

1 **First Report of *Alternaria* spp. Causing Leaf Spot on Sweet Viburnum in China**
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6 Sweet viburnum [*Viburnum odoratissimum* (L.) Ker Gawl] is an evergreen shrub
7 mainly cultivated along roadsides in urban landscapes and also in parks and
8 residential areas. A foliar disease occurred on about 40% of sweet viburnum plants
9 near Anhui Grand Theatre, Anhui Province of China in June 2019. In early stages of
10 sweet viburnum infection, the symptoms appeared as small brown spots ranged in
11 length from 2 to 3 millimeters on the leaves. The spots developed on the upper,
12 middle, and lower leaves of the plant, however, the upper leaves got more severely
13 affected. As the disease develops, the spots enlarged and became rectangular or oval,
14 brown to dark-brown, and their centers became ashen gray. In later stages of infection,
15 the diseased leaves became wilting. Diseased leaves were surface disinfested and
16 three small sections (2-3 mm²) were cut from the margin of the lesions. Sections were
17 placed in 1.5% NaClO for 2 min, submerged in three changes of sterilized distilled
18 water for 1 min each, placed onto potato dextrose agar (PDA) medium amended with
19 50 µg/ml of ampicillin and kanamycin, and incubated at 25 °C for 3 days. The
20 mycelium from the leading edge of colonies growing from the tissue was sub-cultured
21 onto a PDA plate for 3 days, followed by spore induction (Simmons 2007) and single
22 spore isolation to obtain a pure culture of the putative pathogen. Colonies of one

23 single spore isolate HF0719 were rounded, grayish white with dense aerial mycelium
 24 viewed from above and dark brown viewed from below. On potato carrot agar (PCA)
 25 medium, conidiophores were branched or occasionally unbranched. On branched
 26 conidiophores, conidia were in dwarf tree-like branched chains of 2-5 conidia. On
 27 unbranched conidiophores, conidia were simple or in chains of 2-8 conidia. Conidia
 28 were light brown or dark brown, ovoid, ellipsoidal to fusiform, and ranged in size
 29 from 7 to 26.5×4.5 to $11 \mu\text{m}$ with an average size of $16 \times 7 \mu\text{m}$ based on 500 spore
 30 observations, with one beak and 1-7 transverse, 0-3 longitudinal, and 0-3 oblique
 31 septa. Beaks were ranged in $(1.5\text{--})2\text{--}10\text{--}(16) \mu\text{m}$ long. Based on cultural and
 32 morphological characteristics, isolate HF0719 was identified as *Alternaria* spp.
 33 (Simmons 2007). For molecular identification, total genomic DNA was isolated from
 34 mycelia collected from 7 day-old colonies of isolate HF0719 using the fungal
 35 genomic DNA extraction kit (Solarbio, Beijing, China). Fragments of five genes,
 36 including those encoding glyceraldehyde-3-phosphate dehydrogenase (*gpd*), plasma
 37 membrane ATPase, actin, calmodulin, and the *Alternaria* major allergen (*Alt a1*)
 38 regions of isolate HF0719 were amplified and sequenced using primer pairs
 39 *gpd1/gpd2* (Berbee et al. 1999), *ATPDF1/ATPDR1*, *ACTDF1/ACTDR1*,
 40 *CALDF1/CALDR1* (Lawrence et al. 2013), and *Alt-for/Alt-rev* (Hong et al. 2005),
 41 respectively. The obtained nucleotide sequences were deposited into GenBank as
 42 accession numbers: *gpd*, MT614365; ATPase, MT614364; actin, MT614363;
 43 calmodulin, MN706159; and *Alt a1*, MN304720. Phylogenetic tree using a maximum
 44 likelihood bootstrapping method based on the five-gene combined dataset in the

