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Evidence that *Eutypa lata* and other diatrypaceous species occur in New South Wales vineyards

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Abstract. Eutypa dieback, caused by the fungus *Eutypa lata* is a serious disease of grapevines that affects vineyard productivity and longevity. Grapevines displaying foliar symptoms typical of Eutypa dieback or evidence of dead spurs, cankers, or discoloured vascular tissue, were surveyed from 77 vineyards throughout New South Wales (NSW), Australia. Fungal cultures were tentatively identified based on cultural morphology, before further identification using sequence analysis of rDNA internal transcribed spacer regions. *E. lata* and several other species from the Diatrypaceae including *Cryptovalsa ampelina*, and species of *Eutypella* and *Diatrypella* were isolated from diseased grapevines. Eutypa dieback was found to be more widespread in NSW than first thought, with confirmation that the disease is present both in the Central Ranges and southern NSW districts, regions recognised for their cooler climates and higher annual rainfall, both of which favour the growth of *E. lata*.

Introduction

The ascomycete fungus *Eutypa lata* (=*E. armeniaceae*), first described from apricot, is a significant pathogen of grapevines, both in Australia and abroad, where it is responsible for the vascular disease Eutypa dieback (Carter 1957; Carter *et al*. 1983). While both the anamorph (*Libertella blepharis*) and teleomorph are produced on dead wood, the pathogen is disseminated solely by ascospores that are released from mature perithecia for up to 36 h after rainfall (Dubos 1987). Infection commences when ascospores germinate on fresh pruning wounds or other areas of the vine that have been damaged by vineyard operations (Sosnowski *et al*. 2004). *E. lata* subsequently colonises the xylem tissue, cambium and phloem and eventually girdles the vine as a result of canker formation around infected wounds (Munkvold and Marois 1995). Wounds are most susceptible to infection in early winter, becoming less so as the temperature rises later in the year, owing to increased competition from other natural wound colonisers and the initiation of sap flow as the growth cycle commences in spring (Dubos 1987; Chapuis *et al*. 1998). Foliar symptoms, which are caused by toxins produced by the fungus, include stunted shoots with shortened internodes and small distorted, chlorotic leaves with cupped or tattered margins (Mahoney *et al*. 2005). A characteristic wedge-shaped zone of dead wood is also common in the trunks and cordons of infected vines (Moller and Kasimatis 1981). Without intervention, vines may die within several years, thereby reducing vineyard productivity, longevity and economic sustainability (Wicks and Davies 1999; Siebert 2001).

Diagnosis of the disease can be difficult as infection is followed by a period of latency. This delay in symptom expression as the fungus colonises the vascular tissue may be as brief as 1 year (Tey-Rulh *et al*. 1991), but generally occurs over a period of 3–8 years following infection (Carter 1978). As a result, infected vines may appear asymptomatic (Loschiavo *et al*. 2007). Similarly, the expression of foliage symptoms may fluctuate from season to season (Sosnowski *et al*. 2007*b*), can be influenced by grapevine cultivar and fungal isolate (Sosnowski *et al*. 2007*a*) and can be confused with other diseases or disorders or masked by the growth of healthy foliage from neighbouring vines (Lardner *et al*. 2005). In culture, where diagnosis is based on the anamorph, the situation is equally difficult as hyphae lack diagnostic characters; conidial production may be inconsistent and morphological features of the anamorph are insufficient to distinguish *E. lata* from other ascomycetes, especially other genera of the Diatrypaceae possessing *Libertella* anamorphs and including *Eutypella*, *Diatrypella*, *Diatrype*, *Cryptosphaeria* and *Cryptovalsa* (Glawe and Rogers 1984; Acero *et al*. 2004; Mostert *et al*. 2004). Furthermore, the fungus is often overgrown by faster growing species cohabitating the same piece of wood (Rolshausen *et al*. 2004).

Eutypa dieback is widespread throughout many of the premium wine-growing areas of Australia (Sosnowski *et al*. 2007*b*), and has been reported to affect more than 60% of vines in some South Australian vineyards (Highet and Wicks 1998). A recent survey showed that Eutypa dieback is widespread in the Adelaide Hills region, despite the majority of vines being planted within the past decade (Loschiavo *et al*. 2007). This suggests that the fungus can establish rapidly in new grapevines planted under favourable conditions. With premium wines now being produced from cooler climate regions of New South Wales

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(NSW) having climatic conditions not unlike those found in the Adelaide Hills, the status of Eutypa dieback in NSW is of increasing concern. This study aimed to determine the incidence and distribution of Eutypa dieback throughout NSW. Other diatrypaceous fungi isolated from diseased grapevines were also documented as some of these species have previously been implicated in the death and decline of grapevines (Mostert *et al*. 2004; Luque *et al*. 2006).

