Pathogenicity of Diatrypaceous Fungi on Grapevines in Australia

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Abstract

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In addition to *Eutypa lata*, which causes Eutypa dieback, numerous other fungi in the Diatrypaceae family have been isolated from diseased grapevines (*Vitis vinifera*) and other woody hosts. Pathogenicity trials comprising 70 strains of diatrypaceous fungi representing nine species in six genera were conducted to determine whether these fungi, collected in Australia, were pathogenic to grapevines. When inoculated into wounded trunks of 'Cabernet Sauvignon', eight species, including *E. lata*, *E. leptoplaca*, *Cryptovalsa ampelina*, *C. rabenhorstii*, *Eutypella citricola*, *E. microtheca*, *Diatrypella vulgaris*, and a *Diatrype* sp. produced necrotic lesions significantly longer than on controls. In addition, all nine species (including a *Cryptosphaeria* sp.)

were reisolated from the margins of developing lesions and at varying distances above and below the point of inoculation. Diatrypaceous fungi were frequently isolated from asymptomatic or otherwise healthy tissue several centimeters ahead of the disease margin. Methods to control diseases associated with diatrypaceous fungi must take into account their propensity to colonize woody tissues ahead of or in the absence of visible symptoms. Current recommendations for the management of Eutypa dieback using remedial surgery and pruning wound protection appear sufficient for the control of the other diatrypaceous fungi included in this study.

Eutypa lata (Pers.) Tul. & C. Tul., syn. *E. armeniaca* Hansf. & M.V. Carter, was originally discovered on apricot (*Prunus armeniaca* L.) in South Australia (SA), where it was thought to cause the gummosis and dieback symptoms observed in orchards in the Light's Pass and Angaston districts, approximately 80 km northeast of Adelaide. Described by Carter (2) and named for its host, *E. armeniaca* was eventually reduced to synonymy with *E. lata* (21), but not before being recognized worldwide as a major cause of disease on apricot and, thereafter, as a pathogen of other cultivated species including apple (*Malus domestica* Borkh.), pears (*Pyrus communis* L.), lemon (*Citrus limon* (L.) Burm. F.), and, ultimately, grapevines (*Vitis vinifera* L.) (3). On grapevines, where it is responsible for Eutypa dieback (17), the pathogen is disseminated by ascospores (3), with infection generally taking place via fresh pruning wounds, after which the fungus slowly kills the host, destroying vascular tissue via a cocktail of enzymes and secondary metabolites (16,27). Foliar symptoms, which are caused by phytotoxins produced by the fungus, include stunting of shoots, shortening of internodes, and chlorosis, necrosis, and cupping of leaves (16), although canker formation around pruning wounds and wedge-shaped lesions in the trunks and cordons of infected vines are also characteristic of the disease (17).

Although *E. lata* was once thought to be responsible for the dieback and decline of grapevines throughout California (33), as well as the dead and dying vines routinely observed in the Hunter Valley, New South Wales (NSW), Australia (4,5), surveys conducted more recently confirmed that members of the Botryosphaeriaceae family are the principal cause of grapevine decline in both regions (20,36). However, under closer scrutiny, studies also showed that not only is *E. lata* more widespread in Australia than first thought (14,19) but other diatrypaceous fungi, some previously unrecorded or undescribed, occur on grapevines and other hosts in these

regions (31,32), often in higher numbers and to a greater extent geographically than *E. lata* (19,31).

In the United States, *Eutypa leptoplaca* (Mont.) Rappaz has been shown to be a pathogen of grapevine (28), and species of *Diatrypella* and *Diatrype* were isolated from diseased grapevines by Rolshausen et al. (22). In Michigan, and subsequently in Texas, *Eutypella vitis* (Schwein.) Ellis & Everh. (= *E. aequilinaris* (Schwein.) Starbäck) was also isolated from diseased grapevines (13,35), and Trouillas et al. (33) described as many as 11 species of Diatrypaceae from grapevines in California, including species of *Cryptosphaeria*, *Diatrype*, and *Eutypella*. Likewise, *Cryptovalsa ampelina* (Nitschke) Fuckel was isolated from shoots and canes of diseased Shiraz in SA and was shown to be pathogenic in grapevines (18). Pitt et al. (19) confirmed the presence of *C. ampelina* and other diatrypaceous species in vineyards throughout NSW and, along with Trouillas et al. (31), embarked on a major study of these fungi on grapevines and other hosts in Australia, subsequently documenting 12 species, including new records of *Eutypella*, *Diatrypella*, and *Diatrype*.

Although Trouillas and Gubler (29) provided evidence of the pathogenicity of other diatrypaceous genera in grapevines, the pathogenicity of diatrypaceous fungi recently described in Australia remains unknown. Hence, the objective of this study was to determine the pathogenicity to grapevines of diatrypaceous species collected in Australia and to compare the pathogenicity of these species with that of *E. lata*.

Materials and Methods

Fungal strains. Seventy fungal strains representing nine species in six genera within the family Diatrypaceae (Table 1), collected and identified during prior surveys of viticultural regions throughout NSW, SA, and Western Australia (WA) (19,31) and stored at 4°C on agar slants, were transferred to 8.5-cm-diameter petri dishes containing potato dextrose agar (PDA; Oxoid Ltd.) amended with streptomycin sulfate (PDA-strep; Sigma-Aldrich) at 100 mg/liter. Fungal strains were cultured for 7 days at 23°C under fluorescent light (Phillips TLD 36W/865 cool daylight) with a 12-h photoperiod, and selections thereof were used in two pathogenicity trials.

