tul. (31)
7. *Glomus microcarpum* Tul. & Tul. (32)
8. *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe (24)
9. *Glomus occultum* Walker (30)

1. SPORE COLOR
   - Hyaline [1, 4, 7, 9]
   - White [1, 9]
   - Yellow [2, 3, 4, 7, 8]
   - Yellowish-brown to brown [2, 3, 4, 5, 6, 7, 8]
   - Reddish-brown [5, 6]

2. SPORE SIZE AT MATURITY
   - 10-35μm [7, 9]
   - 35-60μm [4, 7, 9]
   - 60-95μm [1, 3, 4, 6, 9]
   - 95-120μm [1, 2, 3, 4, 5, 6, 8, 9]
   - 120-155μm [1, 2, 3, 4, 5, 6, 8]

2. SPORE SIZE AT MATURITY (cont.)

155-180μm
[1, 2, 3, 5, 8]
180-205μm
[2, 8]
205-230μm
[2, 8]
230-255μm
[2, 8]
255-280μm
[2, 8]
280-305μm
[8]
>305μm
[8]

3. SPORE WALL STRUCTURES

A single-unit wall
[4, 7, 9]
A single-laminated wall
[4]
Two walls: outer wall hyaline, inner wall laminate or nonlaminate
[1, 2, 3, 6, 8]
Two wall: outer wall hyaline and evanescent; inner wall laminate
[1, 3, 9]
Two walls: unit walls of nearly equal thickness
[1]
Three walls: unit, laminated & membranous
[5]

4. SPORE WALL THICKNESS AT MATURITY

0.5-3μm
[1, 8, 9]
3-6μm
[2, 3, 4, 5, 7, 8, 9]
6-9μm
[2, 3, 4, 5, 6, 8]
9-12μm
[2, 3, 4, 5, 6]
12-15μm
[2, 3, 4, 5, 6]
15-18μm
[2, 4, 5]

5. PORE CLOSURE AT SPORE BASE

Open
[1]
Septμm
[2, 5, 9]
Inner wall thickening at spore base
[3, 4, 6, 7]
Collapse or loss of subtending hyphae
[1, 3]
Curved septμm in subtending hyphae
[1, 2, 3, 8]

6. DIAMETER OF HYPHAL ATTACHMENT

3-5μm
[1, 4, 7, 9]
5-8μm
[1, 3, 4, 7, 9]
8-11μm
[1, 3, 4, 5, 7, 9]
11-14μm
[1, 2, 3, 4, 5, 6]
14-17μm
[1, 2, 3, 4, 5, 6]
17-20μm
[2, 3, 8]
20-23μm
[2, 8]
23-25μm
[2, 8]
25-50μm
[8]

7. WALL THICKNESS AT POINT OF HYPHAL ATTACHMENT

0.5-1μm
[1, 7, 9]
1-2μm
[1, 7, 9]
2-4μm
[2, 3, 4, 6, 8]
4-6μm
[2, 3, 4, 6, 8]
6-8μm
[2, 3, 4, 6, 8]
8-10μm
[2, 4, 5, 8]
10-13μm
[4, 5]
13-16μm
[4, 5]
16-20μm
[5]
20-25μm
[5]

8. ALIGNMENT OF HYPHAL ATTACHMENT TO SPORE AXIS

Straight
[1, 2, 3, 4, 5, 6, 7, 8, 9]
Curved
[3, 5, 9]

9. SHAPE OF HYPHAL ATTACHMENT

Cylindrical
[1, 2, 4, 6, 7]
Constricted
[1]
Funnel-shaped
[1, 2, 3, 5, 8, 9]

10. LENGTH OF SUBTENDING HYPHAE

Short
[3, 5, 7, 9]
Long
[2, 4, 5, 6, 8, 9]
Collapsed or absent
[1, 2, 3, 5]

11. MACROCHEMICAL REACTION IN COTTON BLUE

No reaction
[3, 4, 5, 6, 8]
Cyanophilous
[1, 9]
Unknown
[2, 7]

12. MACROCHEMICAL REACTION IN MELZER'S REAGENT

No reaction
[3, 4, 5, 6, 8]
Pink to orange-red
[1, 9]
Unknown
[2, 7]