Materials and methods

Isolation and morphological identification

Between November 2006 and April 2008 field surveys were conducted from 77 vineyards across NSW, encompassing seven major grape-growing regions (Table 1), *viz*. Big Rivers (growing season spatial mean average temperature from 1 October 1971 to 30 April 2000, ~21.1°C), Central Ranges (~18.9°C), Hunter Valley (~20.7°C), Southern NSW (~18.3°C), South Coast (~17.8°C), Northern Rivers (~20.8°C), Northern Slopes (~18.6°C). A total of 1846 wood samples were collected from the cordons or trunks of grapevines with evidence of dieback, including dead spurs or cordons, cankers or bleached and discoloured tissue. Five 2-mm^2 portions of wood from each sample, were excised from the margin of diseased and healthy tissue, surface sterilised in 8% bleach (active ingredient 1.0% w/v sodium hypochlorite; LabServ by Biolab Australia Ltd, Clayton, Vic., Australia) for 2 min and transferred to potato dextrose agar (PDA; Oxoid Ltd, Basingstoke, Hampshire, England). Cultures were incubated in the dark at 25°C for 5–7 days before being transferred onto fresh PDA to create pure cultures. Foliar symptoms typical of Eutypa dieback were encountered infrequently during the surveys, but in such cases wood samples were extracted and prepared for fungal isolation as described previously. Diatrypaceous species were tentatively identified based on gross cultural morphology (Glawe and Rogers 1984; Glawe and Jacobs 1987; Carter 1991; Mostert *et al*. 2004). In December 2008, additional surveys and collections were conducted from grapevines both in the Hunter Valley and Tumbarumba (NSW). Foliage symptoms of Eutypa dieback were not observed, at either location, and in contrast to the previous collections, isolations were made from fruiting bodies collected on dead wood from aged vines or debris from the vineyard floor. Isolations of diatrypaceous fungi from these samples were made directly from ascospores as described by Trouillas and Gubler (2004), with species tentatively identified based on morphology of the teleomorph (Glawe and Rogers 1984).

DNA extraction, amplification and sequencing

Before extraction of DNA, selected Diatrypaceae isolates were pure cultured by hyphal tip, before being transferred by colonised agar plug, to 50-mL Falcon tubes containing 20 mL of potato dextrose broth (Oxoid). Broth cultures were incubated on a Sartorius Certomat BS-1 (Goettingen, Germany) orbital shaker at 90 rpm (=0.4779*g*, or approximately 0.5*g*; the orbital mechanism has a rotational radius of 5 cm) for 7 days at 25°C. Mycelia were harvested by filtration, lyophilised and DNA extracted using the Qiagen Plant Mini Kit according to the manufacturer's instructions (Qiagen Pty Ltd, Clifton hills, Vic., Australia). Molecular identification of *E. lata* and other diatrypaceous species was achieved via amplification and comparison of rDNA internal transcribed spacer (ITS) regions (ITS1, 5.8S and ITS2) using the oligonucleotide primers ITS1 and ITS4 (White *et al*. 1990).

Each PCR reaction contained 0.1 volume of 10× buffer (containing 15 mM $MgCl_2$, Qiagen), 200 μM each of dNTP, 0.15 μM of each primer, 1 unit of HotStart *Taq* DNA polymerase (Qiagen), ~50 ng of DNA template, and were adjusted with sterile nanopure water to a total volume of 50 μL. PCR reactions were performed using an Eppendorf Master Thermocycler (Hamburg, Germany). Amplification was achieved by an initial step of 15 min at 95°C, followed by 40 cycles of 30 s at 94°C, 45 s at 55°C, and 1.5 min at 72°C, with a final extension of 5 min at 72°C. PCR products were separated by electrophoresis on 1% agarose containing 0.5× Tris-borate-EDTA buffer, and photographed under UV light after staining with ethidium bromide (0.5 mg/L).

For sequencing, PCR products were purified using the QIAquick PCR purification kit (Qiagen). ITS regions were sequenced in both directions by the Australian Genome Research Facility (University of Queensland, St Lucia, Qld, Australia), and identification of diatrypaceous species confirmed by comparison of ITS sequences of our isolates with those available in GenBank. Individual sequences were compiled in BioEdit sequence alignment editor (Hall 1999) and aligned for comparison using ClustalX (Thompson *et al*. 1997).

Isolates of *E. lata*, *Cryptovalsa ampelina*, *Eutypella* and *Diatrypella* used in this study are maintained on PDA agar slopes at 4°C in the collection at the National Wine and Grape Industry Centre (Charles Sturt University, Wagga Wagga, NSW, Australia), and representative isolates of each species were deposited in the Australian Scientific Collections Unit (NSW Department of Primary Industries, Orange, NSW, Australia). DNA sequences of representative isolates used in this study were submitted to GenBank (Table 1).

Results

A total of 73 isolates developing white, cottony mycelium and conforming to gross morphological descriptions of the Diatrypaceae (Glawe and Rogers 1984; Carter 1991; Luque *et al*. 2006) were isolated from diseased grapevines. *E. lata* was isolated from ~0.65% of grapevines surveyed (12 isolates), with the fungus being reported for the first time both in the Central Ranges and southern NSW regions (Fig. 1). In each case, foliar symptoms typical of Eutypa dieback were present including stunted shoots with shortened internodes and cupped leaves with necrotic margins, but the teleomorph was not observed and identification of the anamorph was accomplished via sequencing and comparison of rDNA ITS sequences. Other diatrypaceous species isolated from initial field collections included 14 isolates of *Eutypella* (0.76% of samples, Fig. 2), 23 isolates of *Diatrypella* (1.25%, Fig. 3), and 21 isolates of *C. ampelina* (1.14%, Fig. 4), which were tentatively identified according to morphological descriptions (Glawe and Rogers 1984). In all cases specimens were isolated from cankers from diseased trunks or cordons. Further isolations from fruiting bodies