Pathogenicity studies. In trial I, conducted at the South Australian Research and Development Institute (SARDI), Adelaide, SA,

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Table 1. Origin and source of diatrypaceous fungi used in pathogenicity studies

Species	Strain	Host	Origin ²	Collector
<i>Cryptosphaeria</i> sp.	NSW01PO	<i>Populus alba</i>	Adelong, NSW	F. P. Trouillas
<i>Cryptosphaeria</i> sp.	NSW03PO	<i>P. balsamifera</i>	Khancoban, NSW	F. P. Trouillas
<i>Cryptosphaeria</i> sp.	NSW05PO	<i>P. balsamifera</i>	Khancoban, NSW	F. P. Trouillas
<i>Cryptovalsa ampelina</i>	AD100	<i>Vitis vinifera</i>	Adelaide, SA	F. P. Trouillas
<i>C. ampelina</i>	AD101	<i>V. vinifera</i>	Adelaide, SA	F. P. Trouillas
<i>C. ampelina</i>	ADSC200	<i>Schinus molle</i> var. <i>areira</i>	Adelaide, SA	F. P. Trouillas
<i>C. ampelina</i>	ADSC201	<i>S. molle</i> var. <i>areira</i>	Adelaide, SA	F. P. Trouillas
<i>C. ampelina</i>	AH01	<i>Acer macrophyllum</i>	Adelaide Hills, SA	F. P. Trouillas
<i>C. ampelina</i>	B10-15A	<i>V. vinifera</i>	Barossa Valley, SA	M. R. Sosnowski, A. Loschiavo
<i>C. ampelina</i>	B5-17A	<i>V. vinifera</i>	Barossa Valley, SA	M. R. Sosnowski, A. Loschiavo
<i>C. ampelina</i>	DO4	<i>V. vinifera</i>	Murrumbateman, NSW	W. M. Pitt, S. Hackett
<i>C. ampelina</i>	DO6	<i>V. vinifera</i>	Murrumbateman, NSW	W. M. Pitt, S. Hackett
<i>C. ampelina</i>	HVVIT04	<i>V. vinifera</i>	Hunter Valley, NSW	F. P. Trouillas, W. M. Pitt
<i>C. ampelina</i>	KC6	<i>V. vinifera</i>	Book Book, NSW	W. M. Pitt, S. Hackett
<i>C. ampelina</i>	RGA05	<i>Fraxinus angustifolia</i>	Adelaide Hills, SA	F. P. Trouillas
<i>C. ampelina</i>	SAPN03	<i>P. nigra italic</i>	McLaren Flat, SA	F. P. Trouillas
<i>C. ampelina</i>	TUUP01	<i>Ulmus procera</i>	Tumbarumba, NSW	F. P. Trouillas, W. M. Pitt
<i>C. rabenhorstii</i>	WA07CO	<i>V. vinifera</i>	Cowaramup, WA	F. P. Trouillas
<i>C. rabenhorstii</i>	WA08CB	<i>V. vinifera</i>	Cowaramup, WA	F. P. Trouillas
<i>Diatrype</i> sp.	ADEL400	<i>U. procera</i>	Adelaide, SA	F. P. Trouillas
<i>Diatrype</i> sp.	ADEL400A	<i>U. procera</i>	Adelaide, SA	F. P. Trouillas
<i>Diatrype</i> sp.	C001	<i>Acacia longifolia sophorae</i>	Coorong, SA	F. P. Trouillas
<i>Diatrype</i> sp.	C002	<i>A. longifolia sophorae</i>	Coorong, SA	F. P. Trouillas
<i>Diatrype</i> sp.	CG14	<i>V. vinifera</i>	Tumbarumba, NSW	F. P. Trouillas, W. M. Pitt
<i>Diatrypella vulgaris</i>	CG7	<i>V. vinifera</i>	Tumbarumba, NSW	F. P. Trouillas, W. M. Pitt
<i>D. vulgaris</i>	CG8	<i>V. vinifera</i>	Tumbarumba, NSW	F. P. Trouillas, W. M. Pitt
<i>D. vulgaris</i>	HVFRA02	<i>F. angustifolia</i>	Hunter Valley, NSW	F. P. Trouillas, W. M. Pitt
<i>D. vulgaris</i>	HVFRA04	<i>F. angustifolia</i>	Hunter Valley, NSW	F. P. Trouillas, W. M. Pitt
<i>D. vulgaris</i>	HVGRF03	<i>Citrus paradisi</i>	Hunter Valley, NSW	F. P. Trouillas, W. M. Pitt
<i>D. vulgaris</i>	HVPT01	<i>S. molle</i> var. <i>areira</i>	Hunter Valley, NSW	F. P. Trouillas, W. M. Pitt
<i>Eutypa lata</i>	ADSC300	<i>S. molle</i> var. <i>areira</i>	Adelaide, SA	F. P. Trouillas
<i>E. lata</i>	ADSC400	<i>S. molle</i> var. <i>areira</i>	Adelaide, SA	F. P. Trouillas
<i>E. lata</i>	B003	<i>V. vinifera</i>	Barossa Valley, SA	M. L. Creaser
<i>E. lata</i>	DO5	<i>V. vinifera</i>	Murrumbateman, NSW	W. M. Pitt, S. Hackett
<i>E. lata</i>	OR039	<i>V. vinifera</i>	Coonawarra, SA	M. R. Sosnowski
<i>E. lata</i>	RGA01	<i>F. angustifolia</i>	Adelaide Hills, SA	F. P. Trouillas
<i>E. lata</i>	SACEA01	<i>Ceanothus</i> sp.	Adelaide, SA	F. P. Trouillas
<i>E. lata</i>	SAPN01	<i>P. nigra italic</i>	McLaren Flat, SA	F. P. Trouillas
<i>E. lata</i>	VT14	<i>V. vinifera</i>	Canowindra, NSW	W. M. Pitt
<i>E. lata</i>	WB052	<i>V. vinifera</i>	Eden Valley, SA	M. R. Sosnowski
<i>E. leptoplaca</i>	ABA200	<i>F. angustifolia</i>	Barossa Valley, SA	F. P. Trouillas
<i>E. leptoplaca</i>	ABA300	<i>F. angustifolia</i>	Barossa Valley, SA	F. P. Trouillas
<i>E. leptoplaca</i>	ADFIC100	<i>Ficus macrophyllum</i>	Adelaide, SA	F. P. Trouillas
<i>E. leptoplaca</i>	ADFIC101	<i>F. macrophyllum</i>	Adelaide, SA	F. P. Trouillas
<i>E. leptoplaca</i>	ADSC500	<i>S. molle</i> var. <i>areira</i>	Adelaide, SA	F. P. Trouillas
<i>E. leptoplaca</i>	RGA02	<i>F. angustifolia</i>	Adelaide Hills, SA	F. P. Trouillas
<i>E. leptoplaca</i>	RGA04	<i>F. angustifolia</i>	Adelaide Hills, SA	F. P. Trouillas
<i>E. leptoplaca</i>	SACOO2	<i>P. nigra italic</i>	Coonawarra, SA	F. P. Trouillas
<i>E. leptoplaca</i>	SAPA01A	<i>P. alba</i>	Adelaide, SA	F. P. Trouillas
<i>E. leptoplaca</i>	SAPN04	<i>P. nigra italic</i>	McLaren Flat, SA	F. P. Trouillas
<i>E. leptoplaca</i>	TUPN02	<i>P. nigra italic</i>	Tumbarumba, NSW	F. P. Trouillas, W. M. Pitt
<i>E. leptoplaca</i>	TUQU01	<i>Quercus palustris</i>	Tumbarumba, NSW	F. P. Trouillas, W. M. Pitt
<i>Eutypella citricola</i>	ADEL100	<i>U. procera</i>	Adelaide, SA	F. P. Trouillas
<i>E. citricola</i>	ADEL101	<i>U. procera</i>	Adelaide, SA	F. P. Trouillas
<i>E. citricola</i>	ADSC100	<i>S. molle</i> var. <i>areira</i>	Adelaide, SA	F. P. Trouillas
<i>E. citricola</i>	ADSC100A	<i>S. molle</i> var. <i>areira</i>	Adelaide, SA	F. P. Trouillas
<i>E. citricola</i>	HVGRF01	<i>C. paradisi</i>	Hunter Valley, NSW	F. P. Trouillas, W. M. Pitt
<i>E. citricola</i>	HVOT01	<i>C. sinensis</i>	Hunter Valley, NSW	F. P. Trouillas, W. M. Pitt
<i>E. citricola</i>	HVVIT03	<i>V. vinifera</i>	Hunter Valley, NSW	F. P. Trouillas, W. M. Pitt
<i>E. citricola</i>	HVVIT07	<i>V. vinifera</i>	Hunter Valley, NSW	F. P. Trouillas, W. M. Pitt
<i>E. citricola</i>	WA01SV	<i>V. vinifera</i>	Swan Valley, WA	F. P. Trouillas
<i>E. citricola</i>	WA02BO	<i>V. vinifera</i>	Mount Barker, WA	F. P. Trouillas
<i>E. microtheca</i>	ADEL200	<i>U. procera</i>	Adelaide, SA	F. P. Trouillas
<i>E. microtheca</i>	ADEL300	<i>U. procera</i>	Adelaide, SA	F. P. Trouillas
<i>E. microtheca</i>	HVGRF02	<i>C. paradisi</i>	Hunter Valley, NSW	F. P. Trouillas, W. M. Pitt
<i>E. microtheca</i>	HVVIT05	<i>V. vinifera</i>	Hunter Valley, NSW	F. P. Trouillas, W. M. Pitt
<i>E. microtheca</i>	T20R4S2	<i>V. vinifera</i>	Hunter Valley, NSW	W. M. Pitt
<i>E. microtheca</i>	YC16	<i>V. vinifera</i>	Hunter Valley, NSW	W. M. Pitt
<i>E. microtheca</i>	YC17	<i>V. vinifera</i>	Hunter Valley, NSW	W. M. Pitt
<i>E. microtheca</i>	YC18	<i>V. vinifera</i>	Hunter Valley, NSW	W. M. Pitt

