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Systemic infection of *Medeola virginiana* (Liliaceae) by the fungus *Medeolaria farlowii* (Ascomycota: Leotiomycetes)

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Abstract

Primers were designed to specifically amplify ITS rDNA regions of the fungus *Medeolaria farlowii*. The fungus was shown to be present not only in stem lesions but in apparently uninfected leaves, stems and rhizomes of the host plant, *Medeola virginiana*. Since the plant reproduces clonally it is likely that the infection is carried in populations of the host plant through systemic infection of vegetative plant parts. The growth patterns of the plant are reviewed and examples are given of long-term perpetuation of the fungus in populations of the plant.

Key words: clonal growth, Indian cucumber, R. P. Korf, R. Thaxter, W. G. Farlow
Introduction

Medeolaria farlowii Thaxter (1922), a distinctive ascomycete parasite of Medeola virginiana L., was described from material collected from Magnolia, Massachusetts; Chochorua, New Hampshire; and Gerrish Island, Kittery Point, Maine. Although Farlow had noted the fungus in 1902 it was not until Thaxter found it in September 1904 on the hillside near Farlow’s summer home in Chochorua that fully mature material was available for study. The fungus is little more than a hymenium composed of asci and paraphyses that forms on fusiform swellings below and/or between the shortened internodes of the host plant (Fig. 1). Asci have no organized opening as seen with the light microscope; the ascospores are large, fusiform to naviculate, with a dark outer wall layer that is striate (Fig. 2). We have neither succeeded in obtaining ascospore germination nor have we be able to grow the fungus from excised tissue; no anamorph is known. Thaxter (1922) suggested that the spores recalled those of Wynnea americana or Choanephora cucurbitarum.

The reduced morphology and the distinctive ascospores defied definitive taxonomic placement. In his thorough and well-documented description, Thaxter (1922) placed M. virginiana among the Protodiscineae of Schroeter conceding that this was a heterogeneous assemblage. No other more satisfactory placement was offered until Richard Korf undertook a study of the fungus in the preparation of his chapter in The Fungi: An advanced treatise (1973). In this important work he treated the genus as the only member of the family Medeolariaceae in the order Medeolariales; subsequently, Korf validated both names (Eriksson 1982). Our analysis (LoBuglio and Pfister 2010) of
the genus placed *Medeolaria* among the Leotiomycetes but with no clear alliance, in part
we believed because of the lack of taxon sampling within the class.

*Medeolaria farlowii* collected at Chochorua by Thaxter in 1922 is represented in
the widely distributed Reliquiae Farlowianae as number 639, presumably gathered from
the same hillside where it had been collected in 1904. From correspondence it is clear
that both Farlow and Thaxter knew that the fungus was undescribed and that Farlow had
intended to describe it but by his death in 1919 he had not done so. Thaxter’s (1922)
description was based primarily on collections from Kittery Point and Chochorua.

To better understand this fungus Korf went to Chochorua, with the senior author
of this paper, then his graduate student at Cornell University, in October of 1970. Often
with rare and elusive species returning to the site of a previous collection yields
disappointment but not new material. Korf had researched the Chochorua location and
had determined the exact location of Farlow’s house through contact with mycologist
Edith Cash who provided directions. We were able to collect infected *M. virginiana* on
the hillside where, nearly 70 years before, Thaxter had made his collection in 1904. The
persistence in a particular location is a feature of this fungus that we have now
demonstrated in other locations.

After relocating to New England the senior author continued to search for infected
individuals of this quite common plant of wet woodlands. He also encouraged others,
mycologists and botanists alike, to search for *Medeola* and its parasite. Only a few
additional sites have located, including at Mount Monadnock in western New Hampshire,
Newfield, Oxford County, Maine near the New Hampshire border and not far from
Chochorua (Pfister 1983), and at Mount Wachusetts in central Massachusetts.
We have observed that only small pockets of affected individuals are present even in large populations of the host. Diligent examination of many plants is necessary in order to find infected individuals and then there are often several in close proximity. It is also clear that the infections are recurrent in these pockets. The recurrence of infected plants at Chochorua in notable as is the site at Mount Monadnock that has been visited periodically for nearly thirty years with positive results. On each visit to this site infected plants have been located in the same area. Because of the clonal spread of this plant (Bell 1974, Cook 1988) and the recurrence of the parasite at particular locations, we undertook a study to determine if *M. farlowii* was present in stem, rhizome, tuber and leaf tissue. A recent collection of *Medeolaria farlowii* by Jason Karakehian significantly extended the range of this fungus. This collection was made in autumn of 2012 from in the Monongahela National Forest, West Virginia. He collected infected plants, including rhizomes and tubers. With these specimens we were able to test our hypothesis that the fungus was present not just in and around the lesions but throughout the plant.

**Materials and Methods**

*Medeolaria Specific Primers*

PCR primer sequences specific to *Medeolaria* were identified using the program “Primer-Blast” from NCBI ([http://www.ncbi.nlm.nih.gov/tools/primer-blast/](http://www.ncbi.nlm.nih.gov/tools/primer-blast/)). This program selected potential PCR primer sequences that were unique to *Medeolaria* (GenBank GQ406809), and suitable for PCR reactions, after screening the Ascomycete ITS-28S rDNA sequence database.