Table 1. Diatrypaceous species isolated from grapevines throughout New South Wales

Region (# vines sampled), location	Cultivar	Age	Species (# isolated)	Accession numbers[A]
Central Ranges (246)				
Orange	Chardonnay	9	*Eutypella* sp. (1)	–
	Grenache	10	*Diatrypella* sp. (3)	–
			Cryptovalsa ampelina (1)	–
	Sauvignon blanc	14	*Eutypa lata* (2)	EU835160, DAR79045
			C. ampelina (2)	–
	Cabernet sauvignon	14	*Diatrypella* sp. (2)	–
			C. ampelina (1)	–
Canowindra	Verdello	14	*Diatrypella* sp. (1)	–
			C. ampelina (4)	–
	Chardonnay	13	*E. lata* (2)	EU835166, DAR79048
				EU835167, DAR79049
Cowra	Chardonnay	15	*C. ampelina* (2)	–
Mudgee	Chardonnay	8	*C. ampelina* (1)	–
Northern Slopes (300)				
Bendemeer	Shiraz	12	*Eutypella* sp. (1)	–
Armidale	Pinot grigio	8	*Eutypella* sp. (1)	–
Deepwater	Semillon	12	*Eutypella* sp. (3)	–
			Diatrypella sp. (2)	–
Inverell	Shiraz	13	*Diatrypella* sp. (2)	–
Southern New South Wales (525)				
Murrumbateman	Shiraz	37	*E. lata* (2)	EU835162, DAR79046
				EU835163, DAR79047
			C. ampelina (4)	EU835150, DAR79050
				EU835151, DAR79051
				EU835152, DAR79052
	Cabernet sauvignon	36	*Eutypella* sp. (1)	–
		29	*E. lata* (1)	–
Young	Chardonnay	19	*C. ampelina* (2)	–
			Diatrypella sp. (1)	–
			Eutypella sp. (1)	–
Berridale	Riesling	24	*Diatrypella* sp. (1)	–
Tumbarumba	Pinot noir	28	*Diatrypella* sp. (2)	–
		16	*Diatrypella* sp. (3)	–
		20	*Diatrypella* sp. (1)	–
	Chardonnay	–	*Diatrypella* sp. (2)	–
			Eutypa lata (1)	EU835164, DAR79128
	Sauvignon blanc	–	*C. ampelina* (2)	–
South Coast (250)				
	–	–	–	–
Big Rivers (427)				
Wagga Wagga	Shiraz	11	*Diatrypella* sp. (1)	–
Book Book	Shiraz	12	*C. ampelina* (1)	EU835154, DAR79054
Griffith	Shiraz	39	*E. lata* (3)	EU835157, DAR79040
	Traminer	27	*C. ampelina* (1)	–
	Ruby Cabernet	33	*E. lata* (1)	EU835156, DAR79043
	Chardonnay	41	*Eutypella* sp. (6)	–
Northern Rivers (62)				
Port Macquarie	Pinot noir	22	*C. ampelina* (1)	–
Hunter Valley (36)				
Pokolbin	Semillon	22	*Diatrypella* sp. (2)	–
			C. ampelina (1)	FJ800509, DAR79966
			Eutypella sp. (4)	FJ800513, DAR79970
				FJ800514, DAR79971
				FJ800515, DAR79972
				FJ800520, DAR79977

[A]GenBank number and DAR (Australian Scientific Collections Unit, NSW Department of Primary Industries, Orange, NSW, Australia) herbarium number, respectively.