45 following order: *gpd*, ATPase, actin, calmodulin, *Alt al* of HF0719 and standard
46 strains representing 120 *Alternaria* species (Lawrence et al. 2013) was constructed.
47 Isolate HF0719 formed a separate branch. On the basis of morphological
48 characteristics and phylogenetic pattern, isolate HF0719 was identified as *Alternaria*
49 spp. A pathogenicity test was performed by rubbing 32 healthy leaves of six
50 5-year-old sweet viburnum plants with a cotton swab dipped in spore suspension
51 containing 2.6×10^6 spores/ml, following leaf surface disinfection with 70% ethanol
52 in the open field. Sterilized distilled water was used as control. The average air
53 temperature was about 28°C during the period of pathogenicity test. Eleven days after
54 inoculation, 100% of inoculated leaves showed the leaf spot symptom identical to
55 symptoms observed in the field. Control leaves were symptomless. The experiment
56 was done three times. The re-isolated pathogen from the leaf lesion had the same
57 morphological and molecular characteristics as isolate HF0719, thus satisfying
58 Koch's postulates. The genus *Alternaria* has been reported to cause leaf spot on sweet
59 viburnum in Florida, USA (Alfieri et al. 1984). To our knowledge, this is the first
60 report of *Alternaria* spp. causing leaf spot on sweet viburnum in China, a highly
61 valued ornamental plant. Our findings will contribute to monitoring and adopting
62 strategies for manage leaf spot disease on sweet viburnum.

63 **References:**

- 64 **Alfieri S. A.** et al. 1984. Florida Dept. Agric. and Consumer Serv., Div. Plant Ind.
65 Bull. 11:1-389.
- 66 **Hong S. G.** et al. 2005. Fungal Genetics Biology, 42:119.

Lawrence D. P. et al. 2013. *Mycologia*, 105:3, 530.

Simmons E. G. 2007. *Alternaria: An Identification Manual*. CBS Fungal

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Figure legends:

Figure 1. Symptoms of leaf spot disease on sweet viburnum leaves caused by *Alternaria* spp. and morphology of *A. spp.* A: Diseased leaves occurring naturally; B and C: Symptomless leaves and leaf spot appearance 11 days after inoculation with water and isolate HF0719, respectively; D: Colony grown on PDA after 7 days at 25 °C (top and bottom of colony); E: Conidia grown on PCA.

Figure 2. Clustering analysis of conserved regions of isolate HF0719 and standard strains of 120 *Alternaria* species. The phylogenetic tree was constructed using the maximum likelihood method, based on the conserved overlapping regions of five-gene combined dataset. Bootstrap values from 1000 replications > 50 are shown at the nodes.

>MT614365 (gpd)

CAACGGCTTCGGTCGCATTGGCAGGATTGTCTTCGTAACGCGTCAGTTCAATTCCT

89 TTTATCACCCGTCTTCTGTTACCATTGCTGACGTAGCTTCGCAGCATCGAGCACGAT
90 GATGTCCTCATCACCGCCGTCAACGACCCCTTCATTGAGCCCCACTACGCCGTAAG
91 CGCCCTTTTGCTCTTTCTTGCCCTCTACATGCTCCGCGAGAGCCGTATGGTATGCAT
92 CGACGAACGCCTGGCTAACAAGCGCGTAGGCCTACATGCTCAAGTACGACAGCAC
93 CCACGGCCAGTTCAAGGGCACGATCGAGGTTGACGGCAACAACCTCAAGGTCAAT
94 GGCAAGACCATCAAGTTCTACACCGAGAAGGACCCAGGCAACATCCCATGGAGCGA
95 GACTGGTGCCTACTACGTCGTTGAATCCACCGGTGTTTTACCACCACCGACAAGG
96 CCAAGGCTCACTTGAAGGGTGGTGCCAAGAAGGTCGTCATCTCTGCTCCCTCCGCT
97 GACGCTCCTATGTTTCGTCATGGGTGTGAACGAGGAGACCTACAAGTCCGATATCGA
98 GGTTCTCTCCAACGCCTCTTGACACAACCAACTGCTTGGC
99 **>MT614364 (ATPase)**
100 ATCGTCTCCATGACCGAGTTCGATGGCATGGACGAGTACACGGCCCTGCAAAAAGTA
101 CATTCTTTTCTACCGCGACACAAAGACCAACCCTCACGCCAACTCCGACGGCAACA
102 AGAGCAAGCAGTGGTGGCAGTTCTGGAAGTCTGGCAGCGCGACCGCCGTCGAGCC
103 CATTGAGGATGCTGGAGCCGTTCCCGATGATGTAGGTTTTCTTCATTTTCTTCTTCT
104 TCGTCGTCATCGTCGACATCGTCGACATCACCCTGGTCTCCCATCGGTGCCATTG
105 TCGCCGATGTCGCCGTGCGGGCCGTCACCGACGAATCAAACAAACAAATTTTTTC
106 GGACATCTCGCTGACTTTTATCCTCTCTGTAGCAATTGAACACTGATCTTCGTGCCG
107 GTTTGACCAGCGCCGATGTGGACCGCCGCGCAAGATCTATGGCTTCAACGAAATC
108 ACCACGGAAAAGACCAACTGGTTGAAGCAGTTCCTCAGCTACTTCCAGGGTCCTATT
109 CTCTACGGTATGTATTCACCATTCGACATTCATTGTGGCAACTCAGCTAACGATCT
110 CGCTAGTCATGGAAATTGCTGCTCTTCTCGCTGCTGGTCTTGGTGACTGGATTGATT

