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Pathogenicity of *Botryosphaeria* species isolated from declining grapevines in sub tropical regions of Eastern Australia

S. Savocchia[1)], C. C. Steel[1)], B. J. Stodart[2)] and A. Somers[1, 3)]

[1)] National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, NSW, Australia
[2)] School of Agriculture and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia
[3)] New South Wales Department of Primary Industries, CB Alexander College, Paterson, NSW, Australia

Summary

From 2002 to 2004, vines from 11 vineyards in the Hunter Valley region and from 4 vineyards in Mudgee (New South Wales, Australia) were inspected for foliar and wood symptoms of Eutypa dieback and *Botryosphaeria* canker. *Eutypa lata* was not isolated, however species of *Botryosphaeria* were frequently isolated from dead and declining spurs, cordons and trunks. Two species conforming to the anamorphs of *Diplodia* and *Fusicoccum* were isolated. Cultures on PDA producing a yellow pigment, and hyaline aseptate conidia (14-23.5 x 8-13 µm in size) were identified as *B. lutea*. Cultures with dark brown, 1-septate, ornamented conidia (15-25 x 8-14 µm in size) at maturity were identified as *B. obtusa*. Pathogenicity tests were conducted on detached green shoots, detached one-year-old canes and glasshouse-grown grapevines. In all situations, disease symptoms were reproduced and Koch's postulates were fulfilled. The results demonstrate the potential of *B. obtusa* and *B. lutea* as primary pathogens of *Vitis vinifera*.

K e y w o r d s : Grapevine decline, trunk diseases.

Introduction

The Hunter Valley region (Upper and Lower) located in the state of New South Wales (NSW), is Australia's oldest wine region with the first grapevine plantings being established in the 1820s (MacQuitty 1990). During the growing season, the region is hot with mean daily maximum temperatures reaching 30 °C and mean relative humidity ranging from 42 to 70 % throughout the day. The average annual rainfall is 742 mm (Bureau of Meteorology 2006). Frost, hail and thunderstorms are common. Mudgee is located west of the Hunter Valley in the Central Ranges region of NSW with grapevines being established in the 1950s. The growing season is also warm to hot with mean daily maximum temperatures reaching 31 °C and mean relative humidity ranging from 37 to 66 % throughout the day. Summer rainfall is common but tends to be less than in the Hunter Valley. The average annual rainfall for Mudgee is 675 mm (Bureau of Meteorology 2006). Declining and dying grapevines have been observed in the Hunter Valley and Mudgee for many years, and the problem appears to be most prevalent in *Vitis vinifera* cvs Semillon and Chardonnay. In the past, these symptoms were thought to be due to infection by *Eutypa lata*, however, there are no published reports of this fungus having been isolated from grapevines exhibiting decline and dieback symptoms from these regions (Castillo-Pando *et al*. 2001).

Species of *Botryosphaeria* are reported to cause cankers, dieback, fruit rots and other symptoms on a wide range of host plants (Davison and Tay 1983, Latorre and Toledo 1984, Maas and Uecker 1984, Milholland 1988). A number of species have been isolated and found to be associated with dieback of grapevines worldwide (Shoemaker 1964, Milholland 1988, Phillips 1998, Larignon *et al*. 2001, Auger *et al*. 2004, van Niekerk *et al*. 2004). *B. dothidea* was found to be pathogenic on grapevines causing bleaching of the outer bark, cracking of canes, dieback of shoots and bud mortality, while *B. stevensii* and *B. obtusa* were found to be wound pathogens, causing small lesions and minimal damage to the host (Phillips 1998). In South Africa, *B. australis* was found to be most pathogenic causing rotting and streaking symptoms in mature canes and mature wood of *Vitis vinifera* 'Periquita'.