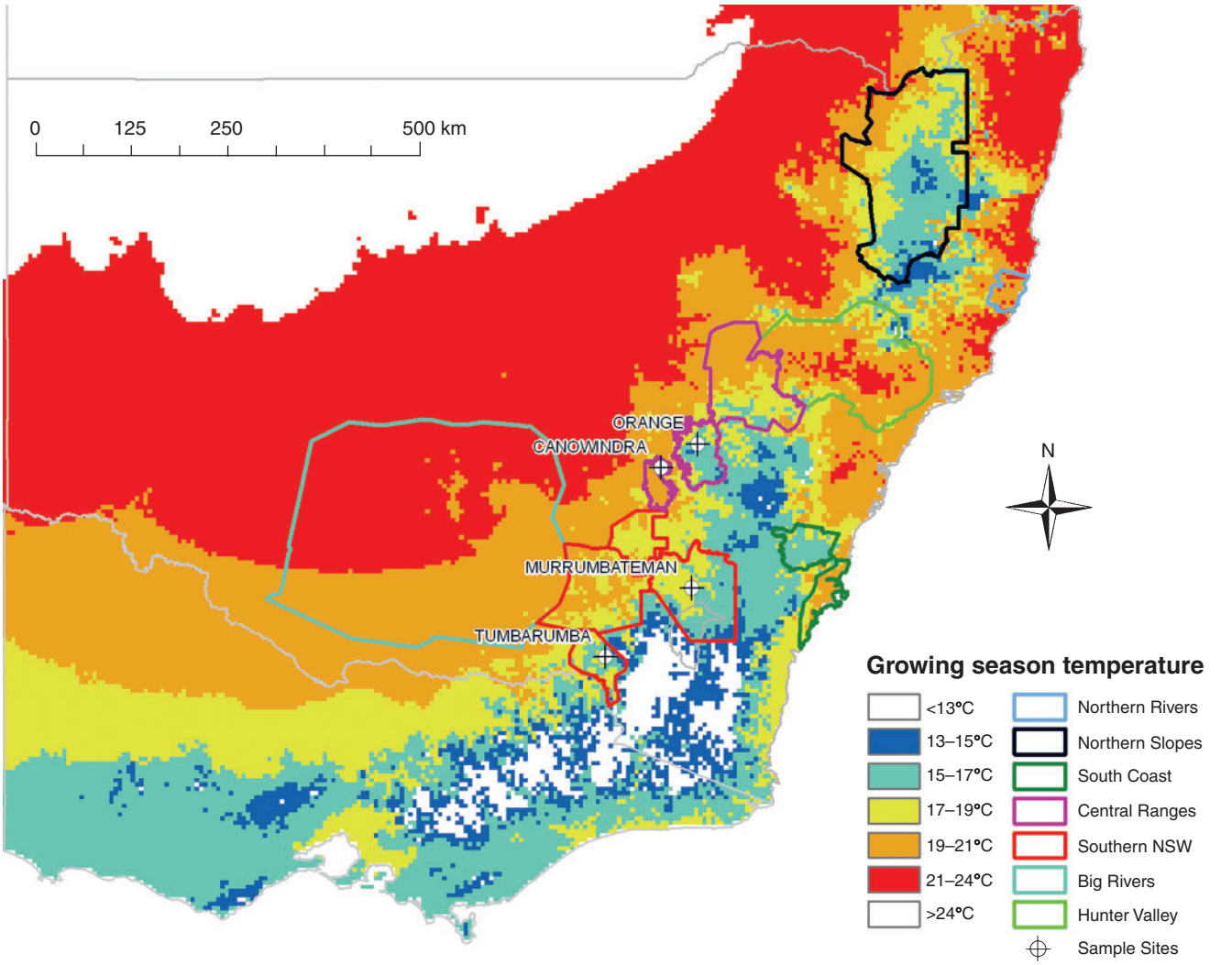

Fig. 1. Growing season mean average temperature distribution bands across New South Wales (1 October 1971–30 April 2000) and the major grape-growing regions, including sites of isolation of *Eutypa lata.* Data obtained from the Australian Government Bureau of Meteorology (2005), and map produced following protocol of Hall and Jones (2009).

collected in the Hunter Valley tentatively identified an additional four isolates of *Eutypella* and one isolate of *C. ampelina* based on features of the stromata and teleomorph, but no foliar symptoms of Eutypa dieback were observed, nor was *E. lata* isolated from the samples collected. Molecular identification of isolates from this latter survey subsequently confirmed the identity of *C. ampelina* and revealed the presence of several different species of *Eutypella*, but neither these isolates nor isolates of *Diatrypella* could be identified at the species level based on available data from GenBank. No diatrypaceous species were isolated from the South Coast region of NSW.

Discussion

The generic concept in the Diatrypaceae is principally based on stromatic characters, such as the degree of stromatal development, configuration of perithecial necks and type of host tissue in which stromata occur (Glawe and Rogers 1984). In general, diatrypaceous asci are clavate to spindle-shaped, long stipitate with a truncate or blunt apex, often containing cytoplasmatic strands in the apex and frequently possess a thicker walled region above the ascospores (Carmaran *et al*. 2006). Differentiation of the various genera and species within the Diatrypaceae is difficult, as many are indistinguishable, possessing few if any unique taxonomic features. Apart from *Cryptovalsa* and *Diatrypella*, which can be clearly separated on the basis of their polysporous asci, the number of ascospores being the only aspect of the ascus regularly used for diagnostic purposes (Carmaran *et al*. 2006), the other common species, *viz. Cryptosphaeria*, *Diatrype*, *Eutypa* and *Eutypella* are indistinguishable, and all comprise octosporous asci, with differentiation instead relying on the use of molecular tools (Rolshausen *et al*. 2004).

Several genera of the Diatrypaceae are known to occur on grapevines throughout the world (Farr *et al*. 1989). Until recently *E. lata* was thought to be responsible for dead and declining

