Novel *Seimatosporium* Species from Grapevine in Northern California and Their Interactions with Fungal Pathogens Involved in the Trunk-Disease Complex

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Abstract

Seimatosporium spp. and closely related “pestalotioid fungi” have been isolated from vineyards worldwide, but their ecological status in grapevine wood is unclear. To determine their involvement in the grapevine trunk-disease complex, we tested the pathogenicity of Californian isolates obtained from vines with general symptoms of Botryosphaeria, Eutypa, and Phomopsis diebacks. Multilocus phylogenetic analyses revealed three species: *Seimatosporium vitis* and two newly described and typified species, *S. luteosporum* sp. nov. and *S. vitifusiforme* sp. nov. Inoculations to woody stems of potted grapevines of both isolates of *S. vitis* and one isolate of *S. vitifusiforme*, but not *S. luteosporum*, were associated with significantly

larger lesions than those of noninoculated controls. Coinoculations with trunk pathogens (*Cryptovalsa ampelina*, *Diaporthe ambigua*, *Diatrypella verruciformis*, *Diplodia seriata*, and *Eutypa lata*), coisolated from the same wood cankers in the field, brought about increased lesion lengths for *S. vitifusiforme* paired with *D. seriata*, and *S. luteosporum* paired with *Diaporthe ambigua*. In contrast, there were no differences in lesion lengths of *S. vitis* and *Diatrypella verruciformis* or *S. vitis* and *E. lata*, inoculated alone or together. Our findings suggest that *Seimatosporium* spp. are involved in the grapevine trunk-disease complex, and their virulence may depend on or affect that of trunk pathogens.

Grapevine trunk diseases are among the main depreciable drivers of vineyard longevity and productivity worldwide. The grape industry suffers large economic losses due to cumulative yield losses and limited profitable lifespans (Kaplan et al. 2016), premature replanting of vineyards (Sipiora and Cuellar 2014), and impacts on wine quality (Lorrain et al. 2012). Trunk diseases are caused by a diverse community of fungal pathogens (Bruez et al. 2016; Travadon et al. 2016), which compromise the permanent woody structure of the vine through the combined actions of cell-wall-degrading enzymes and secreted toxins (Massonnet et al. 2018; Morales-Cruz et al. 2015). The collective impact of the mixed infections on symptom expression are determined, in part, by the diverse virulence factors among trunk pathogens, the expression of which varies depending on fungal species composition (Morales-Cruz et al. 2018). Beyond identifying the species composition of mixed infections, it is critical to characterize the fungal interactions, which can impact wood colonization and disease development (Boddy and Hiscox 2016).

Several “pestalotioid fungi” belonging to the family Sporocadaceae (within the class Sordariomycetes) (Jaklitsch et al. 2016) have been reported as components of the grapevine trunk-disease complex: *Neopestalotiopsis* Maharachch., K. D. Hyde & Crous, *Pestalotiopsis* Steyaert, *Seimatosporium* Corda, and *Truncatella* Steyaert

(Arzanlou et al. 2013; Jayawardena et al. 2015; Maharachchikumbura et al. 2017; Úrbez-Torres et al. 2009). They are named pestalotioid fungi because their anamorphic state produces conidia that are multi-septate and more-or-less fusiform, with apical or basal appendages (Maharachchikumbura et al. 2014). Generic boundaries based on morphology are easily confused, due to intermediate conidial morphologies of closely related genera. Of the three *Seimatosporium* spp. (*Seimatosporium botan* Sat. Hatak. & Y. Harada, *S. vitis* Y. P. Xiao, Camporesi & K. D. Hyde, and *S. hysterooides* (Fuckel) Brockmann) reported from grapevines in Chile, Hungary, Italy, Iran, and Australia (Díaz et al. 2012; Mehrabi et al. 2017; Senanayake et al. 2015; Sergeeva et al. 2005; Váczy 2017), only *S. botan* has been evaluated in standard pathogenicity tests on trunks and canes of field vines (Díaz et al. 2012). *S. vitis* colonization was evaluated on green shoots and detached canes but this is not conclusive evidence of its pathogenicity to woody stems of intact plants, which are the types of tissues this species was originally isolated from in the field (Díaz et al. 2012; Váczy 2017).

The genus *Seimatosporium* was erected with *S. rosae* Corda as the type species and consists predominately of saprobes and endophytes of woody plants (Goonasekara et al. 2016; Norphanphoun et al. 2015; Perera et al. 2016; Wijayawardene et al. 2016), although some species are pathogens (e.g., Leaf spot of eucalypts) (Barber et al. 2011). The genus is characterized by producing holoblastic or anellidic conidigenous cells within acervuli or pycnidia that produce multiseptate conidia with pigmented median cells, a truncate basal cell with or without an eccentrically positioned appendage, and with or without a centrally positioned unbranched apical appendage (Nag Raj 1993). *Seimatosporium* spp. are heteromorphic, with species producing divergent conidial shapes. For example, conidia can vary from fusiform (*S. rosae*) to obovoid (*S. vitis*) to acerose (*S. biseptatum* (H. J. Swart & M. A. Will.) P. J. Barber & Crous). Septation can vary from 2 to 3 to 5 septa with continuous or occasional septal constrictions, and with or without an apical or basal appendage (Barber et al. 2011), thus making species-level assignment challenging.

From symptomatic vines in northern California vineyards, we isolated multiple isolates with morphological characteristics of *Seimatosporium* spp. along with several causal fungi of the following trunk diseases: Botryosphaeria dieback (*Diplodia seriata* De Not.) (Úrbez-Torres and Gubler 2009), Eutypa dieback (*Cryptovalsa ampelina* (Nitschke) Fuckel, *Diatrypella verruciformis* (Ehrr.) Nitschke, and *Eutypa lata* (Pers.) Tul. & C. Tul.) (Trouillas et al. 2010), and Phomopsis dieback (*Diaporthe ambigua* Nitschke) (Lawrence et al. 2015). These fungal

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species can theoretically exhibit antagonistic, neutral, or synergistic interactions while exploiting the same resource (Begon et al. 2006). Antagonism (negative effects on each other's development) may occur through competition for nutrients or space, toxin production, or induced host resistance. Species that are antagonistic would, furthermore, rarely cooccur. In contrast, synergism (promotion of each other's development) would be supported by frequent cooccurrence from the same ecological niche. Because of our frequent isolations from wood cankers of both *Seimatosporium* spp. and trunk pathogens, we tested the hypothesis that these fungal species are synergistic. Our objectives were to (i) characterize the diversity of *Seimatosporium* spp. associated with symptoms of trunk diseases, based on morphological and molecular analyses, and pathogenicity testing, and (ii) evaluate in vitro and in planta interactions between *Seimatosporium* spp. and trunk pathogens isolated from the same wood cankers.

Materials and Methods

Grapevine sampling and fungal isolation. Isolates originated from two winegrape vineyards in Napa and Solano Counties of northern California (Table 1), which were surveyed and sampled for trunk-disease pathogens (Travadon and Baumgartner 2015).

Wood samples were collected from mature grapevines (>10 years old) with dead spurs, dead cordons, and dead canes, which are symptoms shared by the dieback-type trunk diseases (*Botryosphaeria*, *Eutypa*, and *Phomopsis* diebacks). Fungi were isolated from wood cankers (Fig. 1A to D) following the detailed protocol of Baumgartner et al. (2013). Isolation plates were incubated at 25°C in darkness for up to 21 days. Fungal colonies that exhibited typical *Seimatosporium* characteristics (namely, a white or pale yellow, cottony morphology with a mostly smooth margin) were subcultured to potato dextrose agar (PDA) and incubated in darkness for 7 days. Fungi that exhibited cultural characteristics of other known grapevine trunk pathogens—members of the families *Botryosphaeriaceae* (fast-growing colony, which darkens with age), *Diaporthaceae* (medium-growing colony with a shiny, reflective margin), and *Diatrypidae* (medium-growing white colony with a whirled margin)—were also subcultured. In this way, in total, 25 *Seimatosporium* isolates and 15 trunk pathogen isolates were hyphal-tip purified to fresh PDA plates for molecular and morphological characterization.

