Notes

Biscopyran, a Phytotoxic Hexasubstituted Pyranopyran Produced by *Biscogniauxia mediterranea*, a Fungus Pathogen of Cork Oak

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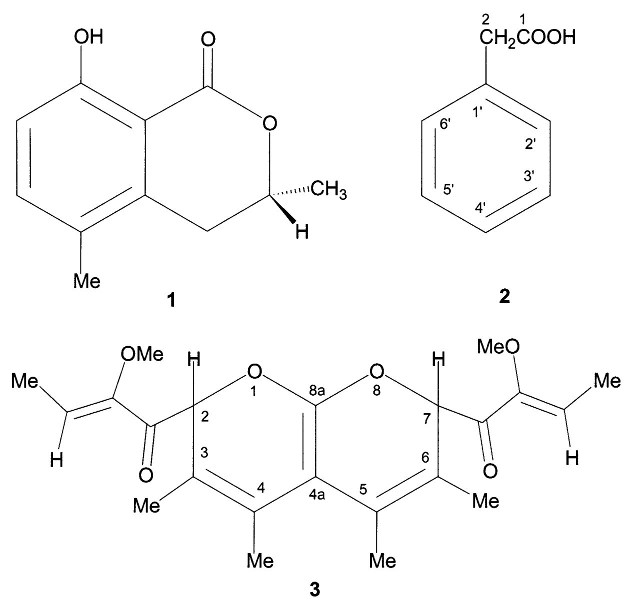
A new phytotoxic hexasubstituted pyranopyran, biscopyran (**3**), was isolated together with phenylacetic acid (**2**) and previously isolated 5-methylmellein (**1**) from the liquid culture filtrates of *Biscogniauxia mediterranea*, a major fungal pathogen involved in oak decline in Sardinia. Biscopyran was characterized by spectroscopic methods as a new (*Z*)-2-methoxy-1-[7-((*Z*)-2-methoxybut-2-enoyl)-3,4,5,6-tetramethyl-2*H*,7*H*-pyrano[2,3-*b*]pyran-2-yl]but-2-en-1-one. Biscopyran assayed at 0.26−0.026 mM concentration range caused epinasty on cork oak cuttings. On a nonhost plant, tomato, biscopyran caused wilting. Phenylacetic acid, assayed at the same concentration, was toxic *to Q. suber*, while on tomato cuttings it induced internal tissue collapse on the stem.

Biscogniauxia mediterranea (De Not.) O. Kuntze is an endophytic fungus, widespread in Sardinian oak forests, and considered one of the main causes of cork oak (*Quercus suber* L.) decline.[1] This fungus can survive as an endophyte in all of the aerial organs of the oak plants and can act as an opportunistic pathogen when the oaks suffer prolonged periods of stress. The fungus induced discoloration of the woody tissues, dieback, and stem and branch cankers, progressing to the appearance of characteristic black carbonaceous stromata on dead organs.[2]

These symptoms suggested that the fungus produced phytotoxic metabolites, as also observed for isolates of *Hypoxylon mammatum* (Wahlenberg) Miller, the causal agent of the poplar canker disease.[3] Indeed, the role of 5-methylmellein, a well-known metabolite produced by Xylariaceae ascomycetes such as *B. mediterranea*,[4,5] has been extensively studied in relation to its role in disease induced on the host plant.[6]

This paper describes the isolation and chemical and biological characterization of phenylacetic acid and of biscopyran, the main phytotoxic metabolite produced by *B. mediterranea*. The structure of the latter was determined by spectroscopic methods, essentially MS and NMR techniques.

The crude oily residue (3.85 g) obtained by EtOAc extraction of the culture filtrates (10 L) of *B. mediterranea* was fractioned using a combination of column chromatography and thin-layer chromatography as described in the Experimental Section. Three phytotoxic metabolites were isolated by this process: the previously described 5-methylmellein (**1**)[6] and two other metabolites, the most polar of which was identified by means of its spectroscopic properties as phenylacetic acid (**2**, 16.6 mg, 1.66 mg/L), a low molecular weight acid, isolated for the first time as toxic metabolite from this fungus. The third metabolite representing the main toxin was called biscopyran (**3**, 21.4 mg, 2.14 mg/L) on the basis of its structural features described below. The latter (**3**) was obtained as white needles by crystallization from EtOAc, and many attempts to determine its structure by X-ray analysis failed.