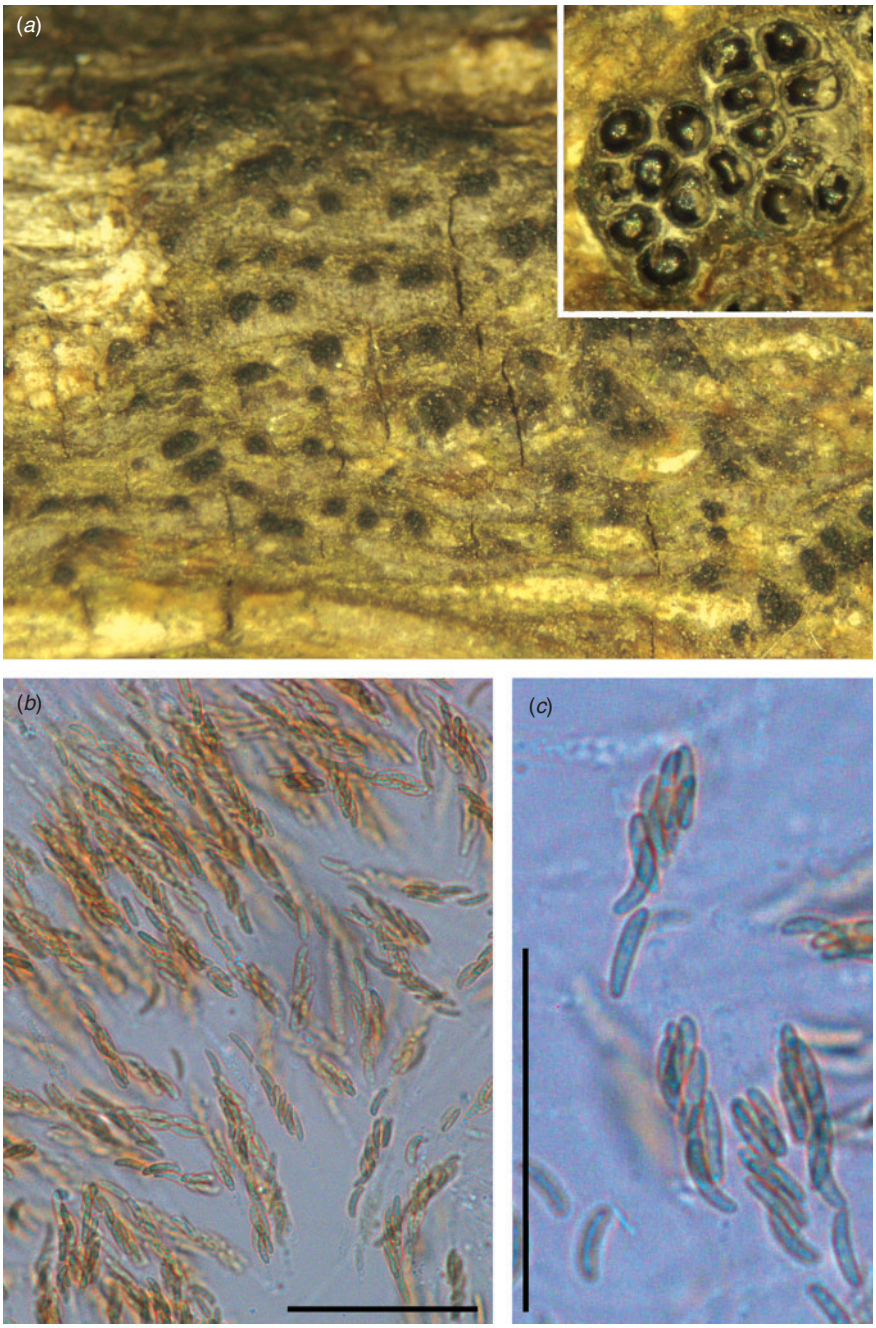

Fig. 2. *Eutypella* sp. from *Vitis vinifera*, (*a*) poorly developed stromata embedded in bark with perithecial necks erumpent in groups (inset: close-up of perithecia embedded in mixture of fungal and host tissue), (*b*) squash mount of asci, and (*c*) close-up of clavate to spindle-shaped, long stipitate, octosporous ascus comprising eight allantoid to moderately curved, subhyaline to subolivaceous ascospores (bar = 50 µm).

vines routinely observed in the Hunter Valley region of NSW. However, foliar symptoms of the disease have not been observed, nor has the fungus been isolated from material collected during extensive surveys of the region (Castillo-Pando *et al*. 2001; Creaser *et al*. 2003; Qiu *et al*. 2006; Savocchia *et al*. 2007). Recently, *E. lata* was reported in the Riverina region of NSW for the first time (Pitt *et al*. 2007). Although prominent in South Australian vineyards, Eutypa dieback had not been reported north of Wentworth (latitude 34°2′S, longitude 141°5′E) in NSW, which is on the Victorian border (T. Wicks, pers. comm.), and until now, no definitive records of the fungus exist from the wine-growing regions of south-eastern NSW. Extensive surveys conducted throughout NSW have now shown that *E. lata* is more widespread than first thought, with new isolations from Canowindra and Orange in the Central Ranges and Murrumbateman and Tumbarumba in southern