Phylogenetic analyses. Total genomic DNA was isolated from mycelium (scraped with a sterile scalpel from the surface of a 14-day-old culture) using the DNeasy Plant kit (Qiagen, Valencia, CA), following

Table 1. *Seimatosporium* isolates and those of taxonomically related genera isolated from grapevine and other woody hosts^a

Species	Isolate ^c	Geographic origin	Host	GenBank accession ^b			
				ITS	LSU	BT	TEF1- α
<i>Adiscio yakushimense</i>	NBRC 104194 ^T	Japan	<i>Symplocos prunifolia</i>	AB594789	AB593721	...	...
<i>Cryptovalsa ampelina</i>	Wint015	Solano County, CA	<i>Vitis vinifera</i>	KY849963	...	...	...
<i>Diaporthe ambigua</i>	Wint904	Solano County, CA	<i>V. vinifera</i>	KM669923	...	...	KM669779
<i>Diatrypella verruciformis</i>	Napa016	Napa County, CA	<i>V. vinifera</i>	KY849964	...	...	...
<i>Diplodia seriata</i>	Napa810	Napa County, CA	<i>V. vinifera</i>	KY849966	...	...	...
<i>Discostroma fuscillum</i>	NBRC 32625	Unknown	<i>Rosa canina</i>	AB594794	AB593726	...	...
<i>D. stoneae</i>	NBRC 32690 ^T	Unknown	Unknown	AB594797	AB593729	...	...
<i>D. tostum</i>	TC2-029 ^T	Unknown	Unknown	AB594795	AB593727	...	...
<i>Eutypa lata</i>	Napa003	Napa County, CA	<i>V. vinifera</i>	KY849965	...	...	...
<i>Immersidiscosia eucalypti</i>	NBRC 104195	Japan	<i>Quercus myrsinifolia</i>	AB594790	AB593722	...	...
<i>I. eucalypti</i>	NBRC 104196	Japan	<i>Q. myrsinifolia</i>	AB594791	AB593723	...	...
<i>I. eucalypti</i>	NBRC 104197	Japan	<i>Ardisia japonica</i>	AB594792	AB593724	...	...
<i>I. eucalypti</i>	NBRC 242781	Japan	Decayed leaves	AB594793	AB593725	...	...
<i>Sarcostroma lomatiae</i>	CBS 118144	South Africa	<i>Restio</i> sp.	DQ278921	DQ278926	...	...
<i>Seimatosporium biseptatum</i>	CPC 13584	New South Wales, Australia	<i>Eucalyptus cypellocarpa</i>	JN871199	JN871208	...	...
<i>S. botan</i>	HHUF 27946 ^T	Japan	<i>Paeonia suffruticosa</i>	AB594799	AB593731	...	...
<i>S. botan</i>	HMUC-sei-302PD	Chile	<i>V. vinifera</i>	JN088482	...	...	...
<i>S. cornii</i>	MFLUCC 14-0467 ^T	Italy	<i>Cornus</i> sp.	KT162918	KR559739	...	...
<i>S. discosioides</i>	NBRC 104201	Japan	<i>Punica granatum</i>	AB594800	AB593732	...	...
<i>S. elegans</i>	NBRC 32674	Japan	<i>Melaleuca ericifolia</i>	AB594801	AB593733	...	...
<i>S. eucalypti</i>	CPC 156	South Africa	<i>E. smithii</i>	JN871200	JN871209	...	...
<i>S. falcatum</i>	CPC 13578	New South Wales, Australia	<i>Eucalyptus</i> sp.	JN871204	JN871213	...	...
<i>S. ficeae</i>	MFLUCC 15-0519 ^T	Italy	<i>Rubus</i> sp.	KR092800	KR920686	...	...
<i>S. foliicola</i>	NBRC 32676	Japan	<i>Juniperus phoenicea</i>	AB594802	AB593734	...	...
<i>S. glandigenum</i>	NBRC 32677	Japan	<i>Fagus sylvatica</i>	AB594803	AB593735	...	...
<i>S. grevilleae</i>	ICMP 10981	South Africa	<i>Protea</i> sp.	AF405304	AF382372	...	...
<i>S. hakeae</i>	NBRC 32678	Japan	<i>Pteridium aquilinum</i>	AB594804	AB593736	...	...
<i>S. hypericinum</i>	NBRC 32647	Japan	<i>Hypericum</i> sp.	AB594805	AB593737	...	...
<i>S. lichenicola</i>	NBRC 32680	Japan	<i>Ribes</i> sp.	AB594806	AB593739	...	...
<i>S. lichenicola</i>	MFLUCC 14-0052 ^T	Italy	<i>Rosa canina</i>	KT005515	KT005514	...	...
<i>S. luteosporum</i>	Napa754	Napa County, CA	<i>Prunus persica</i>	KY706283	KY706308	KY706258	KY706333
<i>S. luteosporum</i>	Wint754/CBS 142599 ^{T*}	Solano County, CA	<i>V. vinifera</i>	KY706284	KY706309	KY706259	KY706334
<i>S. mariae</i>	NBRC 32681	Japan	<i>Correa reflexa</i>	AB594807	AB593740	...	...
<i>S. obtusum</i>	CPC 12935 ^T	New South Wales, Australia	<i>Corymbia henryi</i>	JN871206	JN871215	...	...
<i>S. parasiticum</i>	NBRC 32682	Japan	<i>Physocarpus amurensis</i>	AB594808	AB593741	...	...

(Continued on next page)

^a GenBank accession numbers for sequences of four loci—ribosomal DNA (rDNA) internal transcribed spacer region (ITS), rDNA large subunit (LSU), nuclear locus β -tubulin (BT), and translation elongation factor 1- α (TEF1- α)—were generated by this study (in bold) or from other studies.

^b Sequences from isolates in our collection are highlighted in bold.

^c T = type cultures and * indicates isolates used for morphological characterization and pathogenicity assays.

the manufacturer's instructions. Ribosomal DNA (rDNA) fragments, including the intervening internal transcribed spacer (ITS) regions and 5.8S rDNA, were amplified using primers ITS1 and ITS4 (White et al. 1990). Three additional loci were amplified for all *Seimatosporium* isolates. Amplification of ribosomal large subunit fragments (LSU) utilized primers LR0R (Rehner and Samuels 1994) and Un-Lo28S1220 (Bala et al. 2010), with the following polymerase chain reaction (PCR) program: initial denaturation (94°C, 4 min); followed by 35 cycles of denaturation (94°C, 60 s), annealing (55°C, 30 s), and extension (72°C, 120 s); and a final extension (72°C, 10 min). Amplification of translation elongation factor 1- α (TEF1- α) fragments utilized primers EF1-728F and EF1-986R (Carbone and Kohn 1999), and amplification of the β -tubulin (BT) locus utilized primers T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass and Donaldson 1995), both with the following PCR program: initial denaturation (95°C, 5 min); followed by 35 cycles of denaturation (95°C, 30 s), annealing (57°C, 30 s), and extension (72°C, 60 s); and a final extension (72°C, 10 min). PCR products were visualized on a 1.5% agarose gel (120 V for 25 min) to validate presence and size of amplicons, purified via Exonuclease I and recombinant Shrimp Alkaline Phosphatase (Affymetrix, Santa Clara, CA), and sequenced in both directions on an ABI 3730 Capillary Electrophoresis Genetic Analyzer (College of Biological Sciences Sequencing Facility, University of California, Davis).