When assayed on test plants, **2** and **3** showed phytotoxic activity. Both substances were shown to be nonselective toxins. Phenylacetic acid, assayed at concentrations ranging from 0.26 to 0.026 mM, was toxic *to Q. suber*. Necrotic lesions appeared on the leaves within 5 days after absorption of the toxic solutions. Cork oak cutting wilted within 10 days at 0.26 mM. When **2** was assayed on tomato cuttings, phytotoxicity was observed at 0.26−0.13 mM, inducing internal tissue collapse on the stem. When biscopyran was assayed at concentrations ranging from 0.26 to 0.026 mM on cork oak cuttings, epinasty was observed. On a nonhost plant, tomato, biscopyran caused wilting at 0.13 and 0.26 mM.

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Table 1. ^1H and ^{13}C NMR Data of Biscopyran (**3**)[a]

C	δ^b	^1H δ	*J* (Hz)	HMBC
2	91.4 (d)	6.10 s		3.91
3	129.7 (s)			6.10, 1.89
4	126.9 (s)			
4a	101.9 (s)			6.10, 1.94
8a	165.1 (s)			1.94
C=O	166.0 (s)			6.10, 3.91
CH=*C*	160.0 (s)			6.70, 6.10, 1.84
*C*H=C	129.7 (d)	6.70 (q)	(7.0)	1.84
Me-CH=C	14.2 (q)	1.84 (d)	(7.0)	6.70
MeO	56.0 (q)	3.91 (s)		
Me-C(3)	12.1 (q)	1.89 (s)		
Me-C(4)	8.6 (q)	1.94 (s)		

[a] Each carbon and proton signal, except those of carbon C-4a and C-8a, represents two carbons and two protons, respectively. The chemical shifts are in δ values (ppm) from $CDCl_3$. 2D $^1H,^1H$ (COSYand TOCSY) and 2D $^{13}C,^1H$ (HSQC) NMR experiments delineated the correlations of all protons and the corresponding carbons. [b] Multiplicities determined by DEPT spectrum.

Studies on the antifungal activity of **2** and **3** are in progress in order to understand the competition between *B. mediterranea* and other fungal endophytes within the host.

Biscopyran has a molecular weight of 388, corresponding to a molecular formula of $C_{22}H_{28}O_6$, which is consistent with the presence of nine degrees of unsaturation. These results together with the signal pattern observed in both the ^1H and ^{13}C NMR spectra (Table 1) as well as in the EI and ESI mass spectra suggested a symmetrical structure for this toxin. In particular, the ^1H NMR spectrum showed a signal typical of a pentasubstituted 2H-pyran ring and those of a 2-methoxybut-2-enoyl side chain and two vinylic methyl groups. The proton (H-2) of a 2H-pyran ring resonated as a singlet at δ 6.10 and showed homoallylic coupling ($J < 1.0$ Hz)[7,8] in the COSY spectrum[9] with the C-4 methyl group, which resonated as a singlet at δ 1.94. In the same spectrum the olefinic proton of the 2-methoxybut-2-enoyl side chain was observed as a quartet ($J =$ 7.0) at the typical chemical shift value of δ 6.70 being coupled with the geminal methyl group (Me-C=CH), which, in turn, resonated as a doublet ($J = 7.0$ Hz) at δ 1.84.[8] The ^1H NMR spectrum also showed the presence of a singlet at δ 3.91, due to the methoxy group of the side chain, as well as that at δ 1.89 assigned to the C-3 methyl of the 2H-pyran ring.[8]

The presence of these partial structures in **3** was confirmed by the correlations observed in the HSQC spectrum.[9] On the basis of these results the protonated carbons of both the pyran ring [C-2, Me-C(3), and Me-C(4)] and the side chain at C-2 (*C*H=C, *Me*-CH=C, and MeO) were readily assigned to the signals appearing in the ^{13}C NMR spectrum (Table 1) at the expected chemical shift values of δ 91.4, 12.1, and 8.6, and 129.7, 14.2, and 56.0, respectively.[10] Furthermore, the ^{13}C NMR spectrum showed the presence of six quaternary carbons, which were also attributed on the basis of the correlations observed in the HMBC spectrum.[9] The carbonyl and quaternary olefinic carbons of the side chain at C-2 were present at δ 166.0 and 160.0 (C=O and CH=*C*) as well as those of the 2H-pyran bearing a methyl group at δ 129.7 and 126.9 (C-3 and C-4), while the two bridgehead carbons resonated at δ 165.1 and 101.9 (C-8a and C-4a).[10]

These structural features were also consistent with the typical absorption band observed for conjugated carbonyl and triene systems in the IR[11] and UV[12] spectra.