² NSW, SA, and WA = Australian states of New South Wales, South Australia, and Western Australia, respectively.

Australia, 1-year-old 'Cabernet Sauvignon' (clone SAVII01) rooted cuttings (rootlings) obtained from Vinewright were planted in plastic bags (120 × 120 × 300 mm; Poly Products Co. Pty. Ltd.) filled with pasteurized potting mix comprising four parts hydrated coco peat and one part sharp river sand (SARDI), and transferred to a shade house, where they were hand watered as required. In February 2009, a single wound penetrating as far as the pith was made in the trunks of healthy grapevine rootlings using a sterile 5-mm-diameter drill bit. Each rootling was then inoculated with a 7-day-old fungal culture on PDA-strep. Agar plugs (5 mm in diameter) were removed from the margins of actively growing cultures using a sterile cork borer and inserted into each wound, which was then sealed with petroleum jelly and wrapped with Parafilm M laboratory film (American National Can). Sterile noncolonized plugs of PDA-strep were used as controls.

In December 2009, 9 months after inoculation, grapevines were removed from the bags, the roots were excised, and soil and debris were removed by rinsing thoroughly with tap water. Using a knife, bark was removed from each rootling to expose any staining or discoloration of the wood (lesions), and the total length of the lesion caused by each fungal strain was assessed by cumulating the distance of staining above (acropetal) and below (basipetal) the point of inoculation. To determine the extent of colonization, each rootling was then surface disinfested in 2.5% NaOCl for 10 min and rinsed in sterile distilled water. Wood pieces (approximately 2 mm thick) were excised with sterile secateurs at 10-mm intervals

up to 100 mm above and below the inoculation point and transferred to PDA-strep. Following 7 to 10 days of incubation at 23°C under fluorescent light as described above, diatrypaceous species were identified using descriptions of colony morphology (29,31,32). The furthest distance at which fungi were reisolated, both acropetal and basipetal to the point of inoculation, was then recorded and cumulated to determine total colonization distances.

In trial II, conducted at the National Wine and Grape Industry Centre (NWGIC), Wagga Wagga, NSW, Australia, 1-year-old 'Cabernet Sauvignon' (clone SAVII01) rootlings obtained from Vinewright were placed in 330-by-265-mm plastic pots (Garden City Plastics) containing 15 liters of pasteurized premium potting mix comprising three parts bark fines, four parts hardwood sawdust, two parts blue metal grit, and one part mushroom compost (Loam and Stone Landscape Supplies). Pots were placed in a temperature-controlled glasshouse with an overnight low of 20°C, a daily maximum of 32°C, and relative humidity of 40%. Pots were watered to run-off once daily. In June 2010, rootlings were inoculated with diatrypaceous fungi as described above and, 18 months later, in November 2011, removed from the soil, and the total lesion length and degree of colonization of the wood assessed for each fungal strain, as described previously.