Forward and reverse nucleotide sequences were assembled, proof-read, and edited in Sequencher (v. 5; Gene Codes Corporation, Ann Arbor, MI) and deposited in GenBank (Table 1). Isolates morphologically

resembling trunk pathogens (i.e., species belonging to the families Botryosphaeriaceae, Diaporthaceae, and Diatripaceae) were identified via BLASTn searches of the curated molecular repository for species identification of grapevine trunk pathogens, TrunkDiseaseID.org (Lawrence et al. 2017). BLASTn searches of the National Center for Biotechnology Information and extensive literature review (cited within) resulted in the collection of 51 ITS sequences with high similarity from type and nontype *Seimatosporium* isolates ($n = 11$ and 40, respectively), which were included in our analyses for phylogenetic reference. Multiple sequence alignments were performed in MEGA v. 6 (Tamura et al. 2013) and manually adjusted where necessary in Mesquite v. 3.10 (Maddison and Maddison 2016). Alignments were submitted to TreeBASE under accession number S20203.

The datasets were analyzed using two different optimality search criteria, maximum-likelihood (ML) and maximum-parsimony (MP), in MEGA v. 6 (Tamura et al. 2013). For ML analyses, MEGA was used to infer a model of nucleotide substitution for each dataset, using the Akaike Information Criterion (AIC). Each ML analysis utilized the nearest-neighbor-interchange heuristic method and branch stability was determined by 1,000 bootstrap replicates. For MP analyses, heuristic searches with 1,000 random sequence additions were implemented with the tree-bisection-reconnection algorithm, with gaps treated as missing data. Bootstrap analyses with 1,000 replicates were used to estimate clade support. The following outgroups closely related to *Seimatosporium* sensu lato were selected based on previous phylogenetic analyses (Tanaka et al. 2011; Watanabe et al. 2016):

Table 1. (Continued from previous page)

Species	Isolate ^c	Geographic origin	Host	GenBank accession ^b			
				ITS	LSU	BT	TEF1- α
<i>S. pezizoides</i>	71TB	Unknown	Unknown	KF573991	...	...	...
<i>S. physocarp</i>	MFLUCC 14-0625 ^T	Russia	<i>P. opulifolius</i>	KT198722	KT198723	...	...
<i>S. pistaciae</i>	CBS 138865 ^T	Iran	<i>Pistacia vera</i>	KP004463	KP004491	...	...
<i>S. pseudorosarum</i>	MFLUCC 14-0466	Italy	<i>Rosa canina</i>	KT284775	KT281912	...	...
<i>S. quercina</i>	MFLUCC 14-1198 ^T	Germany	<i>Q. robur</i>	KU974965	KU974964	...	...
<i>S. restionis</i>	CBS 118154	South Africa	<i>Restio filiformis</i>	DQ278922	DQ278924	...	...
<i>S. rhombisporium</i>	MFLUCC 15-0727 ^T	Italy	<i>Vaccinium myrtillus</i>	KR092792	KR092780	...	...
<i>S. rosae</i>	MFLUCC 14-0621 ^T	Czechoslovakia	<i>Rosa canina</i>	KT198726	KT198727	...	...
<i>S. sorbi</i>	MFLUCC 14-0469 ^T	Italy	<i>Sorbus torminalis</i>	KT284774	KT281911	...	...
<i>Seimatosporium vitifusiforme</i>	Wint753/CBS 142600 ^{T*}	Solano County, CA	<i>Vitis vinifera</i>	KY706296	KY706321	KY706271	KY706346
<i>S. vitifusiforme</i>	Napa751	Napa County, CA	<i>V. vinifera</i>	KY706289	KY706314	KY706264	KY706339
<i>S. vitifusiforme</i>	Napa767	Napa County, CA	<i>V. vinifera</i>	KY706290	KY706315	KY706265	KY706340
<i>S. vitifusiforme</i>	Napa768*	Napa County, CA	<i>V. vinifera</i>	KY706285	KY706310	KY706260	KY706335
<i>S. vitifusiforme</i>	Napa780	Napa County, CA	<i>V. vinifera</i>	KY706286	KY706311	KY706261	KY706336
<i>S. vitifusiforme</i>	Napa783	Napa County, CA	<i>V. vinifera</i>	KY706291	KY706316	KY706266	KY706341
<i>S. vitifusiforme</i>	Napa785	Napa County, CA	<i>V. vinifera</i>	KY706292	KY706317	KY706267	KY706342
<i>S. vitifusiforme</i>	Napa786	Napa County, CA	<i>V. vinifera</i>	KY706293	KY706318	KY706268	KY706343
<i>S. vitifusiforme</i>	Napa795	Napa County, CA	<i>V. vinifera</i>	KY706294	KY706319	KY706269	KY706344
<i>S. vitifusiforme</i>	Napa796	Napa County, CA	<i>V. vinifera</i>	KY706287	KY706312	KY706262	KY706337
<i>S. vitifusiforme</i>	Napa797	Napa County, CA	<i>V. vinifera</i>	KY706288	KY706313	KY706263	KY706338
<i>S. vitifusiforme</i>	Wint755	Solano County, CA	<i>V. vinifera</i>	KY706297	KY706322	KY706272	KY706347
<i>S. vitifusiforme</i>	Napa798	Napa County, CA	<i>V. vinifera</i>	KY706295	KY706320	KY706270	KY706345
<i>S. vitis</i>	MFLUCC 14-0051 ^T	Italy	<i>V. vinifera</i>	KR920363	KR920362	...	...
<i>S. vitis</i>	Napa759	Napa County, CA	<i>V. vinifera</i>	KY706282	KY706307	KY706257	KY706332
<i>S. vitis</i>	Napa764	Napa County, CA	<i>V. vinifera</i>	KY706273	KY706298	KY706248	KY706323
<i>S. vitis</i>	Napa772	Napa County, CA	<i>V. vinifera</i>	KY706275	KY706300	KY706250	KY706325
<i>S. vitis</i>	Napa774*	Napa County, CA	<i>V. vinifera</i>	KY706276	KY706301	KY706251	KY706326
<i>S. vitis</i>	Napa776	Napa County, CA	<i>V. vinifera</i>	KY706277	KY706302	KY706252	KY706327
<i>S. vitis</i>	Napa779	Napa County, CA	<i>V. vinifera</i>	KY706274	KY706299	KY706249	KY706324
<i>S. vitis</i>	Napa781	Napa County, CA	<i>V. vinifera</i>	KY706280	KY706305	KY706255	KY706330
<i>S. vitis</i>	Napa782*	Napa County, CA	<i>V. vinifera</i>	KY706278	KY706303	KY706253	KY706328
<i>S. vitis</i>	Napa784	Napa County, CA	<i>V. vinifera</i>	KY706281	KY706306	KY706256	KY706331
<i>S. vitis</i>	Wint752	Solano County, CA	<i>V. vinifera</i>	KY706279	KY706304	KY706254	KY706329
<i>S. walkeri</i>	CPC 17644	Australia Victoria	<i>Eucalyptus</i> sp.	JN871207	JN871216	...	...
<i>Synnemapestaloides rhododendri</i>	MAFF 239201 ^T	Japan	<i>Rhododendron brachycarpum</i>	LC047753	LC047744	...	...
<i>S. rhododendri</i>	TAMA 492	Japan	<i>R. brachycarpum</i>	LC047760	LC047749	...	...

Sarcostroma lomatae (McAlpine) Nag Raj CBS 118144, *Seimatosporium ficeae* Dayar., Phook. & K. D. Hyde MFLUCC 15-0519 and *Seimatosporium rhombisporum* Senan., Camporesi & K. D. Hyde MFLUCC 15-0727 (ITS and ITS + LSU analyses), and *Synnemapestaloides foliicola* (Berk.) Kyoko Wanta., Nozawa, Kaz. Tanaka & Toy. Sato isolate NBRC 32676 (ITS + LSU + TEF1- α + BT analysis).