Other symptoms associated with infection by species of *Botryosphaeria* include necrotic wedge-shaped lesions in cross-sections of wood, similar to those observed with infection by *E. lata*, or half-moon lesions (Castillo-Pando *et al*. 2001, Wood and Wood 2005). In Australia, species of *Botryosphaeria* have also been isolated from grapevine, and pathogenicity studies showed that *B. obtusa* was likely to be responsible for dark streaking in the wood of artificially inoculated Chardonnay (Castillo-Pando *et al*. 2001). In contrast, Taylor *et al*. (2005) concluded that *B. obtusa* was most likely a saprophyte of grapevines. This study reports on the results of a survey conducted in the Hunter Valley and Mudgee regions and the symptoms observed in declining grapevines. Species of *Botryosphaeria* were characterised based on culture and spore morphology, and pathogenicity studies were conducted to determine which species should be regarded as potential pathogens of grapevines in Australia.

Material and Methods

F i e l d o b s e r v a t i o n s , v i n e y a r d s a m p l i n g a n d i s o l a t i o n s o f f u n g i : Grapevines aged from 8 to 122 years old in 15 vineyards located in

Correspondence to: Dr. S. Savocchia, National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW, 2678, Australia. Fax: +61-2-6933-2107. E-mail:ssavocchia@csu.edu.au

the Hunter Valley and Mudgee regions were examined for wood and foliar symptoms associated with trunk diseases from 2002 to 2004. Symptomatic cordons, spurs and trunks were sampled. Approximately 5 wood samples were taken from each vineyard by either collecting sections of cordons and spurs or by drilling into trunks of vines and collecting the wood shavings. The superficial bark tissue was removed from all samples prior to surface sterilisation. Wood samples were surface sterilised in 0.5 % sodium hypochlorite for 3 min and rinsed three times, for 2 min each, in sterile distilled water. Each sample was blotted dry with sterile paper towel. Small pieces of tissue were cut from the margin of necrotic and healthy tissue and placed onto potato dextrose agar (PDA) amended with streptomycin (25 µg ml^{-1}) to inhibit bacterial growth. Samples were incubated at 25 °C in the dark and monitored for fungal growth. Fungi growing from the wood samples were isolated, transferred onto fresh PDA amended with streptomycin (25 µg ml^{-1}) and incubated at 25 °C in the dark.

Morphological and cultural characterisation: All cultures displaying morphological characteristics associated with species of *Botryosphaeria* were incubated at 25 °C, for 4 to 7 d and their colour recorded according to Rayner (1970). Isolates were incubated at 25 °C for 2 to 8 weeks in the dark to encourage the production of pycnidia. Those that did not produce pycnidia were subcultured onto 1 % water agar containing 3 triple-autoclaved *Pinus radiata* needles, to encourage pycnidium production. Species of *Botryosphaeria* were identified by examining the morphological characteristics of 25 conidia from each isolate.

Excised shoot inoculations: Green shoots, 8-10 mm in diameter and 30 cm in length, from healthy mature Chardonnay, were collected from a vineyard at Charles Sturt University (CSU), Wagga Wagga, New South Wales, Australia. All leaves and tendrils were removed and the shoots were surface-sterilised with 70 % ethanol prior to inoculation. Four shoots were inoculated with either one of two isolates of *B. lutea* (1-046a, 3-025) or one of 6 isolates of *B. obtusa* (1-015, 2-027, 2-022, 1-080, 2-116, 1-070). Two different inoculation methods were tested: with or without wounding. For the first method, shoots were wounded 10-15 cm from the top of the shoot by removing the cortex with a sterile 4 mm diameter metal cork borer. A 4 mm diameter inoculum plug of a 7-d-old isolate on PDA was then placed into each hole. Each hole was covered with Parafilm. For the second method, the inoculum plug was placed directly onto the shoot and covered with Parafilm. Control shoots were filled with non-colonised plugs of PDA. Each inoculated shoot was placed into an individual 500 ml plastic container containing 200 ml tap water and covered with individual plastic bags to maintain a humid environment. The shoots were maintained in a glasshouse at 25 °C with natural light. After 7 d the plastic bags were removed and the shoots maintained in the glasshouse for a further 14 d. The water was replaced every 4 d. The length of a resulting lesion (minus diameter of initial wound) was measured after 21 d. To satisfy Koch's postulates, sections of tissue from three replicates per treatment were placed onto PDA and incubated at 25 °C in the dark until pycnidia were observed. Pycnidia were examined for the presence of spores and the identity of the organism confirmed.