Finally, given that each proton and carbon signal, except those of the bridgehead carbons, represents two protons and carbons, respectively, and given all the correlations described above and observed in the COSY, HSQC, HMBC, and TOCSY spectra,[9] the chemical shifts to all protons and carbons (Table 1) are also assigned in agreement with the literature.[8,10]

Table 2. 2D ^1H-NOE (NOESY) Spectral Data Obtained for Biscopyran (**3**)

considered	effects
6.70 (C*H*=C)	1.84 (*Me*-CH=C)
6.10 (H-2)	3.91 (MeO), 1.89 (*Me*-C(3)
3.91 (MeO)	6.10 (H-2), 1.84 (*Me*-CH=C)
1.89 (*Me*-C(3))	6.10 (H-2)
1.84 (*Me*-CH=C)	6.70 (CH=C), 3.91 (MeO)

On this basis, the structure of 2-methoxy-1-[7-(2-methoxybut-2-enoyl)-3,4,5,6-tetramethyl-2*H*,7*H*-pyrano[2,3-*b*]pyran-2-yl]but-2-en-1-one was assigned to biscopyran (**3**).

The symmetrical structure **3** assigned to the toxin was supported by the $^1H,^{13}C$ long-range correlations recorded in its HMBC spectrum (Table 1)[9] and by data of its EI and ESI MS spectra. Indeed, the HREIMS spectrum showed the molecular ion at *m/z* 338.1876 and peaks generated by fragmentation mechanisms typical of 2H-pyran and ether.[8,13] In fact, the parent ion through MeOH loss generated the ion at *m/z* 356, and the latter by alternative losses of H and Me residues yielded the ions at *m/z* 355 and 341. The same spectrum showed the significant peak at *m/z* 194, due to one-half of the symmetrical molecule, and a peak at *m/z* 168 generated by loss of MeOH. The ESIMS spectrum showed the potassium $[M + K]^+$ and the sodium $[M + Na]^+$ clustered and the pseudomolecular ions at *m/z* 427, 411, and 389, respectively, as well as the same adducts corresponding to one-half of the molecule at *m/z* 233, 217, and 195 ($[M/2 + K]^+$, $[M/2 + Na]^+$, and $[M/2 + H]^+$, respectively).

Considering the effect observed in the NOSEY spectrum[9] (Table 2), the bicyclic 2H-pyran moiety of **3** should assume a conformation of two fused half-chairs. In particular, the effect observed between the pyran proton (H-2) and the adjacent methyl group [Me-C(3)] as well as with the methoxy group of the side chain determined H-2 to be pseudoequatorial and consequently the 2-methoxybut-2-enoyl side chain to be pseudoaxial, in one of the possible conformations in a continuous equilibrium with other preferred conformations. The side chain olefinic bond has a *Z*-configuration, as shown by the significant effect observed in the NOESY spectrum between its methyl and methoxy groups. Furthermore, in agreement with the NOESY data (Table 2) and an inspection of a Dreiding model, and considering the optical inactivity of biscopyran, the relative configuration of 2*R*,7*S* (equal to 2*S*,7*R*) could be assigned to the toxin (**3**).

The biscopyran belongs to the family of pyranopyrans, which are well known as synthetic intermediates, but few are known as natural compounds.[14−16] The latter include the brevetoxins[17] and dactomelynes[18] and diplopyrone, the main new phytotoxin recently isolated by our group from *Diplodia mutila*, the causal agent of a canker disease of cork oak.[19]

Isolation of phenylacetic acid as a phytotoxin came as no surprise since it was previously reported as a fungal phytotoxic metabolite produced by *Rhizoctonia* spp.[20] Furthermore, low molecular weight acids, namely, β-nitropropionic,[21] oxalic,[22] fumaric,[23] and 3-methylthiopropionic[24] acids, were previously described as toxins produced by both phytopathogenic fungi and bacteria.