Morphology. Mycelial plugs (5 mm in diameter) were taken from the margin of an actively growing culture and transferred to 90-mm-diameter Petri dishes. Radial growth was measured after 14 days at 25°C in darkness for triplicate plates per isolate by taking two colony diameter measurements perpendicular to each other. Assessments of colony color (Rayner 1970) and morphology were made at 14 days. To induce fruiting bodies, a subset of *Seimatosporium* isolates representing different species (Napa768, Wint753, Wint754, Napa774, and Napa782), based on preliminary phylogenetic analyses were inoculated on grapevine wood segments embedded in PDA. Briefly, 1-year-old grapevine canes (approximately 1 cm in diameter) were collected in the vineyard, cut to 5-cm segments, and autoclaved twice (autoclaving 24 h apart, both times at 122°C for 25 min). Autoclaved segments were then placed in 90-mm Petri dishes (two to three segments per dish) and PDA was poured to embed them. A mycelial plug from an actively growing culture was placed on the surface of the agar, between the wood segments. Petri dishes were incubated at room temperature under a natural photoperiod in December 2016, and fruiting body formation was monitored weekly for 4 weeks.

Fruiting bodies ($n = 30$ per isolate) were characterized in terms of structure and size. Fresh fruiting bodies were transversely sectioned by hand with a razor blade and observed at $\times 100$ (Leica DM500B; Leica Microsystems CMS GmbH, Wetzlar, Germany). No stain was applied, to preserve the natural pigments. Conidial dimensions ($n = 50$ per isolate) were measured at $\times 100$ from 28-day-old cultures (squash mounts in sterile deionized water). Analyses of variance (ANOVA) were used to test the null hypotheses that colony diameters and conidial dimensions were similar among isolates. Homogeneity of variance was evaluated prior to ANOVA. ANOVA was performed using the MIXED procedure in SAS (v. 9.2; SAS Institute, Cary, NC), and the main effect of isolate was treated as a fixed effect. For significant effects ($P < 0.05$), means were compared by Tukey's tests.

Fungal interactions in planta. Dual inoculations to the woody stems of potted grapevines were used to evaluate whether *Seimatosporium* isolates were antagonistic or synergistic with trunk pathogens, based on smaller or larger lesions, respectively, when paired versus alone. Pairs of isolates originating from the same wood cankers in the field were coinoculated (listed in order of *Seimatosporium* isolate and trunk-pathogen isolate): *Seimatosporium vitifusiforme* Wint753 and *C. ampelina* Wint015, *S. luteosporum* Wint754 and *D. ambigua* Wint904, *S. vitifusiforme* Napa768 and *Diplodia seriata* Napa810, *S. vitis* Napa774 and *E. lata* Napa003, and *S. vitis* Napa782 and *Diatrypella verruciformis* Napa016 (Table 1). Inoculations of individual *Seimatosporium* isolates served as pathogenicity tests for our newly described species of *Seimatosporium*. Because intraspecific variation in virulence of some grapevine trunk pathogens is reported—for example, *Diaporthe ambigua* (Lawrence et al. 2015), *Diplodia seriata* (Luque et al. 2009), and *E. lata* (Sosnowski et al. 2007)—individual inoculations of the trunk pathogens provided a means of evaluating our isolates in the context of the published literature. In spite of attempts to produce spores of all 10 isolates in culture, three species of trunk pathogens *C. ampelina* Wint015, *Diatrypella verruciformis* Napa016, and *E. lata* Napa003 never produced spores. As such, mycelial suspensions were used as a uniform source of inoculum for all isolates, to examine in planta interactions. For each isolate, mycelial suspensions were prepared as inoculum (6-day-old potato dextrose broth [PDB] cultures were homogenized with a hand-held disperser), following the protocol of Travadon et al. (2013).

The experimental design consisted of two repeated experiments, which were performed, starting 1 week apart, on two sets of plants propagated in two separate greenhouses. In each experiment, eight plants were inoculated per each of 10 isolates and eight plants were dual inoculated per each of five pairs of isolates. Wounded but non-inoculated controls were wounded once or twice (eight plants per control treatment), to evaluate the effect of one versus two wounds on lesion length in the absence of inoculum. Therefore, there were 17 inoculation treatments, giving a total of 272 plants (eight plants $\times$ 17 treatments $\times$ two experiments). Plants were arranged in a completely randomized design in both greenhouses.

Dormant cuttings of *Vitis vinifera* L. 'Pinot noir' ENTAV-INRA clone 777 were propagated in the greenhouse according to Travadon et al. (2013). Briefly, starting in April 2016, cuttings were callused at

Fig. 1. A to C, Wood cankers from which *Seimatosporium* isolates and trunk pathogens were originally isolated were asymmetric in shape, dark brown to light brown in color, and appeared to grow out from the internal heartwood of the grapevine wood, with black spotting in the surrounding wood closer to the bark. D, Wood cankers were sometimes darker in color, but were still present within the heartwood and surrounded by outer layers of wood with black spotting. Stem lesions that formed on plants in single and dual inoculations with five pairs of species: E, *Seimatosporium vitis* Napa774 and *Eutypa lata* Napa003; F, *S. vitis* Napa782 and *Diatrypella verruciformis* Napa016; G, *S. vitifusiforme* Wint753 and *Cryptovalsa ampelina* Wint015; H, *S. vitifusiforme* Napa768 and *Diplodia seriata* Napa810; and I, *S. luteosporum* Wint754 and *Diaporthe ambigua* Wint904.

30°C and 100% humidity in a mixture of perlite and vermiculite (1:1, vol/vol) for 21 days. Once shoot and root initials emerged from the callus tissue, a power drill was used to produce a wound (2 mm in width by 3 mm in depth) approximately 2 cm below the uppermost node. For dual inoculations, two wounds were made per stem, with each wound approximately 5 mm apart and 2 cm below the uppermost node. Inoculum (20 µl) was pipetted into the wound, which was then sealed with Vaseline (Unilever, Rotterdam, London) and Parafilm (Bemis Co., Neenah, WI) to prevent inoculum desiccation. An equal volume (20 µl) of sterile PDB was pipetted into the wound of noninoculated controls. Cuttings were coated with melted paraffin wax (Gulf Wax; Royal Oak Enterprises, LLC, Roswell, GA), to prevent moisture loss, and potted in sterile potting mix amended with slow-release fertilizer (Osmocote Pro 24-4-9; Scotts, Marysville, OH). Plants were grown in the greenhouse at the University of California Experiment Station in Davis from June 2016 to May 2017 (natural sunlight photoperiod, 25 ± 1 and $18 \pm 3^\circ\text{C}$, day and night, respectively), with some modifications to the temperature conditions (10 ± 2 and $4 \pm 2^\circ\text{C}$, day and night, respectively) during dormancy (for 2 months starting in December 2016). Plants were watered four times per week for 15 min using a drip-irrigation system ($0.5 \text{ liters h}^{-1}$).

The length of internal wood discoloration extending out from the inoculation site up and down the stem (lesion length) was measured approximately 11 months after inoculation in April 2017 during the second growing cycle. First, the newly developed green shoots, roots, and bark of each plant were removed and discarded, and the woody stems were surface sterilized in 1% sodium hypochlorite for 2 min and rinsed with deionized water. The length of each stem was recorded and cut longitudinally to expose wood discoloration, the length of which was measured with a digital caliper. To confirm that the isolate was responsible for wood discoloration in inoculated plants, recovery was attempted by cutting 10 pieces (2 by 5 by 5 mm) of wood from the distal margin of the lesion, followed by surface disinfection in 0.6% sodium hypochlorite (pH 7.2) for 30 s, two 30-s rinses in sterile deionized water, plating on PDA amended with tetracycline (1 mg liter^{-1}), and incubation in the dark at approximately 22°C for 14 to 21 days. Plants that died during the course of the study, which sometimes occurs due to improper rooting, were excluded from analyses of lesion lengths and from recovery attempts.