Data analysis. Trials I and II were conducted using a completely randomized design with 36 and 42 treatments (fungal strains), respectively, including controls. In trial I, three vines per fungal strain were inoculated whereas, in trial II, four vines were inoculated

Table 2. Mean lesion length and colonization distance of diatrypaceous fungi 9 months after inoculation of potted 'Cabernet Sauvignon' grapevines located at the South Australian Research and Development Institute (SARDI), Adelaide, SA, Australia between February and December 2009

Species	Strain	Mean lesion length (mm) ^x	Mean colonization distance (mm) ^y	Asymptomatic colonization (mm) ^z
<i>Eutypa lata</i>	SACEA01	(28.80–) 43.99 (–60.38) a	(23.98–) 38.32 (–61.23) b–g	–5.66
	WB052	(19.53–) 24.77 (–30.88) ab	(42.63–) 61.68 (–89.25) ab	36.91
	ADSC400	(13.73–) 23.47 (–37.14) ab	(34.52–) 51.68 (–77.37) b–e	28.20
	ADSC300	(9.22–) 22.50 (–45.36) ab	(42.63–) 61.68 (–89.25) ab	39.18
	B003	(14.49–) 22.32 (–32.76) ab	(31.84–) 48.33 (–73.35) b–e	26.00
	RGA01	(15.11–) 22.12 (–31.20) ab	(37.18–) 54.98 (–81.31) a–d	32.86
	OR039	(13.48–) 21.26 (–31.86) ab	(30.52–) 46.67 (–71.36) b–e	25.41
	SAPN01	(13.66–) 17.76 (–22.78) ab	(41.24–) 59.98 (–87.23) a–c	42.22
	ADSC100	(6.80–) 27.98 (–67.42) ab	(27.89–) 43.34 (–67.34) b–f	15.36
	ADSC100A	(21.11–) 26.77 (–33.30) ab	(10.96–) 22.99 (–48.23) d–i	–3.78
<i>Eutypella citricola</i>	ADEL101	(11.15–) 22.61 (–40.47) ab	(42.63–) 61.68 (–89.25) ab	39.07
	ADEL100	(13.11–) 20.04 (–29.39) ab	(26.59–) 41.68 (–65.33) b–g	21.64
	ADEL200	(20.94–) 29.76 (–40.40) ab	(20.99–) 37.49 (–66.96) b–h	7.73
	ADEL300	(12.66–) 17.80 (–24.45) ab	(43.97–) 63.31 (–91.15) ab	45.50
<i>Cryptovalsa ampelina</i>	AD100	(31.84–) 34.64 (–37.55) a	(14.00–) 25.00 (–44.66) e–i	–9.63
	AH01	(24.91–) 32.70 (–41.49) a	(20.18–) 33.35 (–55.12) b–i	0.65
	B10-15A	(17.57–) 24.26 (–32.49) ab	(25.54–) 40.33 (–63.68) b–g	16.06
	AD101	(17.70–) 22.45 (–28.03) ab	(24.25–) 38.67 (–61.65) b–g	16.22
	ADSC200	(6.14–) 22.12 (–55.21) ab	(21.43–) 34.99 (–57.13) b–h	12.87
	SAPN03	(15.93–) 21.35 (–27.98) ab	(5.19–) 12.00 (–27.73) i	–9.35
	B5-17A	(4.88–) 16.40 (–42.87) ab	(23.98–) 38.32 (–61.23) b–g	21.92
	ADSC201	(8.82–) 13.49 (–20.08) ab	(66.30–) 90.02 (–122.21) a	76.53
	RGA05	(3.30–) 12.68 (–38.21) ab	(4.85–) 13.00 (–34.83) g–i	0.32
	ADEL400	(11.19–) 25.34 (–47.76) ab	(6.24–) 13.67 (–29.96) hi	–11.67
<i>Diatrype</i> sp.	ADEL400A	(3.83–) 11.23 (–28.69) ab	(21.28–) 45.02 (–95.21) a–g	33.79
	SAPN04	(12.23–) 32.20 (–61.82) a	(16.43–) 28.33 (–48.87) d–i	–3.87
<i>Eutypa leptoplaca</i>	ADFIC100	(18.16–) 25.24 (–33.95) ab	(17.91–) 30.33 (–51.36) c–i	5.08
	RGA02	(15.05–) 21.53 (–29.81) ab	(20.18–) 33.35 (–55.12) b–i	11.82
	ABA200	(8.09–) 18.56 (–37.11) ab	(37.18–) 54.98 (–81.31) a–d	36.42
	SAC002	(9.51–) 15.17 (–23.33) ab	(13.96–) 27.49 (–54.14) b–i	12.32
	ABA300	(7.75–) 11.60 (–17.00) ab	(13.96–) 27.49 (–54.14) b–i	15.90
	RGA04	(4.57–) 11.54 (–26.22) ab	(27.89–) 43.34 (–67.34) b–f	31.80
	ADFIC101	(9.68–) 11.27 (–13.09) ab	(15.20–) 26.66 (–46.75) d–i	15.38
	ADSC500	(6.07–) 10.59 (–17.82) ab	(33.17–) 50.00 (–75.36) b–e	39.41
	SAPA01A	(6.97–) 8.58 (–10.53) ab	(9.31–) 18.34 (–36.11) f–i	9.76
	Co	(4.07–) 6.27 (–9.54) b	0.00 j	–6.27

^x Mean total lesion lengths, with upper and lower 95% confidence intervals in parenthesis. All values are back-transformed to the original scale (millimeters). Means in the same column followed by the same letters are not significantly different according to Tukey's test ($P = 0.05$).

^y Mean total colonization distances, with upper and lower 95% confidence intervals in parenthesis. All values are predictions based on generalized linear regression using a Poisson distribution and log link function, and are back-transformed to the original scale (millimeters). Means in the same column followed by the same letters are not significantly different according to least significant difference ($P = 0.05$).