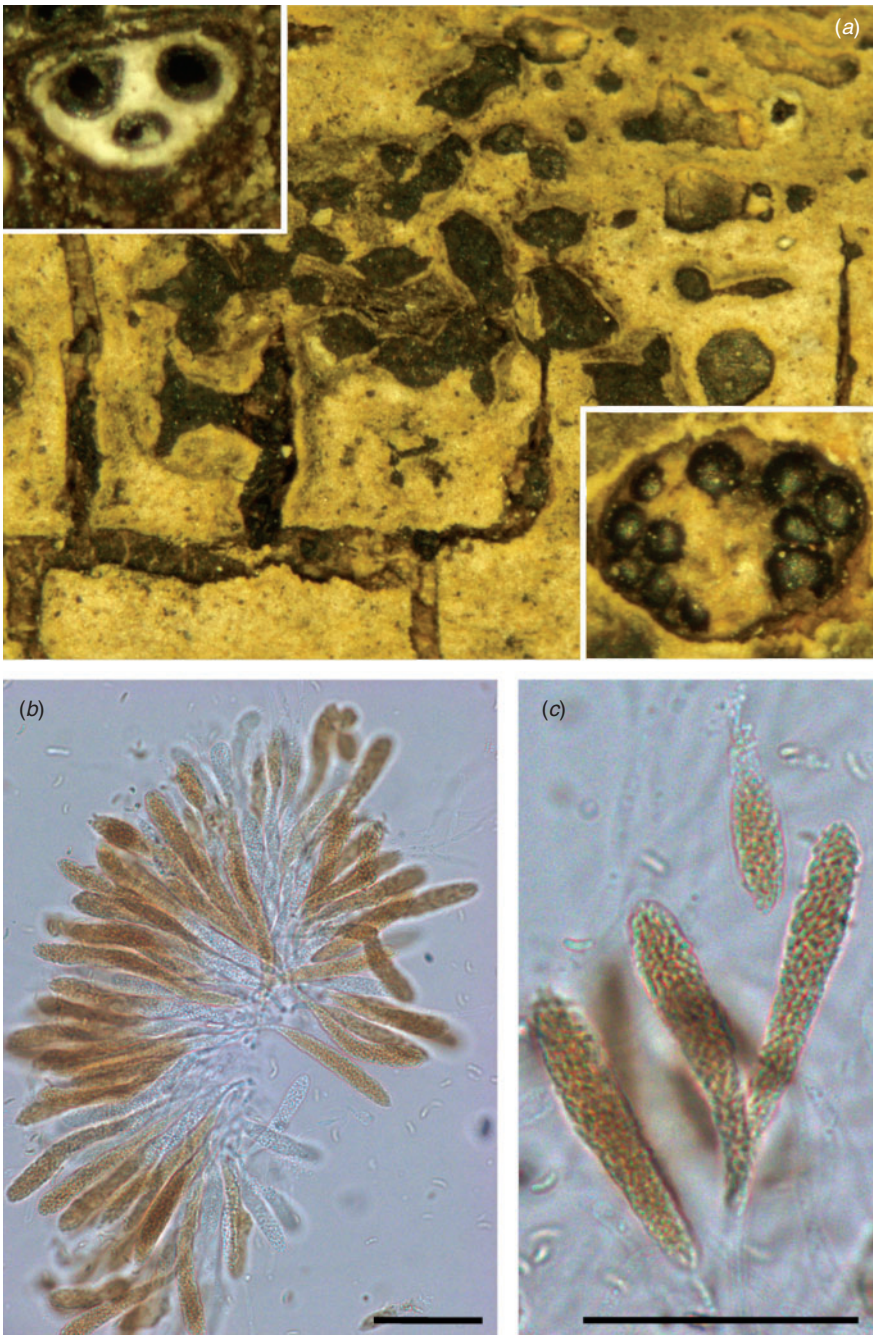

Fig. 3. *Diatrypella* sp. from *Vitis vinifera*, (*a*) well developed stromata, erumpent through bark (inset: close-up of perithecia, surrounded by pseudoparenchymatous tissue), (*b*) squash mount of asci, and (*c*) close-up of spindle-shaped, long stipitate, polysporous ascus comprising ~128 allantoid to moderately curved, subhyaline to subolivaceous ascospores (bar = 50 μm).

NSW (Fig. 1). While Edwards and Pascoe (2004) reported the presence of *Eutypa* from grapevine samples received from Tumbarumba during a routine diagnostic survey, cultures appeared not to have been identified to species, nor characterised at a molecular level. Regardless, these results suggest that *E. lata* may be well suited to the cooler climate regions of NSW where low temperatures and high rainfall favour the growth of the fungus. Certainly, the isolation of *E. lata* from diseased grapevines in Orange (latitude 33°15′S, longitude 149°1′E) represents a small but measurable expansion in the geographic range of the pathogen and the most northerly occurrence of *E. lata* reported in Australia.

While the epidemiology (Petzoldt *et al*. 1981; Carter 1991; Munkvold and Marois 1995) and management (Munkvold and