Lesion lengths among inoculated plants relative to those of noninoculated plants were used as a measure of pathogenicity. Normality and homogeneity of variances were evaluated using normal probability plots and Levene's test, respectively. ANOVAs were used to determine whether there were differences in lesion length among isolates when inoculated alone or in pairs relative to noninoculated plants, and if this was consistent between two replicate experiments. There was a separate ANOVA for each pair of isolates, with all datasets including lesion lengths from the same set of noninoculated plants. ANOVAs were performed using the MIXED procedure in SAS, with inoculation treatment, experiment, and their interaction considered as fixed effects. Means were calculated using the LSMEANS procedure. For significant effects ($P < 0.05$), means were compared by Tukey's tests.

Fungal interactions in vitro. In vitro evaluation of fungal interactions between pairs of *Seimatosporium* isolates and trunk pathogens originating from the same canker was conducted on dual-inoculated PDA plates (three plates per pair of isolates) following a protocol adapted from Kotze et al. (2011). Because *Seimatosporium* isolates grow relatively slowly, they were cultured for 10 days on the pairing plates before adding mycelial plugs of the coisolated trunk pathogens to each plate. Two mycelial plugs (2 by 2 mm) from the margin of a 7-day-old *Seimatosporium* colony were plated 2 cm apart on PDA and incubated for 10 days at 22°C in darkness. After 10 days, two mycelial plugs (2 by 2 mm) from the margin of a 7-day-old colony of each coisolated trunk pathogen were plated 2 cm from the *Seimatosporium* plugs, hence allowing for one self-pairing per *Seimatosporium* isolate and two allo-pairings per pair of isolates on the same plate. Dual-inoculated plates were then incubated for an additional 12 days at 22°C in darkness. Microscopic observations of fungal interactions were observed at $\times 100$ by removing mycelial plugs (2 by

2 mm) from the interaction zone and placing them on a glass slide with sterile deionized water, as described by Kotze et al. (2011).

Results

Phylogenetic analyses. The best-fit model of nucleotide evolution for ML analyses were deduced based on the AIC ($K2 + G$ for ITS, $K2 + G + I$ for ITS + LSU, and $K2 + G$ for ITS + LSU + TEF1- α + BT). Alignment of the two-locus dataset (ITS + LSU) resulted in a 1,364-character dataset (1,100 characters were constant, 127 characters were parsimony uninformative, and 137 characters were parsimony informative). MP analysis produced a single most parsimonious tree of 422 steps and a consistency index (CI) and retention index (RI) of 0.504532 and 0.802410, respectively. ML and MP analyses revealed that 10 of our *Seimatosporium* isolates from grape in California clustered with strong support (96 and 99% ML and MP bootstrap values, respectively) with the ex-type of *S. vitis* isolate MFLUCC 14-0051. Fifteen isolates clustered in a weakly supported clade (60 and 70%) and, within this, was a strongly supported (100 and 100%) subclade of two isolates (Fig. 2). With no apparent type or nontype affiliations for either clade, however, additional analyses were required.

Stronger support for the phylogenetic subdivision of these 15 isolates into two clades was obtained with analyses of ITS-only (Supplementary Fig. S1) and ITS + LSU + TEF1- α + BT datasets (Supplementary Fig. S2). We propose these two clades to be novel taxa, hereinafter identified as *Seimatosporium vitifusiforme* sp. nov. and *Seimatosporium luteosporum* sp. nov. Alignment of ITS sequences resulted in a 562-character dataset (377 characters were constant, 84 characters were parsimony uninformative, and 101 characters were parsimony informative). MP analyses produced two equally most-parsimonious trees of 312 steps and a CI and RI of 0.522449 and 0.815457, respectively. ML and MP analyses of the ITS + LSU dataset (Fig. 2) produced topology and bootstrap values similar to those in the ITS-only analysis, with increased support for the aforementioned newly discovered taxa. The major topological differences between the ITS-only and ITS + LSU analyses were the order of divergence among other phylogenetically close genera, *Adisciso* Kaz. Tanaka, Okane & Hosoya, *Immersidiscosia* Kaz. Tanaka, Okane & Hosoya, *Sarcostroma* Cooke, *Synnemapestaloides* T. Handa & Y. Harada, and fungi previously identified as *S. ficeae*, *S. rhombisporum*, and *S. pezizoides* (Ellis & Everh.) B. Sutton. The latter three taxa require taxonomic revision based on our phylogenetic analyses, because all three cluster distantly from *Seimatosporium* spp.

Alignment of the four-loci dataset (ITS + LSU + TEF1- α + BT) resulted in a 2,229-character dataset (2,023 characters were constant, 91 characters were parsimony uninformative, and 115 characters were parsimony informative). MP analysis produced 10 equally most-parsimonious trees of 206 steps and a CI and RI of 0.968254 and 0.995781, respectively. ML and MP analyses revealed that all three *Seimatosporium* spp. recovered from symptomatic grapevine cankers in northern California are strongly supported (≥ 82 and $\geq 99\%$, respectively) as independent phylogenetic lineages.

Morphology. Average colony growth at 25°C after 14 days was significantly different among the 25 *Seimatosporium* isolates ($P [F_{(21,44)} > 39] < 0.0001$), with *S. vitifusiforme* Napa796 producing the smallest colony (33.5 mm) and *S. vitis* Napa784 the largest colony (75.7 mm). Nonetheless, there was large variation among colony growth within a species (e.g., from 33.5 to 64.8 mm for *S. vitifusiforme*). *S. vitifusiforme* produced a buff, cottony, medium-growing colony with a smooth, lighter colored margin. *S. luteosporum* produced a grayish-white, cottony, medium-fast growing colony with a smooth margin. *S. vitis* produced a light- to medium-brown, cottony, medium-fast growing colony with a smooth, light-colored margin. There were significant differences in conidial length ($P [F_{(2,87)} > 92.28] < 0.0001$) and width ($P [F_{(2,87)} > 129.06] < 0.0001$) among the three species, with *S. vitifusiforme* Wint753 producing, on average, the longest and most narrow conidia as compared with *S. luteosporum* and *S. vitis*, as described below.

Taxonomy. Morphological comparisons, coupled with phylogenetic analyses (ML and MP) of the ITS + LSU + TEF1- α + BT dataset, identified three distinct and strongly supported clades, two of

which have no apparent species names. Thus, we propose the following new species names to properly circumscribe and typify these two unique taxa:

Seimatosporium vitifusiforme D. P. Lawr. & Travadon, sp. nov.
Mycobank number: MB820361; Figures 2 and 3.

Typification. USA, California: Solano County, 38.5249° N, 121.9708° W, 41 m above sea level (masl) isolated from wood canker

of *V. vinifera*, 2010, R. Travadon number Wint753 (holotype BPI 910344, dried culture; ex-type CBS 142600). GenBank accession numbers: KY706296, ITS; KY706271, BT; KY706346, TEF1- α ; KY706321, LSU.

Etymology: The name refers to the host (*V. vinifera*) and to the fusiform shape of the conidia.

Colony diameter of *S. vitifusiforme* isolate Wint753: 51.6 mm in 14 days at 25°C on PDA, medium-growing, slightly raised, wooly

Fig. 2. Single most likely tree (ln likelihood -4439.3212) resulting from the combined analysis of internal transcribed spacer and large subunit sequences from 65 *Seimatosporium* isolates and closely related genera. Numbers represent maximum-likelihood/maximum-parsimony bootstrap values, respectively. Values represented by an asterisk were less than 50%. Scale bar represents the expected number of substitutions per site. Ex-type strains of *Seimatosporium luteosporum* sp. nov. and *Seimatosporium vitifusiforme* sp. nov. are CBS 142599 (Wint754) and CBS 142600 (Wint753), respectively.

light-brown colony with smooth wooly off-white margin. *Hyphae* hyaline, smooth, straight, branched, and septate. *Conidiomata* pycnidoid, mostly solitary some aggregate, dark, globose, 258–(392)–525 μm in diameter ($n = 30$). *Conidiophores* reduced to conidiogenous cells, simple, holoblastic, 11.9–(15.5)–26.4 $\times$ 1.5–(1.9)–2.6 μm ($n = 30$). *Conidia* 18.6–(24.9)–30.3 $\times$ 3.7–(4.2)–5.1 μm ($n = 50$), abundant, single, concolorous median cells, smooth-walled, curved toward apex, three-septate, slight constriction at some septa; basal cell hyaline to pale brown, truncate base, 3.3–(4.4)–5.6 μm with single eccentric, hyaline, unbranched, flexuous appendage 3.9–(9.5)–16.6 μm ; median cells light to medium brown, cylindrical, second cell from base 4.9–(6.1)–7.6 μm , third cell from base 4.3–(7.1)–8.9, together 9.2–(13.2)–16.5 μm , transverse septa thick and dark brown; apical cell hyaline to pale brown, 4–(6.3)–8.1 μm with conical apex, with single, central, hyaline, unbranched, flexuous appendage 7–(10)–12.6 μm . No teleomorph observed. Known distribution: Napa and Solano Counties, California, USA.