One-year-old cane inoculations: Mature one-year-old canes were collected from dormant, healthy Shiraz and Chardonnay vines in a vineyard at CSU. The canes were surface sterilised with 70 % ethanol and cut into 8 cm pieces. A wound was created in each piece of cane by cutting to the pith with a sterile 4 mm diameter metal cork borer. Each piece of cane was inoculated with either one of two isolates of *B. lutea* (1-046a, 3-025) or one of 9 isolates of *B. obtusa* (1-015, 1-050, 2-027, 2-022, 1-080, 2-116, 1-066, 1-068, 1-070) by inserting a 4 mm diameter inoculum plug of a three-d-old isolate on PDA into each hole. Each hole was covered with Parafilm. Control canes were filled with non-colonised plugs of PDA. Each piece of cane was placed into a separate, sterile Petri plate lined with sterile paper towel, moistened with sterile distilled water. The experiment consisted of 5 replicates. Plates were sealed with Parafilm and incubated for 21 d at 25 °C in the dark. After this time, the superficial bark tissue was removed from each piece of cane and the length of any resulting lesion recorded. Small pieces of tissue were cut from the margin of lesions and surface sterilised in 0.5 % sodium hypochlorite for 1 min, and rinsed three times, for 1 min each, in sterile distilled water. Koch's postulates were satisfied according to the procedure described above for shoot inoculations.

Glasshouse-potted grapevine inoculations: Two-year-old Chardonnay rooted cuttings were potted into sterile soil containing coarse river sand: loam: Canadian peat moss (2:2:1) in 28 cm pots and placed in a glasshouse. After 3 months, two lignified shoots and the trunk of each grapevine was surface sterilised with 70 % ethanol, and a wound created by cutting to the pith with a sterile 4 mm diameter metal cork borer. Each shoot and trunk was inoculated with one isolate of *B. obtusa* (2-027) or *B. lutea* (3-025) in replicates of three separate plants by inserting a 4 mm diameter inoculum plug of a three-d-old isolate on PDA into each hole. Each hole was covered with Parafilm. Control shoots and trunks were filled with non-colonised plugs of PDA. Plants were maintained in a glasshouse at approximately 25 °C. After 41 weeks, the inoculated shoots were harvested, cut longitudinally and assessed by measuring lesion lengths. Trunks were assessed similarly, however, the superficial bark was removed first. Koch's postulates were satisfied according to the procedure described above for shoot inoculations.

Data analysis: Data were subjected to an analysis of variance (ANOVA) using the CoStat statistical package (version 6.303, CoHort Software, Monterey, CA, USA). Means were separated by the least significant difference test at $P = 0.05$.

Results

Field observations and isolations: Wood symptoms including blackened areas and cankers around pruning wounds or natural openings in the bark,

longitudinal splitting of canes, bleaching of canes, stunted shoot growth, dead spurs and cordons were observed at each of the vineyards (Fig. 1). No foliar symptoms were evident. Pycnidia were observed on the surface of cordons and trunks. When declining spurs and cordons were cut in cross-section, brown-black staining in the shape of a wedge was observed (Fig. 2). Wood shavings taken from the trunk and cordons were also stained brown-black. Infection of the wood was observed to spread basipetally from a wound site. Isolations from the margin of stained and healthy tissue resulted in the recovery of fungi belonging to the genera *Botryosphaeria*, *Alternaria*, *Pestalotiopsis*, *Epicoccum*, *Aspergillus* and *Penicillium*. The dominant fungi isolated were species of *Botryosphaeria*, with other species being isolated infrequently (1-5 %). A total of 63 isolates of *Botryosphaeria* spp. were obtained in pure culture (Tab. 1).

Fig. 1: Chardonnay showing dieback of cordon and spurs.