^z Distance by which wood colonization exceeded lesion length (millimeters); based on subtraction of mean total lesion length from mean total colonization distance.

per strain. All statistical analyses were performed using GenStat (version 14; VSN International Ltd.). In both trials, data for lesion length were skewed, and a logit transformation was applied to normalize data and eliminate heteroscedasticity. One-way analysis of variance (ANOVA) was used to compare differences in mean lesion lengths among fungal strains and species, with means separated using Tukey's test ($P = 0.05$). For wood colonization, a suitable transformation to normalize data could not be found. Instead, data were subject to generalized linear regression using a Poisson distribution and log-link function. ANOVA was used to compare differences in the distance of wood colonization among strains and species, with means separated by least significant difference ($P = 0.05$). For both data sets, means were back-transformed to the original scale (millimeters) for presentation (Tables 2 and 3; Figs. 1 and 2). Student's t test ($P = 0.05$) was used to test for differences between the total lesion length and colonization distance within each species.

Results

Inoculations of 1-year-old 'Cabernet Sauvignon' grapevines with diatrypaceous fungi seldom resulted in foliar symptoms, with only

three vines, all inoculated with *E. lata*, displaying any foliar symptoms characteristic of Eutypa dieback. However, at sites of infection, inoculations were accompanied by vascular discoloration of grapevine wood, hereafter referred to as "lesions", after a 9-month incubation period. Mean lesion lengths were 9 to 44 mm, with significant differences among strains ($P < 0.001$). However, these differences were between treatments and the control, with individual strains behaving similarly, both within and between species ($P = 0.05$; Table 2). When data were pooled, there were significant differences among the species ($P < 0.001$), with mean lesion lengths ranging from 16 mm for *E. leptoplaca* to 24 mm for *E. lata*. However, differences were once again between treatments and the control ($P = 0.05$), not between the treatments themselves, and all six species in the trial were found to produce lesions significantly longer than the controls.

Diatrypaceous fungi also colonized grapevines, with all strains being reisolated and identified from inoculated wood. Mean colonization distances were 12 to 90 mm. There were significant differences among strains ($P < 0.001$) both within and between species ($P = 0.05$), despite the fact that 25 of the 35 strains behaved

Table 3. Mean lesion length and colonization distance of diatrypaceous fungi 18 months after inoculation of potted 'Cabernet Sauvignon' grapevines located at the National Wine and Grape Industry Centre (NWGIC), Wagga Wagga, NSW, Australia between June 2010 and November 2011

Species	Strain	Mean lesion length (mm) ^x	Mean colonization distance (mm) ^y	Asymptomatic colonization (mm) ^z
<i>Eutypa lata</i>	OR039	(51.81–) 54.67 (–57.80) a	(49.29–) 63.31 (–81.31) a	8.63
	WB052	(30.09–) 44.41 (–59.71) ab	(45.82–) 57.51 (–72.19) ab	13.11
	VT14	(29.35–) 35.53 (–42.24) a–c	(34.87–) 46.67 (–62.46) a–d	11.13
	B003	(26.53–) 34.38 (–43.18) a–c	(39.18–) 50.00 (–63.80) a–c	15.62
	DO5	(17.98–) 20.41 (–23.07) b–f	(26.14–) 34.99 (–46.83) c–g	14.58
<i>Cryptovalsa ampelina</i>	HVVIT04	(25.44–) 32.88 (–41.29) a–d	(28.29–) 37.49 (–49.68) c–f	4.61
	KC6	(17.77–) 32.24 (–51.17) a–d	(37.00–) 47.51 (–61.01) a–d	15.27
	DO4	(22.92–) 26.84 (–31.17) a–e	(34.81–) 45.02 (–58.22) a–e	18.17
	TUUP01	(12.08–) 24.28 (–42.82) b–e	(24.02–) 32.49 (–43.96) d–h	8.21
	DO6	(9.34–) 16.01 (–26.06) c–g	(24.01–) 32.49 (–43.97) d–h	16.49
<i>Eutypa leptoplaca</i>	ADFIC100	(15.81–) 28.83 (–46.63) a–e	(26.39–) 36.67 (–50.95) c–g	7.84
	TUPN02	(17.22–) 26.68 (–38.89) a–e	(32.64–) 42.52 (–55.40) b–e	15.84
	RGA02	(20.97–) 23.42 (–26.06) b–e	(26.14–) 34.99 (–46.83) c–g	11.56
	SAPN04	(13.10–) 17.14 (–22.11) c–f	(28.29–) 37.49 (–49.68) c–f	20.35
	TUQU01	(10.89–) 13.55 (–16.73) c–g	(11.60–) 17.50 (–26.40) i–k	3.95
<i>Diatrype</i> sp.	C002	(13.87–) 23.35 (–36.55) b–e	(13.64–) 20.01 (–29.35) h–k	–3.34
	C001	(15.31–) 21.18 (–28.53) b–f	(13.61–) 20.01 (–29.41) h–k	–1.17
<i>Eutypella microtheca</i>	CG14	(10.28–) 17.65 (–28.60) c–f	(9.61–) 15.00 (–23.40) jk	–2.56
	HVVIT05	(20.75–) 22.70 (–24.78) b–e	(17.71–) 25.00 (–35.30) f–j	2.30
	YC16	(14.15–) 22.58 (–34.03) b–e	(21.91–) 29.99 (–41.07) e–h	7.41
	YC18	(18.32–) 21.59 (–25.26) b–e	(19.79–) 27.49 (–38.20) f–i	5.90
	YC17	(17.99–) 20.41 (–23.07) b–f	(24.01–) 32.49 (–43.97) d–h	12.08
<i>Diatrypella vulgaris</i>	T20R4S2	(14.71–) 17.87 (–21.53) c–f	(12.83–) 20.01 (–31.20) h–k	2.14
	HVGRF02	(9.32–) 13.65 (–19.57) c–g	(11.60–) 17.50 (–26.40) i–k	3.85
	HVGRF03	(27.31–) 30.43 (–33.74) a–e	(26.14–) 34.99 (–46.83) c–g	4.56
	CG7	(16.28–) 20.90 (–26.42) b–f	(15.66–) 22.51 (–32.37) g–k	1.61
	HVPT01	(15.25–) 20.74 (–27.55) b–f	(15.66–) 22.51 (–32.37) g–k	1.78
<i>Cryptovalsa rabenhorstii</i>	CG8	(12.44–) 17.84 (–24.93) c–f	(19.79–) 27.49 (–38.19) f–i	9.65
	HVFRA02	(9.58–) 14.67 (–21.81) c–g	(11.60–) 17.50 (–26.40) i–k	2.83
	HVFRA04	(10.89–) 12.43 (–14.15) d–g	(15.65–) 22.51 (–32.38) g–k	10.08
	WA07CO	(11.85–) 20.90 (–34.20) b–f	(11.60–) 17.50 (–26.40) i–k	–3.41
	WA08BO	(11.37–) 14.90 (–19.30) c–g	(9.61–) 15.00 (–23.40) jk	0.10
<i>Eutypella citricola</i>	WA01SV	(20.72–) 24.34 (–28.37) b–e	(19.79–) 27.49 (–38.20) f–i	3.16
	HVVIT07	(14.81–) 19.15 (–24.40) b–f	(19.79–) 27.49 (–38.20) f–i	8.35
	WA02BO	(14.12–) 19.03 (–25.15) b–f	(11.60–) 17.50 (–26.40) i–k	–1.53
	HVGRF01	(7.76–) 15.61 (–28.90) c–g	(21.90–) 29.99 (–41.07) e–h	14.38
	HVVIT03	(9.02–) 14.37 (–22.13) c–g	(11.59–) 17.50 (–26.42) i–k	3.12
<i>Cryptosphaeria</i> sp.	HVOT01	(8.40–) 11.39 (–15.28) e–g	(9.61–) 15.00 (–23.40) jk	3.61
	NSW01PO	(11.90–) 14.57 (–17.71) c–g	(9.61–) 15.00 (–23.40) jk	0.43
	NSW05PO	(8.64–) 13.82 (–21.37) c–g	(13.62–) 20.01 (–29.38) h–k	6.19
	NSW03PO	(2.98–) 4.98 (–8.21) g	(7.68–) 12.50 (–20.36) k	7.52
Control	Co	(6.74–) 8.57 (–10.83) fg	0.001	–8.57