Seimatosporium luteosporum D. P. Lawr. & Travadon, sp. nov.
Mycobank number: MB820362; Figures 2 and 4.

Typification. USA, California: Solano County, 38.5249° N, 121.9708° W, 41 masl isolated from wood canker of *V. vinifera*, 2010, R. Travadon number Wint754 (holotype BPI 910345, dried culture; ex-type CBS 142599). GenBank accession numbers: KY706284, ITS; KY706259, BT; KY706334, TEF1- α ; KY706309, LSU.

Etymology: The name refers to the characteristic concolorous yellow-orange pigmentation of the two median cells within a conidium.

Colony diameter of *S. luteosporum* isolate Wint754: 64.5 mm in 14 days at 25°C on PDA, medium-fast growing, grayish white, slightly raised, wooly colony with smooth margin. *Hyphae* hyaline,

smooth, straight, branched, and septate. *Conidiomata* pycnidoid, mostly solitary some aggregate, dark, 345–(468)–568 μm in diameter ($n = 30$). *Conidiophores* reduced to conidiogenous cells 12.6–(17.4)–25.1 $\times$ 1.8–(2.3)–2.6 μm ($n = 30$). *Conidia* 16.7–(19.9)–25.4 $\times$ 4.7–(5.3)–5.6 μm ($n = 50$), abundant, single, pale luteous concolorous median cells, smooth-walled, slightly curved at both ends, three-septate, slight constriction at some septa; basal cell hyaline to buff, truncate base, 2.1–(3.2)–4.6 μm with single eccentric, hyaline, unbranched, flexuous appendage 9.8–(16.7)–23.6 μm ; median cells pale luteous, cylindrical, second cell from base 3.8–(5.6)–8.1 μm , third cell from base 4.4–(6.1)–7.5, together 8.2–(11.8)–15.6 μm , transverse septa thick and cinnamon colored; apical cell hyaline to buff, 2.5–(3.7)–4.9 μm with conical apex, with single, central, hyaline, unbranched, elongated flexuous appendage 10.1–(17.9)–24.2 μm . No teleomorph observed. Known distribution: Napa and Solano Counties, California, USA.

Seimatosporium vitis Y. P. Xiao, Camporesi & K. D. Hyde

Index Fungorum number: IF551200; MycoBank number: MB551200

Colony diameter of *S. vitis* isolate Napa782: 59.8 mm in 14 days at 25°C on PDA, medium growing, light brown, slightly raised, wooly colony with smooth margin. *Hyphae* hyaline, smooth, straight, branched, and septate. *Conidiomata* pycnidoid, mostly solitary some aggregate, dark, 112–(221)–368 μm in diameter ($n = 30$). *Conidiophores* reduced to conidiogenous cells 8.1–(12.1)–21.5 $\times$ 1.4–(2.1)–3 μm ($n = 30$). *Conidia* abundant, single, concolorous median cells, smooth-walled, mostly straight some slightly curved, three-septate, slight constriction at most septa 13–(16.1)–19.7 $\times$ 4.9–(5.5)–5.9 μm ($n = 50$); basal cell hyaline to pale brown, truncate base, 2–(3)–4.5 μm with single eccentric, hyaline, unbranched, short flexuous appendage 4.5–(6.7)–10.9 μm ; median cells light to medium brown, doliiform,

Fig. 3. **A,** Morphology of *Seimatosporium vitifusiforme* Wint753, ex-type CBS 142600. Colony cultured on potato dextrose agar at 25°C in the dark for 14 days. **B,** Pycnidia produced on autoclaved grapevine wood. **C,** Conidiogenous cells with appendage-bearing conidia. **D,** Multiseptate conidia with single apical centrally located appendage and single basal eccentrically located appendage. Scale bars: 500 μm (B) and 25 μm (C and D).

second cell from base 2.9–(3.8)–5.5 μm , third cell from base 2.6–(3.8)–5.6, together 8.2–(11.8)–15.6 μm , transverse septa very thick and medium to dark brown; apical cell hyaline to pale brown, 2.8–(3.6)–4.2 μm with conical apex, with single, central, hyaline, unbranched, short flexuous appendage 2.8–(7.3)–12 μm . No teleomorph observed. Known distribution: Napa and Solano Counties, California USA, Hungary, Iran, and Italy. The teleomorph of *S. vitis* was recently described by Mehrabi et al. (2017).

Based on phylogenetic results, we propose the following taxonomic revision to produce a more stable taxonomy, because *Sarcostroma restionis* clearly belongs in *Seimatosporium*:

Seimatosporium restionis (S. J. Lee & Crous) D. P. Lawr. & Travadon comb. nov.

Mycobank number: MB821608; Figure 2.

Basionym: *Sarcostroma restionis* S. J. Lee & Crous (Lee et al. 2006).

Fungal interactions in planta. The wood cankers from which the *Seimatosporium* isolates originated were irregular in shape, with discolored and necrotic wood mostly present within the heartwood, colored various shades of brown, and frequently surrounded by black spotting or occlusions of the wood (Fig. 1A to D). After an 11-month incubation in the woody stems of potted Pinot noir plants, all fungal

isolates inoculated singly and together caused lesions (Fig. 1E to I), which were similar in color and texture to the wood cankers from which they were isolated. No expression of foliar symptoms was observed. Recovery of isolates was confounded by frequent contamination of fast-growing, naturally occurring endophytes, primarily *Trichoderma* Pers. and *Alternaria* Nees. Only the fast-growing species *Diaporthe ambigua* Wint904 and *Diplodia seriata* Napa810 were reisolated from 53 and 63% of inoculated plants, respectively. *Seimatosporium* spp., which grew particularly slowly, were not recovered from any inoculated plants and, thus, their pathogenic status remains unclear.

For each pair of isolates, ANOVA showed that mean lesion lengths of the inoculation treatments (control, single inoculations with either isolate, and dual inoculations with both isolates) were not significantly different between the two replicate experiments (P values of 0.1 to 1.0). The only significant effect was inoculation treatment (P values of 0.001 to <0.0001). Effects of inoculation treatment were consistent between the two replicate experiments, because there was no significant effect of the interaction between experiment and inoculation treatment (P values of 0.1 to 0.4). Lesions associated with single inoculations of *S. vitifusiforme* Napa768 were not significantly different from those of the control, based on the results of

Fig. 4. **A**, Morphology of *Seimatosporium luteosporum* Wint754, ex-type CBS 142599. Colony cultured on potato dextrose agar at 25°C in the dark for 14 days. **B**, Pycnidia produced on autoclaved grapevine wood. **C**, Conidiogenous cells with appendage-bearing conidia. **D**, Multiseptate conidia with single apical centrally located appendage and single basal eccentrically located appendage. Scale bars: 1 mm (B), and 25 μm (C and D).

means comparisons (Fig. 5A). However, *S. vitifusiforme* Napa768 was associated with significantly larger lesions than those of the control when dual inoculated with *D. seriata* Napa810, the latter of which in both single and dual inoculations was associated with the largest lesion lengths in our study. Larger lesions for both *S. vitifusiforme* Napa768 (statistically significant) and *D. seriata* Napa810 (not statistically significant but, nonetheless, larger) suggest that there is a synergistic effect of dual inoculation on lesion length. We found similar increases in lesion lengths in dual inoculations of *S. luteosporum* Wint754 and *Diaporthe ambigua* Wint904, with *S. luteosporum* Wint754 associated with lesions larger than those of the controls only in dual inoculation with *D. ambigua* Wint904 (Fig. 5B). Unlike *S. vitifusiforme* Napa768, plants inoculated with *S. vitifusiforme* Wint753 in single inoculations had larger lesions than the controls (Fig. 5C). Furthermore, dual inoculations of *S. vitifusiforme* Wint753 and *C. ampelina* Wint015 brought about larger lesions for *C. ampelina* Wint015, which was not pathogenic in single inoculations.