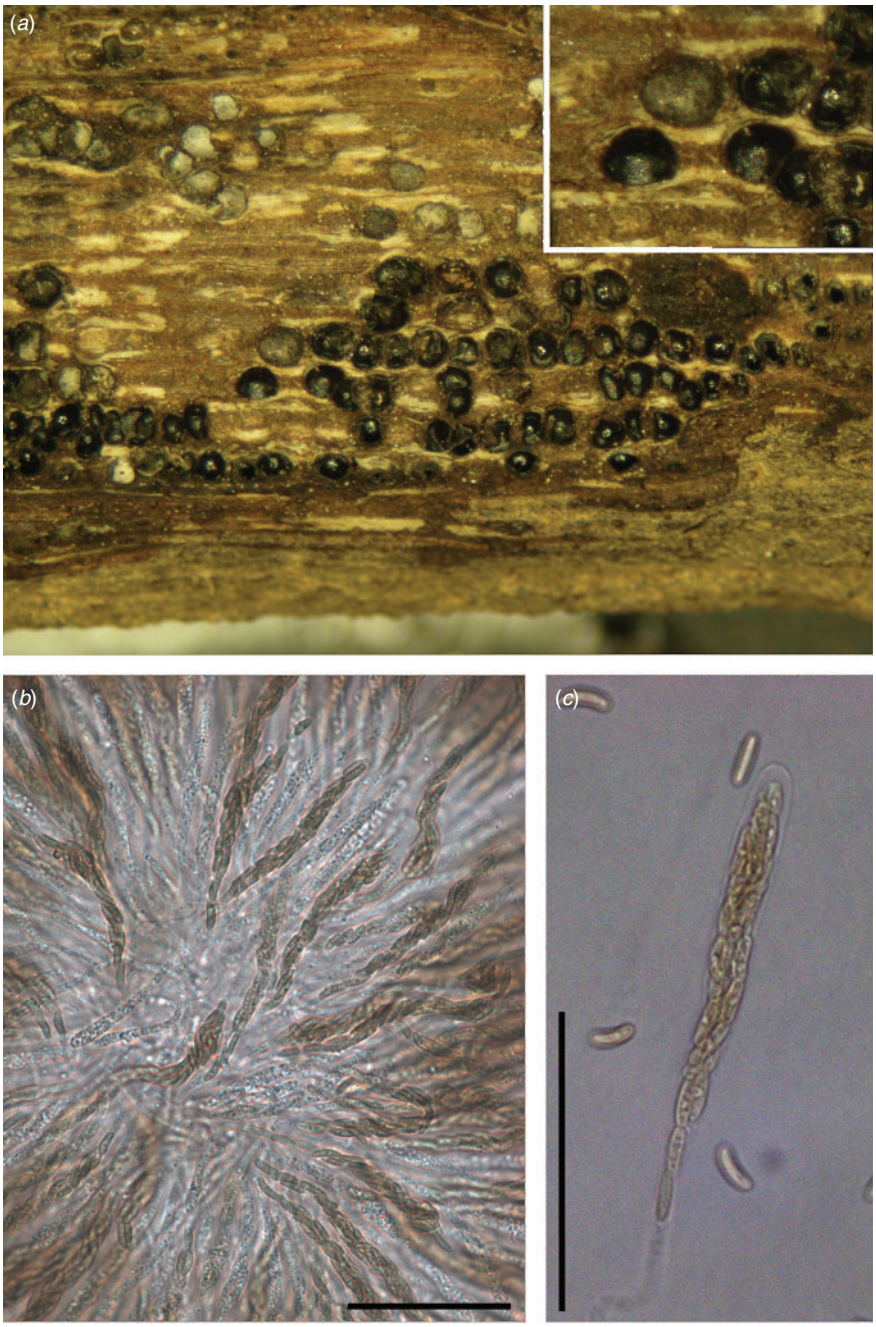

Fig. 4. *Cryptovalsa ampelina* from *Vitis vinifera*, (*a*) poorly developed stromata embedded in decorticated wood (inset: close-up of perithecia, singularly erumpent), (*b*) squash mount of asci and, (*c*) close-up of spindle-shaped, long stipitate, mulitsporous ascus comprising ~32 allantoid, subolivaceous ascospores (bar = 50 μm).

Marois 1993; Weber *et al*. 2007; Sosnowski *et al*. 2008) of *E. lata*, has been studied extensively, little is known about the other members of the Diatrypaceae, many of which are known to occur not only on grapevines but on other hosts including, apples, cherries, pears, olives and poplars (Farr *et al*. 1989). Several species of *Eutypella* and *Diatrypella*, as well as *C. ampelina*, a pathogen in its own right (Mostert *et al*. 2004; Luque *et al*. 2006), were isolated from grapevines from many regions throughout NSW. Interestingly, all three of these genera were present in the Hunter Valley region, and clearly possess a far greater geographic range than *E. lata*. In contrast, *E. lata* was present only in the cooler climate regions of southern NSW, and once again the fungus was not recovered from samples collected from the Hunter Valley, nor was it or any other diatrypaceous species isolated from the South Coast region of NSW. In the latter case, the reasons for this are unknown; however, our failure to isolate *E. lata* from the

Hunter Valley and other regions throughout NSW in no way suggests that these regions are free from the disease. But, it is possible that other genera within the Diatrypaceae are contributing to the dieback phenomenon generally attributed to *E. lata* (Trouillas *et al*. 2001).

Why species such as *C. ampelina*, *Eutypella and Diatrypella* are prominent in the Hunter Valley, seemingly in the absence of *E. lata* remains a mystery. Perhaps the poorly developed stroma of *C. ampelina*, which is embedded in the bark or decorticated wood, as opposed to well developed and erumpent through bark as with *E. lata* (Glawe and Rogers 1984), affords the fungus some measure of protection from the elements, thereby enabling the fungus to survive under a greater range of environmental conditions. In all diatrypaceous genera, stromata are thought to aid in conserving moisture, but in some genera, their function is to aid discharge of ascospores by rupturing the host's bark, thereby exposing perithecial ostioles (Glawe and Rogers 1984). If the reduced stroma composed of both fungal and host tissue preserves less moisture or fails to expose ostioles to the environment, effectively limiting ascospore discharge, fruiting bodies may be better preserved, their survival enhanced, their ability to withstand adverse conditions heightened and geography improved as a function of age. Equally the reverse could be true, or maybe the host ranges of *C. ampelina* and the other more prominent diatrypaceous species are simply larger than that of *E. lata*; as species with larger host ranges often have greater geographic distributions (Glawe and Rogers 1984).