Fig. 2: Cross-section of a Chardonnay trunk showing brown-black staining in the shape of a wedge. *Botryosphaeria obtusa* was isolated from the margin of the healthy and diseased tissue.

Morphological and cultural characterisation: On PDA, cultures of *Botryosphaeria* were initially white, then iron-grey to olivaceous-grey darkening to charcoal-black or leaden-black. All isolates of *Botryosphaeria* produced aerial and high density mycelium on PDA. No zonation was evident in any of the isolates. Black pycnidia were observed after three weeks to two months in some cultures. A yellow pigment, characteristic of *B. australis* and *B. lutea* was observed after two to three d incubation at 25 °C in 22 % of the cultures. The yellow pigment tended to disappear after three to four days. The conidia from these isolates were hyaline, thin-walled, aseptate, fusiform, 14-23.5 x 8-13 µm in size (Tab. 1). One isolate (1-203) was observed to form microconidia, 3.75 x 2 µm in size. These isolates conformed to the *Fusicoccum* anamorphs of the genus *Botryosphaeria* and were identified as *B. lutea* (Pennycook *et al*. 1985, Phillips *et*

Table 1

Species of *Botryosphaeria* isolated from grapevines with decline and dieback symptoms

Region	Cultivar	Year of vine establishment	Number of isolates	Yellow pigment[a]	Average conidial size (µm)[b]	Length/width ratio	Species
Lower Hunter	Chardonnay	1988	2	-	20-25 x 9-10	2.3-2.4	*B. obtusa*
	Semillon	1975, 1983	3	-	20-25 x 9-13	1.8-2.4	*B. obtusa*
			2	+	17.5-23 x 5-7.5	3.3-4.3	*B. lutea*
	Shiraz	1880, 1950, 1983, 1990	7	-	18-25 x 8-12.5	1.9-2.3	*B. obtusa*
			3	+	17.5-23 x 5-13	3.0-3.8	*B. lutea*
Upper Hunter	Cabernet	1969	2	-	16-23 x 10-14	1.2-2.1	*B. obtusa*
			2	+	14-22.5 x 4-5	3.3-4.3	*B. lutea*
	Chardonnay	1973, 1989, 1994	10	-	18-25 x 10	1.8-2.4	*B. obtusa*
			3	+	17.5-22.5 x 5-7.5	3.0-4.5	*B. lutea*
Mudgee	Cabernet	1970	3	-	21-25 x 10-14	1.9-2.3	*B. obtusa*
			3	+	20-25 x 5	4.4-4.6	*B. lutea*
	Muscat	1970	4	-	20-25 x 10	2.1-2.2	*B. obtusa*
	Semillon	1974	3	-	20-25 x 10	2.2-2.4	*B. obtusa*
	Shiraz	1963, 1970	15	-	15-28 x 9-11	2.0-2.6	*B. obtusa*
			1	+	20-22.5 x 5-6.3	4.0	*B. lutea*

[a] A yellow pigment was recorded on PDA after 2-3 d incubation at 25 ºC.
[b] 25 conidia were examined for each isolate.

al. 2002). The remaining 78 % of isolates conformed to the *Diplodia* anamorph of the genus *Botryosphaeria* and were identified as *B*. *obtusa* (Phillips 2002). The conidia from these isolates were hyaline when immature and dark brown at maturity, mostly aseptate but occasionally 1-septate and ornamented on the inner surface, 15-25 x 8-14 µm in size (Tab. 1). On one occasion, *B. obtusa* and *B. lutea* were isolated from the same grapevine sample taken from Cabernet Sauvignon in the Mudgee region. Both species were present in each of the three regions surveyed, however not all species were present in each individual vineyard. Furthermore, species presence was not correlated with cultivar. The origin of isolates used in pathogenicity tests are presented in Tab. 2.