Plants inoculated with *S. vitis* Napa782 in single inoculations had larger lesions than those of the control but dual inoculations with *Diatrypella verruciformis* Napa016 were not associated with lesion length increases for either isolate (Fig. 5D). Both *S. vitis* Napa774 and *E. lata* Napa003 had significantly longer lesion lengths than

those of the controls in single and dual inoculations; however, dual inoculations were not associated with significantly larger lesions for either isolate (Fig. 5E).

Fungal interactions in vitro. In vitro, we observed three types of fungal interactions between different species: no to sparse growth in a band of uncolonized or sparsely colonized media (clearing zone), formation of a band of dark pigmented hyphae (barrage zone), and dense growth of both species in direct contact with each other to sometimes form a “wall” of hyphae. In contrast, there was no evidence of growth inhibition in self-pairings between agar plugs of the same isolate; hyphae originating from both plugs merged into a single colony.

The interactions between *S. vitis* Napa774 and *E. lata* Napa003 and between *S. vitis* Napa782 and *D. verruciformis* Napa016 appeared to be antagonistic in vitro. A clearing zone formed between *S. vitis* Napa774 and *E. lata* Napa003, and the aerial hyphae of *E. lata* Napa003 changed color from white to off-white on the surface of the agar and to orange within the agar (Fig. 6A). Between the clearing zone and the agar plug, each isolate formed a dark, pigmented barrage zone (visible on the underside of the plate), which was particularly thick for *E. lata* Napa003 (Fig. 6A). Microscopic examination showed the hyphae of *E. lata* were swollen near the clearing zone (Fig. 6B). The interaction of *S. vitis* Napa782 and *D. verruciformis*

Fig. 5. In planta interactions after 11 months in the stems of *Vitis vinifera* 'Pinot noir' potted plants between pairs of species originating from the same wood cankers. **A**, *Seimatosporium vitifusiforme* Napa768 and *Diplodia seriata* Napa810; **B**, *S. luteosporum* Wint754 and *Diaporthe ambigua* Wint904; **C**, *S. vitifusiforme* Wint753 and *Cryptovalsa ampelina* Wint015; **D**, *S. vitis* Napa782 and *Diatrypella verruciformis* Napa016; and **E**, *S. vitis* Napa774 and *Eutypa lata* Napa003. Mean lesion lengths for single and dual inoculations each represent 9 to 14 observations, summed across replicate experiments. Mean lesion lengths of control plants (wounded, but noninoculated) represent 29 observations, summed across single and dual control plants, in both replicate experiments. Means with the same letter, within each graph, are not significantly different (Tukey's test, $P < 0.05$, $\alpha = 0.05$).

Napa016 was similar, with a clearing zone between the two isolates and pigmented barrage zones closer to the agar plugs, especially within the colony of *D. verruciformis* Napa016 (Fig. 6C). Microscopic examination revealed *D. verruciformis* Napa016 produced swollen, bulb-shaped hyphae near the clearing zone (Fig. 6D).

The interaction between *S. vitifusiforme* Wint753 and *C. ampelina* Wint015 appeared to be antagonistic in vitro but with less dramatic changes in colony morphology at the interaction zone than described above for pairings with *S. vitis* isolates, and no visible changes in hyphal morphology. The interaction between *S. vitifusiforme* Wint753 and *C. ampelina* Wint015 resulted in the formation of a narrow barrage zone, which appeared as a wall of hyphae on the agar surface and a dark band as viewed on the underside of the plate (Fig. 6E). Both *S. vitifusiforme* Wint753 and *C. ampelina* Wint015 had a high density of hyphae right up to the barrage zone; there was no clearing zone and the hyphae of neither isolate were swollen or misshapen (Fig. 6F).

The interactions between *S. vitifusiforme* Napa768 and *Diplodia seriata* Napa810 and between *S. luteosporum* Wint754 and *Diaporthe ambigua* Wint904 showed the least degree of growth inhibition to either species in each pairings. There was no clearing zone between *S. vitifusiforme* Napa768 and *Diplodia seriata* Napa810 (Fig. 6G). Microscopic examination revealed hyphae of both species were intermixed (Fig. 6H). The interaction of *S. luteosporum* Wint754 and *Diaporthe ambigua* Wint904 resulted in a very narrow clearing zone between the two species (Fig. 6I). Microscopic examination revealed hyphae of each species were restricted with clear separation (Fig. 6J).

Discussion

This study is the first to explore the diversity and pathogenicity of *Seimatosporium* spp. isolated from grapevines with symptoms of grapevine trunk diseases in northern California. Three species of *Seimatosporium* were identified by incorporating reference type and nontype sequences in phylogenetic analyses. Two of the species, *S. luteosporum* and *S. vitifusiforme*, are newly described and typified, whereas the third, *S. vitis*, constitutes a new report from North America. The *Seimatosporium* spp. associated with grapevine and other perennial hosts (e.g., *Eucalyptus*) (Barber et al. 2011) have likely been overlooked, due in part to the lack of type or ex-type sequence data in molecular repositories. Indeed, our species-level identification was restricted mainly to analyses of ITS and LSU sequences, because there is a dearth of potentially more-informative protein-coding loci in GenBank for *Seimatosporium* spp. and allied genera. Therefore, we

amplified BT and TEF1- α in order to provide such data for future analyses by other mycologists and plant pathologists. Future systematic studies of *Seimatosporium* and related genera in the family Sporocadaceae would benefit from the phylogenetic analysis of protein-coding loci such as BT and TEF1- α to better refine organismal relationships.

Seimatosporium and allied pestalotioid genera have several morphological features in common, which confound species identification. Since its inception in 1833, the generic concept of *Seimatosporium* has undergone considerable change. Indeed, from 1964 to 1980, the generic limits were broadened to accept as many as 15 generic synonyms (Nag Raj 1993). Sutton (1980) acknowledged the morphological heterogeneity of members of the genus and suggested reinstatement of certain generic names, to delimit a proper generic concept for *Seimatosporium*. Nag Raj (1993) further subdivided *Seimatosporium* spp. into what he called “five natural groups”, based on conidial characteristics of extant generic names, including (i) *Seimatosporium* sensu stricto, (ii) *Sporocadus* Corda, (iii) *Sarcostroma*, (iv) *Diploceras* (Sacc.) Died., and (v) *Vermisporium* H. J. Swart & M. A. Will. *Seimatosporium* sensu Nag Raj produces conidia with concolorous pigmented median cells, with or without a single apical or basal appendage. However, this narrow morphological concept does not reflect the true natural morphological diversity of *Seimatosporium* spp. and, furthermore, has been refuted by molecular phylogenetic analyses (Barber et al. 2011; Tanaka et al. 2011). Indeed, our phylogenetic analyses showed that *Sarcostroma* and also *Discostroma* Clem. (*Seimatosporium* teleomorph) intermixed within the *Seimatosporium* clade, suggesting that additional taxonomic revisions are necessary. Reassessment of the *Seimatosporium* phylogeny would benefit from additional collections and examination of the holomorphs to help guide morphomolecular taxonomy in this group of fungi.

