



Systemic Infection of *Medeola virginiana* (Liliaceae) by the Fungus *Medeolaria farlowii* (Ascomycota: Leotiomyces)

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

3

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6 Farlow Reference Library and Herbarium of Cryptogamic Botany and Department of
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8

9 Abstract

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

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

19

20 Introduction

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

34 The reduced morphology and the distinctive ascospores defied definitive
35 taxonomic placement. In his thorough and well-documented description, Thaxter (1922)
36 placed *M. virginiana* among the Protodiscineae of Schroeter conceding that this was a
37 heterogeneous assemblage. No other more satisfactory placement was offered until
38 Richard Korf undertook a study of the fungus in the preparation of his chapter in *The*
39 *Fungi: An advanced treatise* (1973). In this important work he treated the genus as the
40 only member of the family Medeolariaceae in the order Medeolariales; subsequently,
41 Korf validated both names (Eriksson 1982). Our analysis (LoBuglio and Pfister 2010) of

42 the genus placed *Medeolaria* among the Leotiomycetes but with no clear alliance, in part
43 we believed because of the lack of taxon sampling within the class.

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

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

59 After relocating to New England the senior author continued to search for infected
60 individuals of this quite common plant of wet woodlands. He also encouraged others,
61 mycologists and botanists alike, to search for *Medeola* and its parasite. Only a few
62 additional sites have located, including at Mount Monadnock in western New Hampshire,
63 Newfield, Oxford County, Maine near the New Hampshire border and not far from
64 Chochorua (Pfister 1983), and at Mount Wachusett in central Massachusetts.

65 We have observed that only small pockets of affected individuals are present even
66 in large populations of the host. Diligent examination of many plants is necessary in
67 order to find infected individuals and then there are often several in close proximity. It is
68 also clear that the infections are recurrent in these pockets. The recurrence of infected
69 plants at Chochorua is notable as is the site at Mount Monadnock that has been visited
70 periodically for nearly thirty years with positive results. On each visit to this site infected
71 plants have been located in the same area. Because of the clonal spread of this plant (Bell
72 1974, Cook 1988) and the recurrence of the parasite at particular locations, we undertook
73 a study to determine if *M. farlowii* was present in stem, rhizome, tuber and leaf tissue. A
74 recent collection of *Medeolaria farlowii* by Jason Karakehian significantly extended the
75 range of this fungus. This collection was made in autumn of 2012 from in the
76 Monongahela National Forest, West Virginia. He collected infected plants, including
77 rhizomes and tubers. With these specimens we were able to test our hypothesis that the
78 fungus was present not just in and around the lesions but throughout the plant.

79 Materials and Methods

80 *Medeolaria* Specific Primers

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

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

88 CCCACCCCATGCGTTTTTC, and the 3' primer is Med3' =
89 GTAGCGAGGGCTGTACTACG.

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

97 Screening for Systemic Medeolaria Infection

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

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

110 Results

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

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

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

122 Discussion

123 In order to explain both the pattern of occurrence of *Medeolaria farlowii* in
124 distinct pockets of the host plant and its reoccurrence in populations it is necessary to
125 further outline the growth dynamics of the host plant. *Medeola virginiana* produces
126 tubers with multiple buds. In spring a shoot normally develops from one to as many as
127 three of these buds. During the growing season rhizomes are formed and at the distal end
128 of the rhizomes new tubers are produced. The plant senesces and dies at the end of the
129 growing season leaving the tuber that was produced during the season. In the spring each
130 tuber will produce one or more shoots. In this way a clonal colony arises (Bell 1974,
131 Cook 1988). It is our contention that in the context of clonal spread the fungus is able to
132 grow within the vegetative parts of the plant – stems, rhizomes and tubers – and thus be

133 manifest as lesions on new shoots. That the internode between the whorls is shortened
134 supports the idea that tissue is infected prior to the full development of the shoot.

135 Since spores are produced in the late fall, often after the plants are senescent, and
136 given plants live for only one season, there is no possibility of direct plant-to-plant
137 transmission involving aboveground parts. Likewise, ascospores are produced after
138 flowering, thus infection of fruits and seeds is not possible through ascospore transfer.
139 Vertical transmission of the fungus seems impossible. Ascospores over-wintering in
140 debris or soil might play a role. Our results clearly show that *M. farlowii* is present in
141 various parts of infected plants but questions remain as to how the primary infection
142 occurs.

143 Whatever the mode of infection, rates of infection must be relatively low since
144 persistent pockets seem limited in size and are geographically widespread. The plant is
145 widespread throughout the eastern United States east of the Mississippi River north of
146 Florida (Utech 2002). Thaxter (1922) suggested that the fungus would likely be “found
147 wherever the host occurs.” Such does not seem to be the case. The bold move by Korf to
148 create an order for this fungus highlighted the special characteristics of *Medeolaria*
149 allowing mycologists and botanists to search for it even if searches were unsuccessful.

150 In contributing this paper to celebrate Professor Korf’s birthday we acknowledge
151 his contributions to mycology and his insightful forays into the fungus world and we are
152 reminded that much remains to be learned.

153 Acknowledgements

154 We wish to thank Jason Karakehian who heard about this fungus and then went out and
155 found it.

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173 Figure captions

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

178 Fig. 2. *Medeolaria farlowii*. A portion of the hymenial surface and ascospores.

179 Illustration by Louis C. C. Krieger from a collection from Chocorua, New Hampshire, 12

180 Sept 1904.