The *Medeolaria* specific PCR primer region selected spans a 396 bp sequence from the 5.8S rDNA to 28S rDNA gene (5’-3’). The 5’ primer identified is Med5’ =
CCCACCCCATGCGTTTTTC, and the 3’ primer is Med3’ = GTAGCGAGGGCTGTACTACG.

The specificity of the Med5’-Med3’ primer pair was tested by attempted amplifications from DNAs of Medeolaria (positive control), Pleospora sp., Colletotrichum acutatum, Cenococcum geophilum, Rickiella edulis, Gelatinopsis sp., and two Maple endophytes (Colletotrichum sp. and Phylosticta sp.) All fungal isolates included in the evaluation were first amplified with primers designed as general fungal primers, namely, ITS1F and ITS4 as well as the Med5’-Med3’ primers. PCR amplifications were as described in LoBuglio & Pfister 2010.

Screening for Systemic Medeolaria Infection

Medeola plants with visible Medeolaria lesions were collected by Jason Karakehian (no. 12082001) in the Monongahela National Forest W VA, 20. Aug. 2012. Samples of Medeola tissue were selected, using a sterile scalpel, from the tuber (4 samples), stem (5 sample) and leaves (1 sample) of the infected Medeola plants. A tissue sample from the Medeolaria lesion was also sampled to serve as a control. DNA was obtained from these tissue samples and PCR amplifications (as described in LoBuglio & Pfister 2010) were carried out using the Medeolaria specific primers Med5’-Med3’.

As a control tissue samples from the rhizome of 4 uninfected Medeola plants collected in MA were also screened for the presence of Medeolaria. Tissue samples were taken from the tuber of visibly uninfected Medeola plants collected by Jason K. (Noon Hill Reservation, Medfield MA, and K. LoBuglio (Paint Mine Conservation Area, Lexington MA).

Results
General primers ITS1F-ITS4 produced amplification from all DNAs tested. The primer combination Med5’-Med3” was successful at amplifying *Medeolaria* DNA and selecting against all of the other fungi tested. The positive PCR amplifications were sequenced with their respective Med5’ and Med3’ primers (as described in LoBuglio & Pfister 2010). Sequencing reactions yielded a single sequence that was identical to the expected *Medeolaria* sequence.

All PCR reactions were positive from the rhizome, stem and leaves of the infected *Medeola*. The BLAST NCBI program determined that sequences from these PCR products were 100% identical with the *Medeolaria* sequence, GenBank GQ406809.

PCR reactions using Med5’-Med3” were unsuccessful at amplifying *Medeolaria* DNA from the rhizome tissue of these uninfected plants.

**Discussion**

In order to explain both the pattern of occurrence of *Medeolaria farlowii* in distinct pockets of the host plant and its reoccurrence in populations it is necessary to further outline the growth dynamics of the host plant. *Medeola virginiana* produces tubers with multiple buds. In spring a shoot normally develops from one to as many as three of these buds. During the growing season rhizomes are formed and at the distal end of the rhizomes new tubers are produced. The plant senesces and dies at the end of the growing season leaving the tuber that was produced during the season. In the spring each tuber will produce one or more shoots. In this way a clonal colony arises (Bell 1974, Cook 1988). It is our contention that in the context of clonal spread the fungus is able to grow within the vegetative parts of the plant – stems, rhizomes and tubers – and thus be
manifest as lesions on new shoots. That the internode between the whorls is shortened supports the idea that tissue is infected prior to the full development of the shoot. Since spores are produced in the late fall, often after the plants are senescent, and given plants live for only one season, there is no possibility of direct plant-to-plant transmission involving aboveground parts. Likewise, ascospores are produced after flowering, thus infection of fruits and seeds is not possible through ascospore transfer. Vertical transmission of the fungus seems impossible. Ascospores over-wintering in debris or soil might play a role. Our results clearly show that *M. farlowii* is present in various parts of infected plants but questions remain as to how the primary infection occurs. Whatever the mode of infection, rates of infection must be relatively low since persistent pockets seem limited in size and are geographically widespread. The plant is widespread throughout the eastern United States east of the Mississippi River north of Florida (Utech 2002). Thaxter (1922) suggested that the fungus would likely be “found wherever the host occurs.” Such does not seem to be the case. The bold move by Korf to create an order for this fungus highlighted the special characteristics of *Medeolaria* allowing mycologists and botanists to search for it even if searches were unsuccessful. In contributing this paper to celebrate Professor Korf’s birthday we acknowledge his contributions to mycology and his insightful forays into the fungus world and we are reminded that much remains to be learned. Acknowledgements We wish to thank Jason Karakehian who heard about this fungus and then went out and found it.


LoBuglio KF, Pfister DH, 2010. Placement of *Medeolaria farlowii* in the Leotiomycets, and comments on sampling within the class. Mycological Progress 9:361-368


Figure captions

Fig. 1. *Medeolaria farlowii* on *Medeola virginiana*. On left, a senescent plant showing the swollen area of the stem below the basal whorl of leaves. On right, a sketch, in pencil, of a cross section of the infected stem. Illustration by Louis C. C. Krieger from a collection from Chocorua, New Hampshire, 12 Sept 1904.

Fig. 2. *Medeolaria farlowii*. A portion of the hymenial surface and ascospores. Illustration by Louis C. C. Krieger from a collection from Chocorua, New Hampshire, 12
Sept 1904.