111 TCGGTGTCATCTGCGGTATTCTCTTGCTTAACGCTGTTGTCGGTTGGTACCAGGAAA
 112 AGCAGGCTGCCGACGTTGTCGCCAGTTTGAAGGGAGACATCGCCATGAAGGCTACT
 113 GTCATTCGTGACGGCGGCCCAATCACCATCCTCGCCCGTGAAATCGTCCCCGGTGA
 114 CATCATCGTCATCGAGGAAGGAAACACTGTCCCCGCTGATGTCCGTCTTATCTGCAA
 115 CTTGACACTCCTGAGGACTACGAGACCTACCTTAAGCTTAAGGAGGAGGACATGT
 116 TCGACGAGTCTGACGACCCCGATAACGAGAAGGATGACGATGACCATGAGGGTCC
 117 CGTCCACGAGGGTGTCTCTCTCATTGCTACCGATCAGTCCGCCATCACCGGAGAGT
 118 CTCTCGCTGTCGACAAGTACATGGGCGAAGTCGCTTACTACACCACTGGTTGCAAG
 119 CGCGGCAAGACCTACGGTATTGCCCTCACCTCTGCTAAGCACTCTTTCGTCGGTCG
 120 CACCGCCAGTCTCGTTGCTGGTGCCAGGATCAGGGTCACTTCAAGGCTATCATGA
 121 ACTCCATCGGA
 122 **>MT614363 (actin)**
 123 TTCGGGTATGTGCAAGGCCGGTTTCGCCGGTGACGATGCACCTCGAGCTGTCTTCC
 124 GTGAGTACCCCGCCACACCATTTTCCAGCCGGAGCTGCATTTGCACTGCACACAAC
 125 AGCGCAATAACCGCAGCTCCTACCGTGTGCTGGATCGTGTTGTGAGCATGCGCTGA
 126 TGAGAATTGTAGCCTCCATTGTCGGTCGCCCCCGTCACCATGGTATCATGATTGGTA
 127 TGGGCCAGAAGGACTCGTATGTTGGCGATGAGGCACAGTCGAAGCGTGGTATCCTT
 128 ACTCTGCGATACCCCATCGAGCACGGTGTGGTCACCAACTGGGACGACATGGAGAA
 129 GATTTGGCATCACACCTTCTACAACGAGCTTCGTGTTGCCCCCGAGGAGCACCCCG
 130 TCTTGCTCACCGAGGCCCCCATCAACCCCAAGAGCAACCGTGAGAAGATGACCCAG
 131 ATTGTCTTCGAGACCTTCAACGCTCCCGCCTTCTACGTCTCCATCCAGGCCGTTCTG
 132 TCCTTGACGCTTCCGGTCGTACCACCGGTATCGTGCTCGACTCCGGTGACGGTGT