Table 2

Origin of isolates used in pathogenicity tests

Region	Cultivar	Year of vine establishment	Isolate
Lower	Semillon	1983	1-015
Hunter	Shiraz	1880	1-046a
	Shiraz	1880	1-050
Upper	Chardonnay	1989	2-022
Hunter	Cabernet Sauvignon	1969	3-025
	Cabernet Sauvignon	1969	2-027
Mudgee	Cabernet Sauvignon	1970	1-080
	Cabernet Sauvignon	1970	2-116
	Shiraz	1970	1-066
	Shiraz	1970	1-068
	Shiraz	1970	1-070

Excised shoots: No lesions were observed in non-wounded excised green shoots. Both *B. lutea* and *B. obtusa* produced dark brown lesions in wounded, excised green shoots of Chardonnay (Tab. 3). Lesions extended to the pith and both upward and downward from the point of inoculation. Lesion lengths varied within and between species. Pycnidia were occasionally observed on the surface of the decaying shoot. *B. obtusa* isolates 2-027, 2-022, 2-116 and 1-070 were the most virulent and differed significantly from the control. Both isolates of *B. lutea* and two isolates of *B. obtusa* (1-015, 1-080) did not differ significantly from the control (LSD = 4.45, $P = 0.05$). Control shoots, produced slight discolouration around the wound site. *B. lutea* and *B. obtusa* were re-isolated from the margin of the brown lesions of each of the replicate shoots whereas *Penicillium* spp. and yeast species were isolated from the control shoots.

One-year-old canes: *B. lutea* and *B. obtusa* produced dark brown to black lesions in one-year-old canes of Shiraz and Chardonnay (Fig. 3, Tab. 4). Lesions extended both upward and downward from the point of inoculation. Pycnidia developed on the surface of canes. When canes were cut in cross-section small, necrotic, wedge-shaped lesions and discolourations extending from the epidermis to the pith were observed. Both species were pathogenic on Chardonnay producing significantly larger

Table 3

Mean lesion length on excised green shoots (*V. vinifera* cv. Chardonnay) 21 d after inoculation with isolates of *Botryosphaeria* species and potato dextrose agar (control)[a]

Treatment	Isolate	Lesion length (mm)[b]	
		Mean	±SE
B. lutea	1-046a	3.7 abcd	1.2
	3-025	1.3 cd	1.3
B. obtusa	1-015	1.5 cd	0.6
	2-027	6.3 a	1.9
	2-022	4.3 abc	2.0
	1-080	2.8 bcd	1.1
	2-116	5.7 ab	1.9
	1-070	3.9 abc	0.4
Control		0.5 d	0.3

[a] Mean lesion length based on 4 replicates of excised green shoots per treatment.

[b] SE = standard error of the mean. Means followed by different letters are significantly different ($P = 0.05$) based on least significant difference.

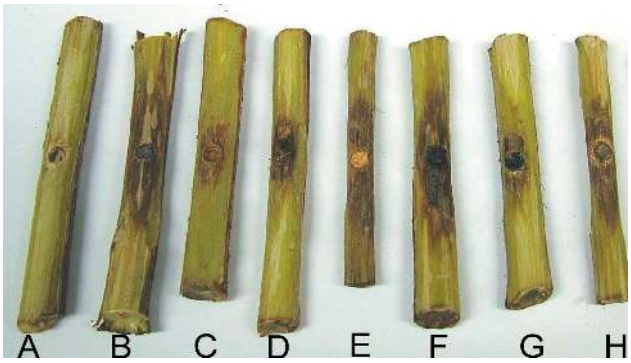

Fig. 3: Pathogenicity of *Botryosphaeria* spp. on 1-year-old canes of Shiraz 21 d after inoculation. **A**: control; **B** to **D**, **F**, **G**: *B. obtusa*; **E**, **H**: *B. lutea.*