The previous isolation of 5-methylmellein from *B. mediterranea* was also no surprise, as this is the most frequent

dihydroisocoumarin isolated from Ascomycetes belonging to different genera (see *Entonaema*, *Hypoxylon*, *Xylaria*, *Resillinia*, *Poronia*, *Podosodaria*, *Hycocopra*, *Daldinia*, *Nummularia*, *Kretzschmaria*, *Camillea*, and *Penzigia*) of the Xylariaceae family, which have also been shown to produce toxic metabolites with different chemical structures such as cytochalasins, naphthalene derivatives, butyrolactones, succinic acid derivatives, chromanones, and diketopiperazines. Several studies have been carried out to use the distribution of such secondary metabolites in conjunction with traditional taxonomic characters in an attempt to develop a better understanding of natural relationships within the family.[4,5,25] Chemical data obtained indicate that there are at least two major divisions within the family, and on the basis of production of 5-methylmellein *B. mediterranea* was grouped together with *Hypoxylon*, *Daldinia*, *Camillea*, and *Entonaema* genera.[5] Furthermore, the phenylacetic acid and biscopyran are specifically biosynthesized by *B. mediterranea*, and although a polyketide origin could be hypothesized for biscopyran as for dihydroisocoumarins,[20] they could be used as exclusive metabolite profiles to recognize the fungus producer.

Experimental Section

General Experimental Procedures. Optical rotation was measured in $CHCl_3$ solution on a JASCO P1010 digital polarimeter; IR and UV spectra were determined neat and in MeCN solution, respectively, on a Perkin-Elmer Spectrum ONE FT-IR spectrometer and a Lambda 25 UV−visible spectrophotometer. ^1H and ^{13}C NMR spectra were recorded at 500 and at 125 MHz, respectively, in $CDCl_3$, on a Varian spectrometer. The same solvent was used as internal standard. Carbon multiplicities were determined by DEPT spectra.[9] DEPT, COSY-45, TOCSY, HSQC, HMBC, and NOESY experiments[9] were performed using Varian microprograms. EI and HREIMS were taken at 70 eV on a Fisons Trio-2000 and a Fisons ProSpec spectrometer, respectively. Electrospray MS were recorded on a Perkin-Elmer API 100 LC-MS; a probed voltage of 5300 V and a declustering potential of 50 V were used. Analytical and preparative TLC were performed on silica gel (Merck, Kiesegel 60, F254, 0.25 and 0.5 mm, respectively) or on reversed-phase (Merck, RP-18, F254, 0.25 mm) plates. The spots were visualized by exposure to UV radiation and/or by spraying with 10% H_2SO_4 in MeOH and then with 5% phosphomolybdic acid in MeOH, followed by heating at 110 °C for 10 min. Column chromatography was performed on silica gel (Merck, Kieselgel 60, 0.063−0.20 mm).

Fungal Strain. The *B. mediterranea* strain used in this study was isolated from stems of infected cork oak (*Q. suber*) trees collected in Sardinia (Italy). A single spore isolate of *B. mediterranea* was grown on malt-agar slants at 25 °C for 7 days and then stored at 5 °C in the fungal collection of the Dipartimento di Proptezione delle Piante, Università di Sassari, Italy (3S).