^x Mean total lesion lengths, with upper and lower 95% confidence intervals in parenthesis. All values are back-transformed to the original scale (millimeters). Means in the same column followed by the same letters are not significantly different according to Tukey's test ($P = 0.05$).

^y Mean total colonization distances, with upper and lower 95% confidence intervals in parenthesis. All values are predictions based on generalized linear regression using a Poisson distribution and log link function, and are back-transformed to the original scale (millimeters). Means in the same column followed by the same letters are not significantly different according to least significant difference ($P = 0.05$).

^z Distance by which wood colonization exceeded lesion length (millimeters); based on subtraction of mean total lesion length from mean total colonization distance.

similarly (Table 2). When data were pooled, mean colonization distances ranged from 22 mm for a *Diatrype* sp. to 53 mm for *Eutypella microtheca* Trouillas, W. M. Pitt & Gubler (Fig. 1), with significant differences ($P = 0.007$) in the response of the various species. Whereas *E. lata* colonized wood to the greatest extent, four of the six species behaved similarly, with differences being largely between treatments and the control.

Diatrypaceous fungi were reisolated from asymptomatic wood at varying distances from the ends of lesions such that, for 29 of the 35 strains, the mean colonization distance exceeded that of lesion length (Table 2). When the means for lesion length were subtracted from those of colonization distance, 11 strains had cumulated colonization distances that exceeded lesion length by more than 30 mm (Table 2). However, the furthest distance at which any fungus was isolated in any direction in advance of staining was 70 mm. Pooled results showed that *E. lata* and *E. microtheca* colonized wood by 29 mm in excess of lesion length whereas, for *E. leptoplaca*, *Eutypella citricola* Speg., and *C. ampelina*, colonization exceeded lesion length by more than 15 mm (Fig. 1). Although differences were not significant for most of the species tested, Student's *t* tests ($P = 0.05$) conducted between the means of lesion length and colonization distance were significant for *E. lata* ($t = 2.44$; t -critical = 1.761, $df = 14$), such that the extent of colonization by the fungus was significantly greater than the lesions it caused.

In trial II, grapevine inoculations performed identically to those in trial I but with a different array of diatrypaceous fungi produced similar results, with lesions of 5 to 55 mm in length, subsequent to an 18-month incubation period. Significant differences among strains ($P < 0.001$), were evident within and among species, as well as between treatments and the control ($P = 0.05$). However, for the most part, these differences were minor, with 36 of the 41 strains producing lesions of similar length (Table 3). When data were pooled, mean lesion lengths ranged from 10 mm for a *Cryptosphaeria* sp. to 36 mm for *E. lata*, and significant differences were evident among species ($P < 0.001$). However, whereas lesions produced by *E. lata* were significantly longer than many of the other species ($P = 0.05$), apart from a *Cryptosphaeria* sp., all bore lesions significantly longer than the control (Fig. 2).

Diatrypaceous fungi were once again reisolated from inoculated grapevine rootlings, with mean colonization distances of 13 to 63 mm. There were significant differences among strains ($P < 0.001$) that were evident both within and between species ($P = 0.05$; Table 3). Whereas individual strains demonstrated greater variability than those observed in trial I, 18 of 41 colonized grapevines to a similar extent, and within species, differences were minimal; such that, for eight of the nine species, all or all but one of the strains therein behaved similarly. When data were pooled, mean colonization distances ranged from 16 mm for a *Cryptosphaeria* sp. to 50 mm for *E. lata* (Fig. 2), with significant differences among species ($P < 0.001$) and in comparison with the control ($P = 0.05$). *E. lata* colonized wood to a significantly greater extent than the other species, followed thereafter by *C. ampelina* and *E. leptoplaca* and, subsequently, *E. microtheca*, *E. citricola*, and *Diatrypella vulgaris* Trouillas, W.M. Pitt & Gubler, which behaved similarly. *Cryptovalsa rabenhorstii* (Nitschke) Sacc. and a *Cryptosphaeria* sp. colonized wood the least.