lesions than the control (LSD = 13.76, $P = 0.05$). Mean lesion lengths for *B. lutea* ranged from 49 to 52.3 mm and for *B. obtusa* from 31.2 to 55 mm, whereas the mean lesion length for the control was 1.4 mm (Tab. 4). A similar observation was made with Shiraz, however two isolates of *B. obtusa* (1-050 and 1-070) were found to not differ significantly from the control (LSD = 15.81, $P = 0.05$). Mean lesion lengths for *B. lutea* ranged from 22.2 to 22.8 mm and for *B. obtusa* from 12.6 to 40.4 mm. A two-way ANOVA indicated a significant difference between the cultivars ($F = 6.58$, $P = 0.001$). Chardonnay was more susceptible to *B. lutea* and *B. obtusa* than Shiraz. Both *B. lutea* and *B. obtusa* were re-isolated from the margin of the healthy and brown lesions of each of the inoculated replicate canes. No fungi were isolated from the control canes.

Glasshouse-grown grapevines: There were no significant differences ($P = 0.42$) between the lesion lengths in the lignified shoot and trunk, therefore the data from these measurements were combined. Both *B. lutea* and *B. obtusa* produced dark brown lesions in the lignified shoots and trunks of the grapevines. There was no

Table 4

Mean lesion length on one-year-old canes (*V. vinifera* cvs. Chardonnay and Shiraz) 21 d after inoculation with isolates of *Botryosphaeria* species and potato dextrose agar (control)[a]

Treatment	Isolate	Mean lesions length (mm)[b]	
		Chardonnay	Shiraz
B. lutea	1-046a	52.3 (5.2) a	22.2 (9.4) bc
	3-025	49 (1.4) ab	22.8 (2.1) bc
B. obtusa	1-015	38.6 (2.9) bc	40.4 (3.6) a
	1-050	50.8 (0.6) a	12.6 (1.2) cd
	2-027	55 (3.0) a	25 (3.9) bc
	2-022	22.4 (1.9) d	25 (7.0) bc
	1-080	31.2 (3.9) cd	35.4 (4.2) ab
	2-116	37.6 (7.0) c	35 (5.5) ab
	1-066	52 (6.5) a	18.2 (6.1) c
	1-068	31.4 (4.8) cd	23 (6.7) bc
	1-070	38 (3.1) bc	12.8 (0.7) cd
Control		1.4 (0.5) e	1.8 (0.4) d

[a] Mean lesion length based on 5 replicates of one-year-old canes per treatment.

[b] Standard errors of the mean are presented in parentheses. Means followed by different letters are significantly different ($P = 0.05$) based on least significant difference.

difference in the lesion lengths produced by *B. lutea* and *B. obtusa*, however both were significantly different to the control treatment (LSD = 5.87, $P = 0.05$; Tab. 5). No foliar symptoms were observed after inoculation with *B. lutea* or *B. obtusa*. Both *B. lutea* and *B. obtusa* were re-isolated from the margin of the brown lesions of each of the replicate canes. No fungi were isolated from the controls.

Table 5

Mean lesion length on glasshouse-potted grapevines (*V. vinifera* cv. Chardonnay) 41 weeks after inoculation with *Botryosphaeria* species and potato dextrose agar (control)[a]

Treatment	Mean lesion length (mm)[b]
B. lutea (3-025)	27.2 (1.5) a
B. obtusa (2-027)	26.2 (2.3) a
Control	3.3 (1.2) b

[a] Mean lesion length based on 3 replicate plants per inoculation. Two shoots and the trunk of each glasshouse-potted grapevine were inoculated with each species.

[b] Standard errors of the mean are presented in parentheses. Means followed by different letters are significantly different ($P = 0.05$) based on least significant difference.