Production, Extraction, and Purification of Phytotoxins. The isolate 3S of *B. mediterranea* was grown in stationary culture in 1 L Roux flasks containing 150 mL of Czapek medium with the addition of 2% yeast and 2% malt extract and adjusted to pH 5.9 with 2 N HCl. Each flask was seeded with 3 mL of a fungal mycelium suspension, grown for 5 days under stirring (150 rpm) in flasks containing 100 mL of malt extract−soya lecithin (0.5%) medium. The flasks were incubated at 25 °C for 30 days in the dark. At harvest, the mycelium mat was removed by filtration. The culture filtrates (10 L, pH 8.2) were concentrated up to 20% of the initial volume under vacuum at 40 °C, acidified to pH 4 with HCl 2 N, and extracted exhaustively with EtOAc. The combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give a red-brown oil residue with high phytotoxic activity. The crude oily residue (3.85 g) was fractioned by column chromatography eluted with petroleum ether−EtOAc (from 100:1 to 1:100). Fractions (15 mL each) were collected and pooled on the basis of their TLC profiles to yield 11 groups of homogeneous fractions (1−11). The residues left by fractions 2−10 showed high phytotoxic activity. The residue left by groups 2 and 3 was combined and purified by successive preparative TLC steps on silica gel [system eluents $CHCl_3$−*i*-PrOH (9:1) and toluene−EtOAc−HCOOH (10:1:1), respectively], yielding 5-methylmellein (**1**, 13.7 mg, 1.37 mg/L) as white needles from EtOAc. The residue left by fraction groups 4 and 5 of the original column was combined (252.2 mg) and purified by column chromatography [eluent petroleum ether−EtOAc (from 100:5 to 0:100)] to give nine fractions (A−I), with fraction C (75.1 mg) and D (68.1 mg) exhibiting activity. Fraction C was further purified by successive preparative TLC steps on silica gel [system eluents $CHCl_3$−*i*-PrOH (9:1) and toluene−EtOAc−HCOOH (10:1:1), respectively], yielding phenylacetic acid (**2**, 16.6 mg, 1.66 mg/L) as white needles from EtOAc [R_f 0.47, 0.46, and 0.70 by silica gel and reversed-phase TLC, system eluents $CHCl_3$−*i*-PrOH (9:1), toluene−EtOAc−HCOOH (10:1:1), and CH_3CN−H_2O (1.5:1), respectively]. Fraction D was further purified by two successive preparative TLC on silica gel and reversed-phase TLC [eluents petroleum ether−*i*-PrOH (7:1) and CH_3CN−H_2O (1.5:1), respectively] to give biscopyran (**3**, 21.4 mg, 2.14 mg/L) as white needles from EtOAc [R_f 0.65, 0.32, and 0.36 by silica gel and reversed-phase TLC, eluent systems $CHCl_3$−*i*-PrOH (9:1), petroleum ether−*i*-PrOH (7:1), and CH_3CN−H_2O (1.5:1), respectively].

Phenylacetic acid (2): UV λ_{max} (log ϵ) 259 (2.07), 205 (4.54) nm; IR ν_{max} 3032, 1700, 1603, 1498, 1454, 1407 cm^{-1}; ^1H NMR δ 12.3 (1H, br s, COOH) 7.36−7.26 (5H, m, Ph−), 3.66 (2H, s, H_2C-2); ^{13}C NMR δ 177.1 (s, C-1), 133.3 (s, C-1′), 129.3 (d, C-2′,6′), 128.6 (d, C-3′,5′), 127.3 (d, C-4′), 41.0 (t, C-2); EIMS *m/z* (rel int) 136 $[M]^+$ (23), 91 $[C_7H_7]^+$ (100); ESIMS (+) *m/z* 175 $[M + K]^+$, 159 $[M + Na]^+$.

Biscopyran (3): colorless oil; $[\alpha]^{25}_D$ 0.0° (*c* 0.45); UV λ_{max} (log ϵ) 329 (3.23), 224 (3.74) nm; IR ν_{max} 1682, 1647, 1617, 1551 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HREIMS *m/z* (rel int) 388.1876 $[M]^+$ (calcd for $C_{22}H_{28}O_6$, 388.1886) (0.2), 356 $[M - MeOH]^+$ (0.2), 355 $[M - MeOH - H]^+$ (0.4), 341 $[M - MeOH - Me]^+$ (0.7), 194 $[M/2]^+$ (2), 168 $[M/2 - MeOH]^+$ (9), 56 (100); ESIMS (+) *m/z* 427 $[M + K]^+$, 411 $[M + Na]^+$, 389 $[M + H]^+$, 233 $[M/2 + K]^+$, 217 $[M/2 + Na]^+$, 195 $[M/2 + H]^+$.

Toxin Bioassays. The culture filtrate, organic extract, chromatographic fractions, and pure substances were assayed for phytotoxicity using cuttings of cork oak and tomato seedlings. The cuttings were taken from cork oak and tomato seedlings (35 and 21 days old, respectively) grown in a growth chamber at 25 °C and 70−80% RH, exposed to a luminous flux of 400 μmol m^{-2} s^{-1} with a 12 h photoperiod. Aliquots of the culture filtrate were assayed after 1:1000 dilution with distilled H_2O. The crude extracts from culture filtrate and residues of column chromatography fractions were assayed at concentrations of 0.26, 0.52, and 1.3 mM. The pure substances were tested at concentrations of 0.26−0.026 mM on cork oak cuttings and 0.26−0.13 mM on tomato cuttings. The toxicity of these solutions was evaluated by placing the test plant parts (cork oak cuttings for 96 h and tomato ones for 48 h) in the assay solutions (3 mL) and then transferring them to distilled water.