As in trial I, the vast majority, 35 of 41 strains, colonized wood beyond the visible lesion margin (Table 3). However, whereas six strains were found to colonize wood by more than 15 mm (cumulated) in excess of lesion length (Table 3), for most, the difference between lesion length and colonization distance was not as great. Nevertheless, when data were pooled, *E. lata*, *C. ampelina*, and *E. leptoplaca* all colonized wood by more than 12 mm in excess of lesion length (Fig. 2), although differences were not significant for any of the species tested ($P = 0.05$). The furthest distance at which any fungus was isolated in any direction in advance of staining was 21 mm.

Discussion

With the exception of a *Cryptosphaeria* sp., eight species, including *E. lata*, *E. leptoplaca*, *C. ampelina*, *C. rabenhorstii*, *E. citricola*, *E. microtheca*, *D. vulgaris*, and a *Diatrype* sp. produced lesions that were significantly longer than on controls. However, all nine species successfully colonized grapevines and were reisolated from developing lesions at varying distances above and below the point of inoculation, such that, despite producing only small lesions, all, including a *Cryptosphaeria* sp., acted as

Fig. 1. Mean total lesion length (gray bar) and colonization distance (white bar) of diatrypaceous fungi 9 months after inoculation of potted 'Cabernet Sauvignon' grapevines located at the South Australian Research and Development Institute (SARDI), Adelaide, SA, Australia between February and December 2009. All values are back-transformed to the original scale (millimeters). Mean lesion lengths followed by the same lowercase letters are not significantly different according to Tukey's test ($P = 0.05$). Mean colonization distances followed by the same uppercase letters are not significantly different based on least significant difference ($P = 0.05$). Bars represent the 95% confidence interval of the mean.

pathogens when inoculated into wounded trunks of 1-year-old 'Cabernet Sauvignon' grapevines.

Whereas inoculations with three strains of *E. lata* were accompanied by foliar symptoms characteristic of Eutypa dieback, only three vines, one per strain, were affected, and no symptoms whatsoever were observed subsequent to inoculations with other diatrypaceous species. That only some strains of *E. lata* caused symptoms and that symptoms were not evident in all vines challenged with these strains was not unexpected, given the variation reported among strains (3,29). However, the lack of foliar symptoms when challenged with other species was fitting, given that, with the exception *E. vitis*, which was reported to cause leaf cupping and chlorosis on 'Concord' grapevines (*Vitis labrusca* L.) (13), no other species in this family have been reported to produce foliar symptoms.

The pathogenicity of *E. lata* on grapevines is well documented (17,24,30), and results of this study have further confirmed the role of *E. lata* as a significant pathogen of grapevines, with this species being the most pathogenic based on lesion length and colonization distance. Although some of the other species produced lesions of a length similar to those of *E. lata*, in both trials, *E. lata* colonized grapevines by the greatest distance. Similarly, Trouillas and Gubler (28) demonstrated the pathogenicity of *E. leptoplaca* to grapevine, and showed that strains of this fungus produced lesions and colonized grapevines much the same as *E. lata* (30). Our results similarly confirm the status of *E. leptoplaca* as a grapevine pathogen, with many of the strains of *E. leptoplaca* used in this study also found to produce lesions and colonize grapevines to an extent similar to *E. lata*.

Eutypella citricola and *E. microtheca* were also pathogenic on grapevines according to this study. The pathogenicity of these species was equivalent to that of *E. lata* in trial I and only slightly less so in trial II. Historically, *Eutypella* spp. are best known as pathogens of maple (*Acer saccharum* Marsh., *A. rubrum* L.) and sycamore (*A. pseudoplatanus* L.) (6,8), although both *Eutypella leprosa* (Pers.) Berl. and *E. vitis* have been isolated from grapevine cankers in the Americas (7,13,35). However, apart from these

records, few definitively link *Eutypella* spp. to grapevine decline, even despite recent studies in both the United States and Australia that saw the isolation of several new species in this genus (31–33). These included *Eutypella australiensis* Trouillas, Sosnowski & Gubler; *Eutypella cryptovalsoidea* Trouillas, W. M. Pitt & Gubler; and four other species that remain unnamed and for which a role in grapevine decline is uncertain. Regardless, this is the first report of the pathogenicity of *E. citricola* and *E. microtheca* to grapevines.

Similarly, this is also the first demonstration of the pathogenicity of *D. vulgaris* and a *Diatrype* sp. on grapevines, two species recently isolated for the first time, the former from dead branches of grapefruit (*Citrus × paradisi* Macfad.), ash (*Fraxinus angustifolia* Vahl), and peppercorn (*Schinus molle* var. *areira* L.) in the Hunter Valley; and the latter from grapevine in Tumbarumba, NSW, and coastal wattle (*Acacia longifolia* subsp. *sophorae* (Labill.) Court) and elm (*Ulmus procera* Salisb.) in SA (31). As with *Eutypella* spp., few studies have reported the occurrence of species in these genera on grapevines. Úrbez-Torres et al. (35) confirmed pathogenicity to grapevines of an unnamed *Diatrypella* sp. in Texas, while Trouillas et al. (33) isolated *Diatrype oregonensis* (Wehm.) Rappaz., *Diatrype stigma* (Hoffm.) Fr., *Diatrype whitmanensis* J. D. Rogers & Glawe, and *Diatrypella verruciformis* (Ehrh.) Nitschke from grapevines in California and, in subsequent work, demonstrated the pathogenicity thereon of *D. stigma* and *D. whitmanensis* (30). Instead, the majority of records occur on tree species, namely alder (*Alnus* Mill.), beech (*Fagus* L.), birch (*Betula* L.), maple (*Acer* L.), and oak (*Quercus* L.) (37,38). However, in this study, both *D. vulgaris* and a *Diatrype* sp. were pathogenic to grapevines, causing lesions of a length similar to *C. ampelina*, *E. leptoplaca*, *E. citricola*, and *E. microtheca*.