Three species of *Seimatosporium* (*S. botan*, *S. hysteroioides*, and *S. vitis*) have been reported from diseased or dead grapevine wood in Australia, Chile, Hungary, Iran, Italy, and the United States (Díaz et al. 2012; Mehrabi et al. 2017; Senanayake et al. 2015; Sergeeva et al. 2005; Váczy 2017). However, there is little information on their pathogenicity. *S. botan* was isolated from symptomatic vines in Chile and was shown to be pathogenic on both detached green shoots (mean lesion length of 2 cm, 14-day incubation) and woody canes and trunks of potted plants (mean lesion lengths of 5 and 4 cm for 7-month incubation in canes and 15-month incubation in trunks, respectively) (Díaz et al. 2012). Váczy (2017) showed that *S. vitis*, isolated from symptomatic vines in Hungary, was pathogenic to the green shoots of potted plants (lesion lengths of 2 to 4 cm). In our study, *S. vitis*

Fig. 6. In vitro interactions on potato dextrose agar (PDA) after 22 days between pairs of species originating from the same wood cankers. **A, C, E, G, and I,** Surface of the PDA (top) and the underside of the plate (bottom). **A,** *Seimatosporium vitis* Napa774 (left) and *Eutypa lata* Napa003 (right); **B,** swollen hyphae of *E. lata* Napa003 at the interaction zone; **C,** *S. vitis* Napa782 (left) and *Diatripella verruciformis* Napa016 (right); **D,** pigmented hyphae of *S. vitis* Napa782 (left) and swollen, bulb-shaped hyphae of *D. verruciformis* Napa016 (right); **E,** *S. vitifusiforme* Wint753 (left) and *Cryptovalsa ampelina* Wint015 (right); **F,** dense hyphae of both *S. vitifusiforme* Wint753 (left) and *C. ampelina* Wint015 (right) at the interaction zone; **G,** *S. vitifusiforme* Napa768 (left) and *Diplodia seriata* Napa810 (right); **H,** dense hyphae of both *S. vitifusiforme* Napa768 (left) and *D. seriata* Napa810 (right) at the interaction zone; **I,** *S. luteosporum* Wint754 (left) and *Diaporthe ambigua* Wint904 (right); and **J,** no mixing of *S. luteosporum* Wint754 (left) and *D. ambigua* Wint904 (right) at the interaction zone. Scale bars: 25 μ m (B), 100 μ m (D), 100 μ m (F), 100 μ m (H), and 100 μ m (J).

produced lesions of similar size on woody stems of potted plants (range of 1- to 3-cm lesion lengths, 11-month incubation) and these were significantly greater than those of the noninoculated controls. Because *S. botan* and *S. vitis* are pathogenic to both green shoots and woody tissues, it is possible that, like other trunk pathogens (e.g., *D. ampelina*) (Wilcox et al. 2015), these species can infect different grapevines tissues through various types of wounds such as the small cracks that form on the surface of rapidly expanding green shoots or thin sections of the cuticle near the peduncle of green berries, in addition to pruning wounds. The presence of wood lesions in the stems of inoculated plants suggests that, during substrate colonization, *Seimatosporium* spp. may damage the wood directly by secreting cell-wall-degrading enzymes (Massonnet et al. 2017; Morales-Cruz et al. 2015) or fungal toxins (Andolfi et al. 2011), as is the case for the grapevine trunk pathogens; further studies are required to test these hypotheses.

It is difficult to compare and contrast in vitro versus in planta results of fungal interactions. Lesion margins in planta likely do not delimit fungal growth, because some trunk pathogens (e.g., *E. lata*) (Sosnowski et al. 2007) are frequently isolated ahead of the lesion margins in the stems of inoculated plants. However, lesion length constitutes a quantitative and repeatable measure (relative to that of the noninoculated controls) of intrahost growth and virulence, an important life history trait for fungal plant pathogens. There were varying interactions between all pairs of species in vitro, the least of which was seen as no to little growth inhibition between *S. vitifusiforme* and *Diplodia seriata*, and *S. luteosporum* and *Diaporthe ambigua*. In planta, dual inoculations with either *S. vitifusiforme* and *Diplodia seriata* or *S. luteosporum* and *Diaporthe ambigua* resulted in larger lesion sizes for the *Seimatosporium* isolates compared with single inoculations (and noninoculated controls), suggesting a synergistic effect of dual inoculation with a trunk pathogen on the virulence of these *Seimatosporium* isolates. It is possible that the relatively mild antagonistic interactions between the two pair of species in vitro are associated with our findings of synergistic effects in planta. Synergistic interactions among fungal plant pathogens are not well characterized but interspecies signaling and metabolic exchange and complementarity are potential mechanisms (Lamichhane and Venturi 2015). Synergistic interactions can also result from the indirect effects of one fungus, including interference with the host immune response or chemical or physical modifications to the substrate, thereby stimulating colonization by another microorganism (Le May et al. 2009; Nomura et al. 2005).

The outcomes of the dual inoculations conducted in this study should not solely be interpreted with regards to the interactions between the pairs of species, because the woody tissues of grapevines typically harbor numerous microbes (Bruez et al. 2016; Travadon et al. 2016). Indeed, we isolated many fast-growing fungi (namely, species of *Trichoderma*) from inoculated plants, which may have prevented recovery of *Seimatosporium* isolates and, thus, completion of Koch's postulates. The "microbial background" in the woody tissues of inoculated plants may have influenced the intrahost growth of inoculated isolates and wood lesion development, in particular because mycoparasitic *Trichoderma* isolates are routinely used as biocontrol agents in cropping systems, including grapevines (Kotze et al. 2011).

The most antagonistic interactions in vitro were between *S. vitis* and *E. lata*, and *S. vitis* and *Diatripella verruciformis*. Both clearing zones and adjacent barrage zones formed between the two pair of species in vitro. These macroscopic changes in colony morphology were also associated with microscopic morphological changes in the hyphae; namely, hyphal swelling for the two diatripaceous fungi which, in wood-colonizing basidiomycetes, have been associated with production of antimicrobial secondary metabolites (Boddy and Hiscox 2016). If such interaction-specific compounds were associated with our observations, they would have been produced by the fungi in both in vitro and in planta assays. In this study, there were no significant changes in lesion lengths for *S. vitis* and *E. lata* or *S. vitis* and *D. verruciformis* (all of which were greater than those of the noninoculated controls) in single versus dual inoculations. Trunk pathogens produce a diverse array of toxins (Andolfi et al. 2011); antibiosis through the release of toxins, such as eutypine produced by *E. lata* (Tey-Rulh

et al. 1991), could limit coinfection and growth, although this hypothesis has not been tested. Therefore, it is possible that the strong antagonistic interactions between the two pair of species in vitro are associated with our findings of no increased virulence in planta.

Grapevine trunk diseases are increasingly recognized as a disease complex resulting from the complex interactions between taxonomically unrelated fungi colonizing grapevine wood (Travadon et al. 2016; Whitelaw-Weckert et al. 2013). Now, modern study tools are available to conduct unifying experiments to examine common versus distinctive virulence factors expressed by pathogens that make up the mixed infections commonly found in the field (Morales-Cruz et al. 2018). It is critical to elucidate fungal interactions in planta for the understanding of microbial pathogenesis, symptom development, and, ultimately, development of disease-management strategies (Lamichhane and Venturi 2015). In particular, some microorganisms colonizing grapevine wood may serve as biological controls (Haidar et al. 2016). Given the perennial nature of the host, the fact that pruning wounds (i.e., infection courts) are created each dormant season throughout the life of a vine, and that the wood infections via these wounds are chronic, research on mixed infections and fungal interactions will help us better understand how to maintain levels of grape yields that can bring profitability for the longest period possible.

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