Discussion

Surveys of vineyards in the Hunter Valley and Mudgee wine grape growing regions revealed grapevines with extensive decline and dieback. The dieback was most prevalent in Semillon and Chardonnay, however, Shiraz, Cabernet Sauvignon and Muscat were also affected. Symptoms on infected grapevines included bleached canes and cankers associated with pruning wounds and natural openings in the bark. These observations reinforce the theory that *Botryosphaeria* spp. are likely to infect grapevines via wounds. Stunted shoot growth and dead spurs occurred along cordons and wedge-shaped lesions of necrotic tissue, similar to those observed in vines infected with *E. lata*, were observed when the dying wood was cut in cross-section. Similar symptoms were previously recorded in a survey of Hunter Valley vineyards, although that study was conducted only on Semillon (Castillo-Pando *et al*. 2001). Symptoms including bleached canes, internal discolouration of wood and wedge-shaped necrosis have also been reported for infection of grapevines by species of *Botryosphaeria* in South Africa and Western Australia (van Niekerk *et al*. 2004, Taylor *et al*. 2005, Wood and Wood 2005). The symptoms observed in the Hunter Valley and Mudgee were not associated with Eutypa dieback, which agrees with previous studies conducted in Australia (Castillo-Pando *et al*. 2001; Wood and Wood 2005). Furthermore, symptoms associated with Eutypa dieback, such as shortened internodes or yellow cupped leaves with tattered and burnt margins (Carter 1988) were not observed in the current study. The absence of Eutypa dieback may be due to climatic influences or the lack of introduction of the fungus into these regions.

Two species, *B. obtusa* and *B. lutea*, were frequently isolated from the margin of the healthy and dying tissue of symptomatic vines suggesting that these fungi are associated with declining grapevines in the Hunter Valley and Mudgee regions. *B. obtusa* and *B. lutea* were isolated from separate wood samples in all isolations except one, where both species were isolated together. The isolation of *B. obtusa* was not specific to vineyard, cultivar or region. *B. lutea* was less frequently isolated and was found in both regions however this species was most prevalent in the Hunter Valley. Furthermore, the species was not isolated from every vineyard or the cultivar Muscat. *B. obtusa* and *B. ribis* have previously been isolated from Semillon grapevines in the Hunter Valley (Castillo-Pando *et al*. 2001), however *B. ribis* was not isolated in our study.

Pathogenicity studies conducted in Western Australia on the table grape cultivar Red Globe revealed *B. australis*, *B. rhodina* and *B. stevensii* to be pathogenic (Taylor *et al*. 2005). *B. obtusa* was reported as a likely saprophyte by Taylor *et al*. (2005), however, after inoculation of rooted cuttings of Red Globe with this species, Auger *et al*. (2004) concluded it to be a pathogen of grapevines in Chile. *B. obtusa* was also found to produce significantly larger lesions in canes of the cultivar Periquita than in controls (van Niekerk *et al*. 2004). Larignon *et al*. (2001) also reported *B. obtusa* to be responsible for dark streaks in one-year-old canes of Cabernet Sauvignon. Similar results were recorded by Castillo-Pando *et al*. (2001) when *B. obtusa* was inoculated onto one-month-old Chardonnay plantlets and potted Chardonnay grapevines, however the study was conducted with only one isolate. Our study expands on the number of isolates tested by Castillo-Pando *et al*. (2001) and indicates that *B. obtusa* is both, a pathogen of Chardonnay and Shiraz. Out of the 6 isolates of *B. obtusa* tested on

excised green shoots, 4 were weakly pathogenic. However, all *B. obtusa* isolates tested were pathogenic on one-year-old canes and glasshouse-potted grapevines. As in the study by Larignon *et al*. (2001), variation in the pathogenicity of *B. obtusa* was recorded amongst the isolates tested and this requires further examination. The conflicting observations made in the literature on the pathogenicity of *B. obtusa* may be due to the differences in grape cultivars tested and the possibility of within-species variability.

Pathogenicity studies on grapevines with *B. lutea* have been limited and further studies are required to ascertain the effects that this species may have on the productivity of grapevines. Our results indicate that the two isolates of *B. lutea* tested were not pathogenic on excised green shoots but were able to cause disease symptoms in one-year-old canes of Chardonnay and Shiraz. Further experiments with additional isolates of *B. lutea* are required to determine the existence of within-species variability with regards to pathogenicity. To our knowledge, this is the first report of *B. lutea* being isolated from grapevines in eastern Australia and furthermore, we have demonstrated the potential pathogenicity of *B. lutea* in wine grape cultivars of *V. vinifera*.

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