Although *C. ampelina* is widespread throughout Australia (18,19), it did "not appear to be a major pathogen of grapevines" according to Luque et al. (15), and was thought to be a "weak pathogen" by Mostert et al. (18). In this study *C. ampelina* produced lesions of a length similar to *E. lata* and colonized grapevine almost to the same extent. Although differences in pathogenicity among individual strains may have contributed to this in-

Fig. 2. Mean total lesion length (gray bar) and colonization distance (white bar) of diatrypaceous fungi 18 months after inoculation of potted 'Cabernet Sauvignon' grapevines located at the National Wine and Grape Industry Centre (NWGIC), Wagga Wagga, NSW, Australia between June 2010 and November 2011. All values are back-transformed to the original scale (millimeters). Mean lesion lengths followed by the same lowercase letters are not significantly different according to Tukey's test ($P = 0.05$). Mean colonization distances followed by the same uppercase letters are not significantly different based on least significant difference ($P = 0.05$). Bars represent the 95% confidence interval of the mean.

congruence, *C. ampelina* appeared to be a more significant pathogen of grapevines than previously reported. Apart from *C. ampelina*, members of the genus are seldom found on grapevines and, for the most part, have been associated with maple (*Acer* L.), holly (*Ilex* L.), and sycamore (*Platanus* L.) in the United States (38); seepweed (*Suaeda fruticosa* (L.) Forsk., syn. *S. nigra* (Raf.) J.F. Macbr.) in the United Kingdom (26); and succulents (*Halosarceia halocnemoides* (Nees) Paul G. Wilson, syn. *Tecticornia halocnemoides* (Nees) K.A. Sheph. & Paul G. Wilson) and mangrove (*Avicennia* L.) in the Asia-Pacific region (11,12). Recent studies by Trouillas et al. (31) uncovered a second species from grapevines in the Cowaramup region of WA, which resembled closely descriptions and illustrations of *C. rabenhorstii*. Like *Eutypella*, *Diatrypella*, and *Diatrype* spp. described previously, *C. rabenhorstii* caused lesions of an equivalent length to *C. ampelina* but did not colonize grapevines to the same extent.

Unlike *Cryptovalsa*, *Diatrypella*, *Diatrype*, *Eutypa*, and *Eutypella* spp., lesions caused by *Cryptosphaeria* spp. isolated from cottonwood (*Populus balsamifera* L.) near Khancoban, NSW, and closely resembling *Cryptosphaeria lignyota* (Fr.) Auersw. (= *C. populina* (Pers.) Sacc.) (31), were not significantly longer than those on controls. This was not surprising given that no records of this species from grapevines are available in the literature and that, historically, *C. lignyota* is best known as the causal agent of *Cryptosphaeria* canker, a major disease of trembling aspen (*P. tremuloides* Michx.) in the western United States (10). However, *C. pullmanensis* Glawe was recently isolated from and shown to be pathogenic to grapevines in California (30,33), and *Cryptosphaeria* spp. in this study did produce lesions from which the fungus was reisolated, regardless of their size.

In addition to causing lesions on grapevine, all 70 diatrypaceous fungi tested in trials I and II were reisolated from grapevines subsequent to a 9- or 18-month incubation. Remarkably, nearly 85% of strains were reisolated from grapevine wood at distances in excess of lesion length. Although Student's *t* tests ($P = 0.05$) of differences between mean lesion length and colonization distance were only significant for *E. lata* and only in trial I, one strain of *C. ampelina* was reisolated from wood as far as 70 mm ahead of the lesion margin. This confirmed prior reports by Sosnowski et al. (24), who reported a similar distance of up to 80 mm following inoculation of grapevines with some strains of *E. lata*. In simple terms, this means that, in most cases, fungi colonize grapevine wood beyond the visible symptoms, and that diatrypaceous fungi are able to colonize grapevines asymptotically.

The ability of *E. lata* to colonize wood ahead of the visible lesion is not completely understood, although *E. lata* has been shown to produce a suite of phytotoxic compounds, mostly acetylenic phenols (1). Although these phenols are responsible for the expression of foliar symptoms and, through the actions of plant phenol oxidases that oxidize phenolic compounds, the darkly stained wedge-shaped lesions characteristic of *E. lata*, toxin production is strain dependent, such that not all strains are capable of producing observable symptoms (16). Furthermore, the symptoms attributed to toxins produced by *E. lata* do not generally appear until 3 to 8 years after infection in mature vines (27) and, by the time symptoms are observed, including stained wood typical of lesion development, the fungus may already have spread throughout the vascular system of the vine. In addition to *E. lata*, many other fungi associated with grapevine trunk diseases are known to produce phytotoxins (1). However, apart from *E. vitis*, which was reported to cause *E. lata*-like symptoms on *V. labrusca*, which were thought to be the result of a toxin (13), this is yet to be demonstrated for other species in the Diatrypaceae family.

Although diatrypaceous fungi are generally thought to be saprophytic, living mostly on the dead or decaying wood of their hosts (28), their ability to colonize wood beyond the disease margin suggests that these species may adopt a hemibiotrophic lifestyle, whereby living tissues are parasitized for a time, after which their lifecycles continue on dead tissues. This delay in symptom expression has important implications for the management of grapevine

trunk diseases. Remedial surgery, which is recommended for the management of both *Eutypa dieback* and *Botryosphaeria canker* (25,34), involves pruning out all infected material. However, due to the propensity of *E. lata* to colonize healthy grapevine tissues in the absence of visible symptoms, current strategies call for the removal of at least 10 cm of additional healthy tissue beyond the margin of disease (24). The results of the current study suggest that this recommendation is sufficient for the management of the other diatrypaceous species included in this study.

Furthermore, pathogenicity tests have shown that strains of *E. lata* and other diatrypaceous fungi collected from native and ornamental species are also pathogenic to grapevines. Trouillas and Gubler (30) speculated that alternate hosts might play a role in the epidemiology of *Eutypa dieback* by acting as reservoirs of inoculum. Although this remains to be established, removal of dead wood of potential hosts of *E. lata* and other diatrypaceous species planted in close proximity to vineyards may help to reduce the incidence of disease. The use of fungicides and paints to protect pruning wounds from infection by *E. lata* has also been successful in reducing the incidence of *Eutypa dieback* (3,23). Recent studies to evaluate fungicides against other diatrypaceous species in vitro have revealed that the majority of fungicides that are effective against *E. lata* are also efficacious against many other diatrypaceous fungi (9). Future studies will continue to evaluate promising agents under field conditions.

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