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References and Notes

(1) Franceschini, A.; Maddau, L.; Marras, F. *IOBC/wprs Bull.* **2002**, *25* (5), 29−36.
(2) Marras, F.; Franceschini, A.; Maddau, L. *IOBC/wprs Bull.* **1995**, *18*(6), 8−13.

(3) Pinon, J.; Manion, P. D. *Eur. J. For. Path.* **1991**, *21*, 202−209, and references therein.
(4) Anderson, J. R.; Edwards, R. L. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2185−2192.
(5) Whalley, A. J. S.; Edwards, R. L. *Can J. Bot.* **1995**, *73*, S802−S810.
(6) Maddau, L.; Ferracane, R.; Franceschini, A.; Cabras, A. *Proceedings of the National Congress "L'endofitismo di funghi e batteri patogeni in piante arboree e arbustive"*; Sassari-Tempio Pausania: Italy, 2002; pp 361−368.
(7) Sternhell, S. *Q. Rev.* **1969**, *23*, 237−269.
(8) Pretsch, E.; Bühlmann, P.; Affolter, C. *Structure Determination of Organic−Tables of Spectral Data for*; Springer: Berlin, 2000; pp 168−174, 202−206, 220−221, 333−338.
(9) Berger, S.; Braun, S. *200 and More Basic NMR Experiments: a Practical Course*; Wiley-VCH: Weinheim, 2004.
(10) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH: Weinheim, 1987; pp 183−293.
(11) Nakanishi, K.; Solomon, P. H. *Infrared Absorption Spectroscopy,* 2nd ed.; Holden Day: Oakland, 1977; pp 17−44.
(12) Scott, A. *Interpretation of the Ultraviolet Spectra of Natural Products*; Pergamon: Oxford, 1964; pp 45−69, 89−124.
(13) Porter, Q. N. *Mass Spectrometry of Heterocyclic Compounds*; John Wiley & Sons: New York, 1985; pp 205−206.
(14) Kozikowski, A. P.; Jaemoon, L. *J. Org. Chem.* **1990**, *55*, 863−870.
(15) Leeuwenburgh, M. A.; Overkleeft, H. S.; Van der Marel, G. A.; Van Boom, J. H. *Synlett.* **1997**, *11*, 1263−1264.
(16) Grotenbreg, G. M.; Tuin, A. W.; Witte, M. D.; Leeuwenburgh, M. A.; Van Boom, J. H.; Van der Marel, G. A.; Overkleeft, H. S.; Overhand, M. *Synlett.* **2004**, *5*, 904−906.
(17) Mori, Y.; Yaegashi, K.; Furukawa, H. *J. Am. Chem. Soc.* **1997**, *119*, 4557−4558.
(18) Aydogmus, Z.; Imre, S.; Ersoy, L.; Wray, V. *Nat. Prod. Res.* **2004**, *18*, 43−49.
(19) Evidente, A.; Maddau, L.; Spanu, E.; Franceschini, A.; Lazzaroni, S.; Motta, A. *J. Nat. Prod.* **2003**, *66*, 313−315.
(20) Turner, W. B.; Aldridge, D. C. *Fungal Metabolites II*; Academic Press: London, 1983; pp 11−13, 55−121.
(21) Evidente, A.; Capretti, P.; Giordano, F.; Surico, G. *Experientia* **1992**, *48*, 1169−1172.
(22) Noyes, R. D.; Hancock, J. G. *Physiol. Plant Pathol.* **1981**, *18*, 123−132.
(23) Mirocha, C. J.; De Vay, J. E.; Wilson, E. E. *Phytopathology* **1961**, *51*, 581−860.
(24) Scala, F.; Evidente, A.; Coppola, L.; Capasso, R.; Zoina, A. *J. Phytopathol*. **1996**, *144*, 325−329.
(25) Stadler, M.; Wollweber, H.; Muhlbauer, A.; Asakawa, Y.; Hashimoto, T.; Rogers, J. D.; Ju, Y.-m.; Wetzstein, H. G.; Tichy, H. V. *Mycol. Res.* **2001**, *105*, 1191−1205.

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