133 CACTCACGTTGTCCCATCTACGAGGGTTTCGCTCTTCCCCACGCCATCTCCCGTG
134 TCGACATGGCTGGTCGTGATTTGACCGACTACCTCATGAAGATCCTTGCTGAGCGC
135 GGTTACACCTTCTCCACCACGGCCGAGCGTGAAATCGTCCGTGACATCAAGGAGAA
136 GCTCTGCTACGTGCGCTTGGACTTCGAGCAGGAGATCCAGACCGCCAGCCAGAGC
137 TCCACTCTCGAGAAGTCCTACGAGCTTCCCGACGGACAGGTCATCACCATTGGCAA
138 CGAGCGTTTCCGTGCCCCCGAGGCTCTCTTCCAGCCCTCCGTCTTGGGTCTCGAGA
139 GCGGCGGTATCCACGTCACCACCTTCAACTCCATCATGAAGTGCGATGTCGATGTC
140 AGGAAAGATCTCTACGGCAACATCGTCATGGTATGTATCACCGAAATCGCTGCCTC
141 GGTACAAAGCTAACAAAAGTAGTCTGGTGGAACCACCATGTACCCCGGTAT
142 **>MN304720 (Alt a1)**
143 GCATTATGCAGTTCACCACCATCGCCTCTCTCTTCGCCGCCGCTGGCCTTGCCGCT
144 GCTGCTCCTCTCGAGTCTCGCCAGGACACCGCATCCTGCCCTGTCACCACTGAGG
145 GTGACTACGTCTGGAAGATCTCCGAATTCTACGGACGCAAGCCGGAAGGAACCTAC
146 TACAACAGCCTCGGCTTCAACATCAAGGCCACCAACGGAGGAACCCTCGACTTCAC
147 CTGCTCTGCTCAGGCCGATAAGCTTGAGGACCACAAGTGGTACTCCTGTGGCGAGA
148 ACAGCTTCATGGACTTCTCTTTGACAGCGACCGCAGCGGTCTGCTCCTGAAGCAG
149 AAGGTTAGCGACGAGTAAGTTACCCTTGTACCTTCGATTACTTCGCAGATTCAGATA
150 TACTAACATATTCCCAGCATCACCTATGTCGCTACCGCCACTCTTCCCAACTACTGC
151 CGCGCTGGCGGTAACGGCCCTAAGGACTTTGTCTGCCAGGGTGTTGCCGACGCCT
152 ACATCACCCCTCGTA
153 **>MN706159 (calmodulin)**
154 TCCTCTGCCATGCCGCGCGGCTGCCTGGTAGCCCTGGGGGCCTGCGCAATCACGA

155 ACATGCAGCTGACGACGTCGTGTTGTAGGACAAGGATGGCGATGGTCAGTACTCTC
 156 CCTCCAAATTCCCTTCCACACACACACTCTCTCTCTCTCCCTCTCTGCCTTCAAAGC
 157 AGTGCCGCATCTCCAGCCTACGCAATCGGCAGAGGGGGCCCGAGCGAGGCTTGCTG
 158 GCTAGGGGTCCAAACCACCGCCACAGCTACAACACCACGACATCCACCCTACTCC
 159 ATAGCAAGCACAACCTGACGACGATGCGCCACAGGTCAAATCACCACCAAGGAGCTA
 160 GGTACCGTTATGCGCTCGCTCGGCCAAAATCCCAGCGAGTCTGAGCTCCAGGACAT
 161 GATCAACGAGGTGCGATGCCGACAACAACGGCACCATTTGACTTCCCAGGTGCGCCTC
 162 TCCCTACCAGTTCAAAGTACCACAGCTAACTTTTCCAGAATTCCTTACCATGATGGC
 163 CCGCAAGATGAAGGACACCGACTCCGAGGAGGAGATCCGGGAAGCCTTCAAGGTC
 164 TTCGACCGCGATAACAACGGTTTCATCTCCGCCGCCGAAGTGGTGCATGAC
 165 TTCTATTGGCGAGAAATTGACCGATGACGAGGTGACGAGATGATCCGGGAGGCTG
 166 ACCAGGACGGTGACGGCCGCATCGACTGTAGGTTGCAGCTGCCTATATCACAAGTG
 167 CAATGCTAATACACACCAGACAACGAGTTC