Other explanations for the geographic disparity among the different members of the Diatrypaceae are speculative at best, although clearly the environmental conditions in the northern regions of NSW, including the Hunter Valley, are less favourable to *E. lata* than to some of the other species in the Diatrypaceae, likely due to stricter rainfall and temperature requirements of *E. lata* (Sosnowski *et al*. 2005, 2007*b*). While the Mediterranean climate common in South Australia where Eutypa dieback is extensive contrasts well with the cooler climate regions of NSW, the subtropical climate of the Hunter Valley region, with higher average daily temperatures appears unfavourable for the establishment of Eutypa dieback, despite the fact that rainfall in the region is suitable for the disease. Notably, in a recent report concerning the seasonal variation in Eutypa dieback symptoms, Sosnowski *et al*. (2005) reported a positive relationship between disease incidence and spring temperatures; the higher the temperature, the lower the incidence of disease, as determined by expression of foliage symptoms. Glawe and Rogers (1984) reported that the teleomorph of the fungus fails to form in regions receiving less than 330 mm of annual rainfall. However, plants infected with *E. lata* have been found in such locations, with long-distance and airborne dispersal of ascospores known to accompany the onset of rainfall (Carter 1957; Ramos *et al*. 1975; Glawe and Rogers 1984). With an average annual rainfall greater than 600 mm (Australian Government Bureau of Meteorology 2008; data for Cessnock), understandably, researchers continue to be baffled by the absence of Eutypa dieback in the Hunter Valley, a disease that for all intensive purposes should be present there. Incidentally, US researchers have also noted large geographical differences in the distribution of *E. lata* with respect to climate (Urbez-Torres *et al*. 2006).

To date, the pathogenicity of *E. lata* (Carter 1991) and *C. ampelina* (Mostert *et al*. 2004; Luque *et al*. 2006) to grapevines has been confirmed, but investigations on the virulence of many of the other diatrypaceous species towards grapevines are limited (Trouillas *et al*. 2001). *Eutypella vitis* has been reported to cause xylem necrosis and foliar symptoms similar to those caused by *E. lata* (Wolf 2006), for which it was suggested as an ulterior cause of Eutypa dieback (Myers 2008). Furthermore, two reports from research conducted in California show that *E. leptoplaca* and *Diatrypella* are regularly isolated from diseased grapevines and that inoculation of fresh pruning wounds with these species can cause disease (Rolshausen *et al*. 2004; Trouillas and Gubler 2004).

As cultures of diatrypaceous fungi are often indistinguishable from one another, the co-occurrence of multiple diatrypaceous fungi in diseased wood of grapevines could also lead to misidentification of the correct agents of disease, especially where multiple species are isolated from the same infection. While this occurred in only two instances in the present study, whereby *C. ampelina* and *Diatrypella* were cultured from the same piece of wood (Central Ranges), more than half of the *C. ampelina* isolates collected in the survey co-occurred with species of the Botryosphaeriaceae, which are also well known trunk disease pathogens of grapevines (van Niekerk *et al*. 2004; Savocchia *et al*. 2007). The frequent occurrence of *C. ampelina* in conjunction with other known grapevine pathogens was thought by Luque *et al*. (2006) to represent a synergistic association by a facultative pathogen, which he then used as an explanation for the moderate virulence of the fungus. However, in recent years, *C. ampelina* has been isolated repeatedly from grapevines both in Australia and overseas, and under suitable conditions has been shown to be a pathogen in its own right (Mostert *et al*. 2004; Luque *et al*. 2006). Nevertheless, competition from other trunk disease pathogens like the Botryosphaeriaceae may play a role in reducing the incidence of the Diatrypaceae. While unlikely with respect to *C. ampelina*, interactions of this nature may contribute to the low incidence of *E. lata* throughout the state, or alternatively to its absence in some regions. To date, no reports of this nature have been published.

This study has shown that many of the other diatrypaceous species are more widespread and abundant than *E. lata* in NSW. While the exact species causing trunk diseases in the vineyard may be inconsequential to many grapegrowers, due to increasing knowledge that the recommended management strategies pertain to many of the grapevine canker causing fungi (van Niekerk *et al*. 2002; van Niekerk *et al*. 2006), correct diagnoses of the causal agent may be essential to predict the severity of the disease and hence the urgency of management. In a major study of the pathogenicity of nine Botryosphaeriaceae species isolated from grapevines in California, Urbez-Torres and Gubler (2009) showed that four species within this family were equivalent to, or greater in virulence than *E. lata*. Other researchers have reported considerable variation in the pathogenicity of isolates of *E. lata* both to grapevines and other hosts, suggesting the existence of two pathotypes, vastly different in virulence (Carter *et al*. 1985). This variation in pathogenicity of individual isolates of *E. lata*, as well as the influence of grapevine cultivar (Sosnowski *et al*. 2007*a*), and the effect of climate on seasonal

disease expression (Petzoldt *et al*. 1981; Sosnowski *et al*. 2007*b*), can greatly influence the severity and perceived importance of such canker diseases. Because it is impossible to discriminate wood symptoms caused by the different trunk disease fungi, the aggressiveness of the pathogen and its identification is of the utmost importance for effective management.

As few of the diatrypaceous species have been studied in detail in Australia, their incidence, distribution and pathogenicity towards grapevines and other cultivated crops requires further research. In the interim, vigilant monitoring, avoiding pruning during and directly after rainfall, protection of pruning wounds, and removal and incineration of dead infected wood from the vineyard remain the best methods of managing Eutypa dieback and other infections that may be caused by the Diatrypaceae.

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