168 **>MN706160 (chitin synthase)**

169 GTTGGATGATTATCTACATCTTCGCAACACCCGTTTTTCGCCTTTGGTCTACCTCTGTA
 170 CTCTTTCTGGTACATGGACGACTTCTCCTGGGGTAACACTCGTGTTGTTACAGGTGA
 171 AAAGGGCCAGAAGATTGTTGTCACGGACGAAGGCAAGTTTGACCCTGCCAGCATCC
 172 CTAAGAAGAGGTGGGAGGAGTACCAAGCAGAGATGTGGGAGCAGCACACCATCCG
 173 TGACGACCGCTCGGAGGTTTCAGGTTACACCTACGCTACCAGGAAGCCGTTTGGA
 174 GCGCATCCGTCGCTGGCTCAGAGTATGGAGGCATGCCACCATCCCGCCCCATGTC
 175 GCACCTCAACCTCCCACGCTACGACAATCACTCACGAATGAGTCTTGCCCAGAGTG
 176 GCTACGGCATGGATAGCATGGAGATGGCGAACCTGCCAAGTGACGATGCTATCTTG

177 ATCGAGATCAAGGATATCTTGTCCAAGTCTGATCTCATGAACGTCACCAAGAAGCAG
178 ATCAAGGCCGAAGTAGAGCAGAGGTATGTTCAATCACATATAATCACACGATCAACA
179 CTAACACGACGGCAGATTTGGATGCGATCTCACGCTCAAGAAGCAGTTCATTGGCT
180 CTGCTGTCGAGTCTTTACTCATCAG
181 **>MN304719 (Tsr1)**
182 GGTCGCCCCTGCGAAACAGAGGCGTTTGCCAAAGGGCACAAAGTAACTACCAGTCC
183 GCATGGTATCTTGACGACATGTCAGACTCAGGTTCTGATCTTGAGGACTTCGACGA
184 GATGGACGTTGAAGACAATCAGAGTCAGCCTGCGCACCCGGCAGACGGTGTAGAA
185 GGCATGGATGTTGATGGCAAGGCCATGACAGAGGGTGGACCATCTGAGTACCCGC
186 AGTCGGAGATGTTCTTAGATCCATCACCAGAGGACGAGATTGCACAGATCGAGGAG
187 TACCGGAGGTCCAGGAAAAGTGAAGAGGATGAAGACCTGGAGTTCCTGATGAGAT
188 CGAATTGCACCCGAATGTTAACGCGCGAGAGCGTCTGATCCGTTACCGAGGATTGA
189 AGAGTCTGAAGAACAGCCATTGGGAAACAGAAGAGGATCGACCATACGAGCCAGAG
190 GAGTGGCAACGGTTGCTCGATATCTCTGACTACAAGCGCACAGCGACCAAGTTCAT
191 GCGCGAAGCCTGGGCTGGTGGTGTCAAGCCTGGAAGTTCGGGTAAGCGTACACATT
192 CGCGGCGTCCCATTACAATTCCAGGAAATTCAATCACGGCCAATGGCCATGTTTTCC
193 CTGCTCAGACACGAGCACAAAGCGCACAGCTTGCAACTACAGCATTCTGCTCAGCTC
194 CGAGCACGAATCGCCGATCAAGTCAAAGACCGAGATGATTGTGCAGTGTGGACCAA
195 GGAGGATAGTGATTAACCCAGTAAGTTACTACGAACGTGTGCTTTACCATCTAGTAC
196 TGACAGTTCCTTAGCTATTCTCGCATGCTGGAAACACACCAAACAACGTGCACAAAT
197 TCTCCCGCTTCCTCCACCCTGGCCAGTCGGCCATTGCGTCTTTCATCGGTCCTCTG
198 ACCTGGGGCAATGTCCCCCTCCTCTTCTTCACACGTGAAACACCCTCTCCCGACCA

199 TCCCTCCTCACTCCGCTTGATTGCGACAGGCACATCTCTCCCACCCTCCACCAACC
200 GCATCATCGCCAAGCGCATCATCTTGACCGGCCACCCCTACAAGATCAACAAGCGC
201 GTCGTGACAGTTCGCTACATGTTCTTCAACGACGGCGACGTCAAGTGGTTCAAGAG
202

Figure 1.



